Commission of the European Communities

EURATOM

RADIATION PROTECTION

PROGRAMME

PROGRESS REPORT

1990-91

EUR 14927 DE/EN/FR

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COMMISSION OF THE EUROPEAN COMMUNITIES
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COMMISSIONE DELLE COMUNITÀ EUROPEE
COMMISSIE VAN DE EUROPESE GEMEENSCHAPPEN
COMISSÃO DAS COMUNIDADES EUROPEIAS

EURATOM

Relación de actividades
Programa
PROTECCIÓN RADIOLÓGICA

Beretning
Program
STRÅLINGSBESKYTTELSE

Tätigkeitsbericht
Programm
STRAHLENSCHUTZ

Έκθεση πεπραγμένων
Πρόγραμμα
ΠΡΟΣΤΑΣΙΑ ΑΠΟ ΑΚΤΙΝΟΒΟΛΙΕΣ

Progress report
RADIATION PROTECTION
Programme

Rapport d'activité
Programme
RADIOPROTECTION

Rapporto d'attività
Programma
RADIOPROTEZIONE

Verslag van de werkzaamheden
Programma
STRALINGSBESCHERMING

Relatório de actividades
Programa
RADIOPROTECÇÃO

1990-91

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TABLE OF CONTENTS

TABLE DES MATIERES

B1 Stochastic Effects of Radiation

B2 Non-stochastic Effects of Radiation

B3 Radiation Effects on the Developing Organism

I

EINLEITUNG

INTRODUCTION

INTRODUCTION

VORWORT

In dem Bericht 1990/1991 über den Stand des Forschungs-und Ausbildungsprogramms der Europäischen Atomgemeinschaft auf dem Gebiet des Strahlenschutzes sind die Ergebnisse aus dem ersten Jahr des Programms 1990-1991 zusammengefaßt. Bei diesem Programm handelt es sich um das erste Strahlenschutzprogramm, das durch multinationale Forschungsvorhaben unter Beteiligung mehrerer Partner durchgeführt wurde. Die Berichte werden daher nach dieser neuen Struktur erstellt. Jeder Bericht enthält einen Überblick über die in der Gruppe erzielten Fortschritte, gefolgt von den individuellen Ergebnissen der einzelnen Gruppenmitglieder. Aus einem Budget von 21,2 Mio. ECU wurden im Rahmen des Programms 137 multinationale Vorhaben unter Beteiligung mehrerer Partner mit insgesamt 347 Einzeltätigkeiten finanziert. Die neue Organisationsstruktur scheint sich bestens zu bewähren; es war eine aktive und begeisterte Zusammenarbeit zwischen den Partnern in den für die Vorhaben verantwortlichen Gruppen zu beobachten. Jede multinationale, mehrere Partner umfassende Gruppe hat einen Koordinator, der für den Kontakt mit den Komissionsdienststellen verantwortlich ist. Das gute Funktionieren der neuen Organisation ist weitgehend der konstruktiven Einstellung der Koordinatoren zu verdanken. An dem Programm war auch Schweden beteiligt; schwedische Wissenschaftler haben an mehreren multinationalen Vorhaben mitgewirkt.

Leider hat sich der Abschluß der neuen Verträge infolge der verspäteten Programmentscheidung um einige Monate verzögert, so daß es nicht möglich war, die Fortschrittsberichte wie sonst üblich zum Jahresende zu erhalten. Sie wurden erst Mitte 1991 eingeholt, als die meisten Vorhaben bereits ein volles Jahr liefen. In einigen Fällen wurden Verträge aus dem Programm von 1985-89 als Einzelverträge verlängert, aber soweit möglich wurden die Berichteüber diese Verträge in diesem Bericht zusammenfassend mit berücksichtigt.

Die Berichte sind nach dem Inhalt des neuen Programms in drei Hauptbereiche gegliedert:

A) Strahlen-und Radioaktivitätsexposition des Menschen

1. Messungen der Strahlendosis und ihre interpretation.
2. Transfer und Verhalten von Radionukliden in der Umwelt.

B) Folgen der Strahlenexposition des Menschen; ihre Abschätzung, Verhütung und Behandlung

1. Stochastische Wirkungen von Strahlen.
2. Nicht stochastische Wirkungen von Strahlen.
3. Strahlenwirkungen auf den sich entwickelnden Organismus.

C) Risiken der Strahlenexposition und ihre Bewältigung

1. Abschätzung der Strahlenexposition des Menschen und ihrer Risiken.
2. Optimierung und Durchführung des Strahlenschutzes.

Die Forschung im Bereich Strahlenschutz umfaßt ein weites Themengebiet, viele verschiedene Disziplinen und reicht von der ersten Grundlagenforschung (Formen der Energiesedimentation, Molekularanalyse von Mutationen und DNA-Reparatur) bis hin zu angewandten Aspekten (Messung von Radonkonzentrationen in geschlossenen Räumen oder Verringerung der

Strahlenbelastung von Patienten in der medizinischen Röntgendiagnostik). Wie die Ergebnisse zeigen, war es trotz des breiten thematischen Spektrums und der begrenzten Haushaltsmittel möglich, ein kohärentes, umfassendes Programm zu wahren. Die Politik zur Förderung des Informationsaustauschs und der Zusammenarbeit zwischen Wissenschaftlern wurde bewußt fortgesetzt: neben 42 Sitzungen von Studiengruppen mit Vertragspartnern und geladenen Sachverständigen wurden 13 internationale Seminare und Workshops veranstaltet. Die 13 Protokolle dieser Veranstaltungen und die Veröffentlichungen über die Vertragsarbeiten sind ein Beweis für die bedeutende Rolle der europäischen Forschung auf dem Gebiet des Strahlenschutzes. Durch die Einführung multinationaler Vorhaben mit mehreren Partnern wurde die bereits durch frühere Programme erfolgreich geförderte Zusammenarbeit zwischen Wissenschaftlern auf dem Gebiet des Strahlenschutzes konsolidiert. Im Rahmen des Programms konnten die Gemeinsamen Absichtserklärungen mit den USA und Kanada weiter erfüllt und die Kontakte zu internationalen Organisationen und Ländern außerhalb der Gemeinschaft fortgesetzt werden. Ein Briefwechsel zwischen der KEG und der Radiation Effects Research Foundation (RERF) in Hiroshima bietet jetzt die Möglichkeit zu einer intensiveren Zusammenarbeit mit japanischen Kollegen.

Ein wichtiger neuer Aspekt des Programms ist die Entwicklung einer koordinierten Bildungspolitik im Rahmen von European Radiation Protection Education and Training (ERPET), die dazu dienen soll, einen repräsentativen Querschnitt an Fachkenntnis zu erhalten und die beruflichen Möglichkeiten junger Wissenschaftler zu fördern, die sich in den Strahlenschutz einarbeiten. In den vergangenen 17 Monaten wurden sieben Ausbildungskurse abgehalten, bei denen es sowohl um den allgemeinen Strahlenschtz als auch um speziellere Themen wie Radioökologie und Bewältigung von nuklearen Störfällen ging.

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PREFACE

The 1990/1991 Progress Report of the Radiation Protection Programme of the Commission of the European Communities summarises the results from the first year of the 1990-1991 programme. This programme was the first radiation protection programme implemented through multi-national, multi-partner research projects and the reports are consequently presented according to this new structure. Each report presents an overview of the progress achieved in the group followed by the individual results from each of the group members. The programme funded 137 multi-national, multi-partner proposals involving 347 individual activities from a budget of 21.2 MECU. The experience with the new type of management has been extremely positive and there has been an active and enthusiastic collaboration between the different partners within the groups. Each multi-national, multi-partner group has a coordinator who is responsible for the contact between the Commission's Services and the new type of management has been successful largely due constructive attitude of the coordinators. Sweden was associated with the programme and Swedish scientists have been integrated in several of the multi-national proposals.

The establishment of the new contracts was unfortunately delayed for some months due to delay in the programme decision, and it has therefore not been possible to collect the progress reports at the end of the year as has been the practice previously. The reports were collected in mid 1991, when the majority of the projects had been running for a full year. In some cases contracts from the 1985-1989 programme were extended as individual contracts but where possible, the reports from these contacts have been grouped for presentation here.

The progress reports are grouped according to the content of the new programme which has three main areas:

A) Human exposure to radiation and radioactivity, having the sectors:

1. Measurement of radiation dose and its interpretation.
2. Transfer and behaviour of radionuclides in the environment.

B) Consequences of radiation exposure to man; assessment, prevention and treatment, having the sectors:

1. Stochastic effects of radiation.
2. Non-stochastic effects of radiation.
3. Radiation effects on the developing organism.

C) Risks and management of exposure, having the sectors:

1. Assessment of human exposure and risks.
2. Optimization and management of radiation protection.

Research in radiation protection covers a wide range of topics, involves many different disciplines and stretches from very basic research, such as patterns of energy deposition, or molecular analysis of mutations and DNA repair, to the more applied aspects, such as measurement of indoor radon concentrations, or reduction of patient exposure in medical diagnostic radiology. In spite of the broad scope and the limited funding it has still been possible to maintain a coherent and comprehensive programme as shown by the results presented here. The programme has

continued its policy of promoting the exchange of information and cooperation between scientists by organising 42 study group meetings with contractors and invited experts and 13 international seminars and workshops. The 13 proceedings of these meetings and the publications originating from the contract work testify to the important role played by European research in the field of radiation protection. The formalisation of multi-national, multi-partner projects has contributed to the consolidation of the cooperation between scientists working in radiation protection which had been so effectively stimulated by previous programmes. The programme has continued to implement the Memoranda of Understanding with the USA and Canada, and has continued its contacts with international organisations and other countries outside the Community. An exchange of letters between the CEC and the Radiations Effects Research Foundation (RERF) at Hiroshima now opens possibilities for more intensive collaboration with Japanese colleagues.

One important new facet of the programme's activities has been the development of a coordinated training policy under ERPET (European Radiation Protection Education and Training) to maintain a cross-section of expertise and promote the career prospects of young scientists entering the field of radiation protection. Seven training courses have been organised during the past 17 months covering general radiation protection as well as more specialised areas such as radioecology and nuclear emergency management.

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PREFACE

Le rapport d'activité 1990/1991 concernant le programme de radioprotection de la Commission des Communautés européennes résume les résultats de la première année du programme 1990-1991. Il s'agit du premier programme de radioprotection à avoir été mis en oeuvre par le biais de projets multinationaux, multipartenaires. La présentation des rapports reflète par conséquent cette nouvelle structure. Chaque rapport contient un aperçu des activités déployées au sein d'un groupe, suivi des résultats personnels de chacun des membres du groupe. Dans le cadre de ce programme, 137 projets multinationaux et multipartenaires représentant 347 activités différentes ont été financés par un budget de 21.2 millions d'écus. La mise en oeuvre de ce nouveau type de gestion a été une expérience très positive et la collaboration a été active et enthousiaste entre les différents partenaires au sein des groupes chargés des divers projets. Chaque groupe multinational, multipartenaires a un coordinateur qui est chargé des contacts avec les services de la Commission et les résultats positifs du nouveau type de gestion sont dus en grand partie à l'attitude constructive des coordinateurs. La Suède a été associée au programme et des chercheurs suédois ont participé à plusieurs projets multinationaux.

L'établissement des nouveaux contrats a malheureusement été retardé de quelques mois en raison du retard avec lequel avait été adoptée la décision arrêtant le programme, de sorte qu'il n'a pas été possible de compiler les rapports d'activité à la fin de l'année comme c'était la coutume antérieurement. Les rapports ont été réunis vers le milieu de l'année 1991, alors que la majorité des projets était en cours depuis au moins un an. Dans certains cas, les contrats se rattachant au programme 1985-1989 ont été prorogés à titre individuel, mais, chaque fois que cela a été possible, les rapports relatifs à ces contrats ont été intégrés au rapport d'activité présenté ici.

Les rapports d'activité sont regroupés en fonction du contenu du nouveau programme, qui couvre trois principaux domaines:

A) Exposition de l'homme aux rayonnements et à la radioactivité:

1. Mesure de la dose d'irradiation et son interprétation.
2. Transfert et comportement des radionucléides dans l'environnement.

B) Conséquences de l'exposition de l'homme aux rayonnements; évaluation, prévention et traitement:

1. Effets stochastiques des rayonnements.
2. Effets non stochastiques des rayonnements.
3. Effets des rayonnements sur l'organisme en cours de développement.

C) Risques et gestion de l'exposition:

1. Evaluation de l'exposition de l'homme et des risques.
2. Optimisation et gestion de la radioprotection.

La recherche en radioprotection couvre une vaste gamme de thèmes, implique de nombreuses disciplines différentes et s'étend de la recherche fondamentale (par exemple modèles de dépôt d'énergie ou analyse moléculaire des mutations et de la réparation de l'ADN) à la recherche appliquée (mesure des concentrations de radon à l'intérieur des locaux ou réduction de

l'exposition des patients en radiodiagnostic médical). En dépit de l'ampleur du programme et de la limitation des crédits, il a encore été possible de maintenir, ainsi que le montrent les résultats présentés ici, un programme cohérent et exhaustif. Dans le cadre de celui-ci, on a poursuivi une politique de promotion des échanges d'informations et de la coopération entre les chercheurs en organisant 42 réunions de groupes d'étude avec des contractants et des experts invités et 13 séminaires et ateliers internationaux. Les 13 comptes rendus de ces réunions et les publications issues des travaux contractuels témoignent de l'importance du rôle joué par la recherche européenne dans le domaine de la radioprotection. L'exécution de projets multinationaux multipartenaires a contribué au renforcement de la coopération entre chercheurs travaillant dans le domaine de la radioprotection qui avait été si efficacement stimulée par les programmes antérieurs. On a poursuivi la mise en oeuvre de la déclaration commune d'intention signée avec les Etats-Unis et le Canada et on a maintenu les contacts avec les organisations internationales et les pays tiers. Un échange de lettres entre la CCE et la Radiations Effects Research Foundation (RERF) d'Hiroshima ouvre maintenant des possibilités de collaboration plus étroite avec les collègues Japonais.

Une nouvelle et importante facette des activités liées au programme a été le développement d'une politique de formation coordonnée dans le cadre d'ERPET (European Radiation Protection Education and Training) en vue de maintenir un échange de connaissances spécialisées et de promouvoir les perspectives de carrière des jeunes chercheurs en radioprotection. Sept cours de formation ont été organisés au cours de 17 derniers mois; ils couvrent la radioprotection en général, ainsi que des domaines plus spécialisés, tels que la radioécologie et la gestion des cas d'urgence nucléaires.

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Recherche en radioprotection

II

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Beratender Verwaltungs- und Koordinierungsausschuss "STRAHLENSCHUTZ"

Members and Experts 1990-91

Management and Coordination Advisory Committee "RADIATION PROTECTION"

Membres et Experts 1990-91

Comité consultatif en matière de Gestion et de Coordination "RADIOPROTECTION"

Mitglieder und Experten 1990-91
Beratender Verwaltungs- und Koordinierungsausschuss "STRAHLENSCHUTZ"

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Management and Coordination Advisory Committee "RADIATION PROTECTION"

Membres et Experts 1990-91
Comité consultatif en matière de Gestion et de Coordination "RADIOPROTECTION"

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III

FORSCHUNGSTÄTIGKEIT STRAHLENSCHUTZ

RESEARCH IN RADIATION PROTECTION

RECHERCHE EN RADIOPROTECTION

III A

EXPOSITION DES MENSCHEN DURCH STRAHLEN UND RADIOACTIVITÄT

HUMAN EXPOSURE TO RADIATION AND RADIOACTIVITY

EXPOSITION DE L'HOMME AUX RAYONNEMENTS ET À LA RADIOACTIVITÉ

Contract: Bi6-026 **Sector: A11**

Title: Collaboration on research and development concerned with the methodology and data in radiation dosimetry.

1 Dennis EURADOS

I. Summary of Project and Global Objectives

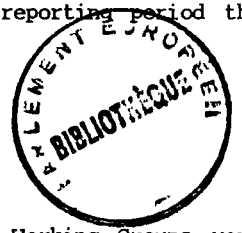
Both generally and specifically within the European Communities the global objectives of this project are:

1. The stimulation of collaborative developments and research into methods and techniques for the evaluation of exposures to and risks from ionising radiations.

2. The harmonization of methods of assessing and researching radiation exposures by means of intercomparisons, workshops, seminars and by active collaboration.

3. The collection and evaluation of physical data relevant to the assessment of the biological effects of ionising radiations and to the assessment of occupational and environmental exposures.

The society operates through Working Groups each composed of 10 to 15 scientists from European laboratories. During the reporting period the following Working Groups actively continued:



Working Group 2. **Skin Dosimetry.**

Working Group 4. **Numerical Dosimetry.**

Working Group 6. **Assessment of Internal Dose.**

In addition to these Working Groups, the following Working Groups were started:

Working Group 7. **Radiation Spectrometry in Working Environments.**

Working Group 8. **Development of Individual Dosemeters for External Penetrating Radiations.**

Working Group 9. **Criticality Accident Dosimetry.**

Working Group 10. **Basic Physical Data and Characteristics of Radiation Protection Instrumentation.**

Both Working Group 8 and Working Group 10 are continuations and extensions of earlier groups.

Head of Project 1: Dr. Dennis

II Objectives for the reporting period

To maintain Working Groups 2,4 and 6 and to bring into effective operation Working Groups 7,8, 9 and 10.

III Objectives for next period

To consolidate and where necessary redirect active scientific collaboration within the Working Groups. Working Group 6 will complete a feasibility study for the formation of European Registries of Internal Dosimetry. Working Group 9 will continue to plan and study for an international intercomparison of criticality dosimetry systems. Working Group 10 will make a comprehensive evaluation of W-values for neutrons to be used with gas detectors in mixed field dosimetry.

IV Progress achieved including publications

Working Group 2. Skin Dosimetry.

Some progress has been made in the preparation of the document "Review of Survey Instruments for the Measurement of Dose Rates in Mixed Fields of Beta and Photon Radiations". It is expected to be completed in draft form by late November 1991. The planning of an international workshop on skin dosimetry was completed. This workshop was jointly organised with the Irish Nuclear Energy Board and the Commission with sponsorship by the US Department of Energy. It will be held near Dublin in Ireland during May, 1991.

Working Group 4. Numerical Dosimetry.

A benchmark study of Bonner spheres for neutron spectrometry was extended. Intercomparison of response functions for these detectors revealed a problem with thermal neutron group cross-sections. A set of these cross-sections for polythene has been compiled. Problems in the practical numerical implementation of voxel phantoms for external and internal dosimetry have been discussed.

Working Group 6. Assessment of Internal Dose.

An experimental programme for using stable isotope tracers to investigate the metabolism of elements of interest to radiological protection has been established.

Progress has been made in setting up a UK Autopsy Registry for cases of internal contamination with radionuclides and a basis for a European registry of internal dose assessment computer models has been investigated using ICRP Task Group proposals.

A joint Task Group has been established with EULEP to produce improved respiratory tract models relating intakes of radionuclides by workers to organ doses and bioassay measurements.

A proposal has been formulated for an intercomparison study of whole body monitoring by European laboratories using a person adventitiously contaminated internally with gamma-emitting radionuclides.

Working Group 7. Radiation Spectrometry in Working Environments.

The objectives and membership of this Working Group have only recently been established. It is expected to start active collaboration during 1991.

Working Group 8. Development of Individual Dosemeters for External Penetrating Radiations.

The results of a joint neutron irradiation of individual dosemeters at PTB, GSF, and PSI were analyzed and published. This is a unique guide to the current performance of etched-track neutron dosemeters.

A joint study of the background and sensitivity characteristics of etch plastics from different sources and manufacturers was completed. The results are being assessed and will be published.

Working Group 9. Criticality Accident Dosimetry.

Spectrometric measurements were made of the leakage spectra from the lead shielded SILENE reactor at Valduc, France. A report of these measurements has been completed and will be submitted for publication. Liaison with IAEA on the possibility of an international intercomparison of criticality dosimetry systems has been established.

Working Group 10. Basic Physical Data and Characteristics of Radiation Protection Instrumentation.

A work programme has been established consisting of:

a). Basic physical data for ionization in gases.
b). Modelling and experimental work on discharge processes in proportional counters.
c). Development of gas ionisation devices for dosimetry.

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Contract: Bi6-322 **Sector: A11**
Title: Quantities, units and measurement techniques for ionizing radiation.

1 Allisy Bureau Intern.des Poids et Mesures

I. Summary of Project and Global Objectives

The International Commission on Radiation Units and Measurements (ICRU), since its inception in 1925, has had as its principle objective the development of internationally acceptable recommendations regarding:

(1) Quantities and units of radiation and radioactivity
(2) Procedures suitable for the measurement and application of these quantities in radiation protection as well as in clinical radiology and radiobiology.
(3) Physical data needed in the application of these procedures, the use of which tends to assure uniformity in reporting.

The ICRU considers and makes recommendations in the field of radiation protection. In this connection, its work is carried out in close cooperation with the International Commission on Radiological Protection (ICRP) and it should be noted that much of the work in the field of clinical radiology is conceived to lead to a direct and considerable dose reduction for the patient.

The ICRU endeavors to collect and evaluate the latest data and information pertinent to the problems of radiation measurement and dosimetry and to recommend the most acceptable values for current use.

The Commission's recommendations are kept under continual review in order to keep abreast of the rapidly expanding uses of radiation and also to provide answers to urgent questions such as those related to environmental monitoring connected with nuclear reactor accidents.

Head of Project 1: Prof. Allisy

II Objectives for the reporting period

Completion of the drafting work on ICRU reports on:

(1) Supplemental information on tissue substitutes in radiation dosimetry and measurement.
(2) Measurement of dose equivalent
(3) Phantoms used in therapy, diagnosis and protection
(4) Stopping powers and ranges for protons and alpha particles

III Objectives for next period

Publication of the reports cited above, initiation of work on beta-ray dosimetry for radiation protection and on determination of body burdens of radionuclides and completion of drafting work on ICRU reports concerned with:

(1) Fundamental quantities and units
(2) Dose specification for reporting external beam therapy with photons and electrons
(3) Dose specification for reporting interstitial therapy

IV Progress achieved including publications

Substantial progress has been achieved toward meeting the goals for the current reporting period.

Following ICRU review and approval of the document providing supplemental information on tissue substitutes in radiation dosimetry and measurements, work was begun on the needed modifications and revisions identified as a result of the review. This is now substantially complete and it is expected that the report can be sent to the printer in the near future. The document, ICRU Report 46, Supplemental Information on Tissue Substitutes in Radiation Dosimetry and Measurement, is a companion volume expanding on the material covered in ICRU Report 44, Tissue Substitutes in Radiation Dosimetry and Measurement. The new document provides specific information on representative sets of tissues that illustrate the effects of tissue composition variation on the pertinent radiation interaction quantities. It includes photon, electron, proton and neutron interaction data for body tissues, covering the age interval from fetus to adult.

The new report on measurement of dose equivalent is to be the third report in the series treating determination of dose equivalent. The report emphasizes principles of measurement, characterization of instruments, calibration and the impact of the new operational quantities on the design of future instruments. The draft report was approved by the Commission at its last meeting, and the printer's manuscript is now being prepared.

Also approved by the Commission at the last meeting was the report on phantoms used in therapy, diagnosis and protection involving ionizing radiation. The report emphasizes organ and body masses and geometries to meet the need for human anatomical data in the development of phantoms and computational models. The influence of age, sex and ethnic origins on human anatomy is treated. Existing types of phantoms and computational models used with photons, electrons, protons and neutrons are reviewed. The specifications of phantoms and computational models are provided. The printers manuscript of the report is being prepared.

The drafting work on a new report concerned with stopping powers for protons and alpha particles was also completed during the current reporting period. ICRU work on stopping powers encompasses the treatment of electrons and positrons (ICRU Report 37), protons and alpha particles, and heavy ions. The report on protons and alpha particles includes discussions of the stopping power formula and corrections, electronic (collision) stopping powers, nuclear stopping powers, comparison of tabulated and experimental stopping powers, energy loss straggling, and methods for stopping power measurements. The report tabulates, for material of interest in radiological physics and biomedical dosimetry: (1) electronic, nuclear and total stopping powers, (2) ranges, and (3) detour factors. Again, the draft report was approved by the Commission and preparation of the printer's manuscript is now underway.

Work continued during the current reporting period on the many other projects aimed at the preparation of ICRU reports. Noteworthy among these are those concerned with:

(1) absorbed dose standards for photon irradiation and their dissemination,
(2) stopping powers for heavy ions
(3) fundamentals of particle counting applied to radioactivity measurements,
(4) in situ gamma spectrometry in the environment
(5) secondary electron spectra resulting from charged particle interactions.

The identification of needed new activities also represents important progress. Here, the determination to begin new efforts concerned with the following topics is noteworthy.

(1) beta-ray dosimetry for radiation protection
(2) determination of body burdens for radionuclides
(3) statistical aspects of environmental sampling
(4) tissue substitutes, characteristics of biological tissue and phantoms for ultrasound

Initiation of work on these new projects will begin in the near future

Progress Report

Contract: Bi6-347a Sector: A11

Title: The implementation of the operational dose quantities into radiation protection dosimetry (NRPB Association)

1	O'Riordan	NRPB
2	Marshall	AEA Technology Harwell Laboratory
3	Lembo	ENEA
4	Chartier	CEA - FAR

I. Summary of Project and Global Objectives

The main objectives of this project are to improve the measurement of spectral and angular distributions of external radiations in the workplace and to examine the implications of these measurements for personal dosimetry.

(1) To develop and compare techniques for measuring spectra and directional distribution of X and γ radiation in the workplace. (NRPB/AEA)

(a) To review methods available for measuring spectra.

(b) To assemble equipment based on G-M detectors, NaI detectors and Ge detectors and compare their utility for measurements in workplaces.

(c) In parallel with the practical work, calculations will be carried out to examine the theoretical utility of the different methods.

(d) To examine unfolding techniques developed for X-ray and neutron spectroscopy, adapt them to obtain energy and angular distributions from the results of measurements.

(e) To examine the implications of measurements and calculations for the estimation of $H^*(10)$ and H_E in the workplace.

(2) Development of methods to measure the spectra of neutron radiations in the workplace. (AEA/ENEA)

(a) To refine existing measurement techniques.

(b) To make measurements in a suitable workplace with a neutron field.

(c) To examine the feasibility of making calculations of neutron spectra in support of measurements.

(3) Calculation and experimental measurements to examine the performance of personal dosemeters. (ENEA/CEA)

(a) Calculation of the fluence and energy of backscatter from the ICRU sphere and other phantoms in ISO beams of X and γ radiation.

(b) Measurements to verify the above calculations.

(c) To examine practical calibration techniques for the new quantities in personal dosimetry and the uncertainties introduced by the use of transfer (tertiary) standards.

(4) To assess the implications of spectral and spatial distribution measurements on personal dosimetry in the workplace and on methods of calibration. (NRPB/AEA/ENEA/CEA)

(a) To review the results of spectral measurements made in the workplace and classify them into energy and spatial bands.

(b) To recommend the best methods of measuring energy and spatial distributions in the workplace.

(C) To recommend appropriate methods of calibration, given the uncertainties due to spectral and angular distribution.

Head of Project 1: Mr O'Riordan

II. Objectives for the reporting period

The co-ordination role involved reviewing the proposals of participating organisations to ensure that the research programme is cohesive (two organisations were incorporated into the project subsequent to the original proposal from NRPB and AEA).

III. Objectives for next period

(i) To develop and compare techniques for measuring energy spectra and directional distribution of photon radiation in the workplace in collaboration with UKAEA.

(ii) To assess the implications of energy spectra and spatial distribution measurements for the calibration of personal dosemeters used in the workplace in collaboration with the other contractors in this project.

IV. Progress achieved including publications

Two co-ordination meetings were arranged and a work programme has been specified and agreed with participants, identifying particular projects and work areas to be tackled. In addition, practical work has been carried out at NRPB to improve techniques for measuring the energy spectra and directional distribution of photon fields. The technique developed by NRPB for these measurements has been extensively reviewed and equipment changes have been made to reduce the risk of contamination in the workplace and to increase the speed of measurement. One of the main aims of the project is to investigate the feasibility of using sodium iodide rather than Geiger-Muller detectors for measurements in the workplace. Preliminary results are encouraging, involving the use of special filters so that NaI detectors can provide spectral and dose rate information at the same time. Investigations are currently being carried out to install suitable collimation for the detectors so that directional information can also be obtained. This work is being carried out in close collaboration with AEA so that unfolding techniques used for examining neutron spectral measurements can be successfully applied to photon field measurements.

Head of Project 2: Dr Marshall

II. Objectives for the reporting period

There has been delay in commencing work as new participants were added to the original UKAEA/NRPB proposal. The main task has been to meet with the new contractors and agree a clear programme of work for each contactor. To this end an initial meeting was held in May 1990 and a further meeting to clarify the position in December 1990.

III. Objectives for next period

(i) To develop and compare techniques for measuring energy spectra and directional distribution of photon radiation in the workplace in collaboration with NRPB.

(ii) To develop methods to measure the spectra of neutron radiations in the workplace in collaboration with ENEA.

(iii) To assess the implications of energy spectra and spatial distribution measurements for the calibration of personal dosemeters used in the workplace in collaboration with all other contractors in the project.

IV. Progress achieved including publications

It is intended that the EGS4 Monte Carlo computer code will be used to determine theoretically the response of photon detection systems as a function of energy and angle. Systems will include: devices which rely on filtration, detector response and collimation to provide simple energy and angular information; and photon energy detectors (NaI and Ge) with appropriate collimation.

Work directly for this project has just started (determining the response of a NaI crystal), but much work has been carried out under a related project to install the EGS4 code on to personal computers (PC's: IBM compatible 286, 386 and 486 machines) and to validate its operation, using various benchmark programs and by comparing the depth dose distribution for electrons with published material. We are confident that the program is operating correctly and that we can apply it to the current problems.

The neutron spectrometry system developed at Harwell has been used recently to measure spectra at the Silene facility in France. While the measurements were made using separate CEC funding, operational improvements have been made under this contract. These have involved transferring the computer programmes used to unfold the raw counter data from an IBM mainframe to a PC, and making these programs easier to use. These improvements will considerably reduce the time required to produce neutron spectra from raw data. In due course the data from the Silene measurements will be compared with spectra obtained elsewhere, as part of a program to provide a library of spectra and to evaluate the accuracy with which measurements can be made for a variety of spectra and situations.

Head of Project 3: Dr Lembo

II. Objectives for the reporting period

Due to late signature of contract documents delay had occurred. To agree a detailed plan of work with other contractors and initiate preliminary calculations.

III. Objectives for next period

(i) To develop methods to measure the spectra of neutron radiations in the workplace in collaboration with UKAEA.

(ii) Calculation and experimental measurements to examine the performance of personal dosimetry in collaboration with CEA.

(iii) To assess the implications of energy spectra and spatial distribution measurements for the calibration of personal dosemeters used in the workplace in collaboration with all other contractors.

IV. Progress achieved including publications

A new version of the MCNP Monte Carlo Code has been written at ENEA Bologna that transports electronics besides neutrons and photons, incorporating the EGS4 electron modules. The code has been implemented on the IBM 3090XA mainframe. The code which is currently being benchmarked, will be used for the calculation of ICRU operational quantities and to calculate parameter which are representative of the radiation fields.

A tissue equivalent ICRU sphere has been brought from the Gottingen University and it is being used in order to determine backscatter factors with different x-ray beams from the ISO catalogue in order to validate MCNP-E in this range of energies.

Until now some preliminary calculations were performed in the kerma approximation in order to determine backscatter factors (versus backscatter angle), mean energy of the backscattered radiation (versus backscatter angle) and energy spectrum of the backscattered radiation on the axis of the bean frontally of the ICRU sphere for ISO narrow spectrum series and BIPM series ranging from 10 kV to 300 kV. The results obtained so far are in a fairly good agreement with those obtained by NRPB (Dimbylow, Francis and Bartlett) and PTB (Grosswendt). A series of reference tables will be produced for a variety of different radiation x-ray spectra used for the calibration of personal dosemeters. The calculations will be extended to the determination of the some quantities for the real phantoms used for calibration, eg, cuboids or slabs, and for the radiation fields reproducing those determined in the work places.

Head of Project 4: Dr Chartier

II. Objectives for the reporting period

Only joined project in late 1990 when contract signed and therefore have only just had a meeting with other contractors to discuss programmes of work.

III. Objectives for next period

(i) Calculation and experimental measurements to examine the performance of personal dosimetry in collaboration with ENEA.

(ii) To assess the implications of energy spectra and spatial distribution measurements for the calibration of personal dosemeters used in the workplace in collaboration with other participants in project.

IV. Progress achieved including publications

In its contribution to the CEC contract, the CEA is involved in the practical implementation of the ICRU quantities for individual monitoring. The practicality of the "ICRU sphere", among other characteristics, has raised serious questions, and an ICRU Working Group has recommended, in the course of 1990, the use of a practical phantom (slab) and the definition of a new operational quantity related to the phantom. In order to take into account the most recent trends of ICRU, modifications have to be brought to the combination of the ENEA/CEA program previously established. A revised program is now under consideration.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

II Univ. Degli Studi di Roma
"Tor Vergata"
Via O. Raimondo
I-00173 Roma

Contract no.: BI6-A-302-I

Head(s) of research team(s) [name(s) and address(es)]:

Prof. C. Franconi
Dipart. di Medicina Interna
II Univ. Degli Studi di Roma
Via O. Raimondo
I-00173 Roma

Telephone number: 06-6131170

Title of the research contract:

Development of high sensitivity spectrometric alpha emitter detector for use in monitoring of environment and radio protection.

List of projects:

1. Development of high sensitivity spectrometric alpha emitter detector for use in monitoring of environment and radio protection.

Title of the project no.: 1

Development of high sensitivity spectrometric alpha emitter detector for use in monitoring of environment and radio protection.

Head(s) of project:

Prof. C. Franconi - Dipartimento di Medicina Interna - II Università degli Studi di Roma - Via O. Raimondo - 00173 Roma - Tel. (06)6131170.

Scientific staff:

Dr. A. Magrini, Dr. G. Izzo, Prof. K.V. Ettinger, A. Canichella, I. Vannucci, P. Necci.

I. Objectives of the project:

The aim of the project was to design and construct a prototype of a reliable high sensitivity alpha particle detector which will complement solid state track detectors in applications which require superior energy resolution and in which the need for etching is inappropriate. the project called for a multiwire proportional chamber of alpha particles. adaptable for samples deposited onto carriers or introduced in a gaseous form. The equipment should be able to operate unattended, under a control of a small computer or microprocessor, and as a possible refinement a remote setting of the operating regime and a remote reporting was considered.

II. Objectives for the reporting period:

The final report covers in fact the whole duration of the project with a particular attention to the work done during the last year. During that time the design of the chamber should be finalized, tested and evaluated with realistic samples. It was also essential to check the operational characteristics of the chamber, optimized the gas gain in comparison with the electronic signal gain. Overall, the most important aim was to put the chamber in actual operation, for significant periods of time. An important characteristic was to measure the resolution and efficiency of detection in the chamber for available alpha activities.

III. Progress achieved:

1. CHOICE OF THE TYPE OF DETECTING SYSTEM

The use of multiwire proportional counter (MWPC) as a detecting element in the high sensitivity spectrometric alpha emitter detection system was a consequence of its superior characteristics when used with large surface area radiation sources. Following the assumption that the radionuclide to be detected will be deposited on the filter paper, adhesive strip or similar carrier and not dissolved or ashed, at least during the initial assay, there are the following alternative techniques available:

a. Contact or near-contact scintillation counting using a block of bare scintillator made of non hygroscopic material,

b. Spark counting using a multiwire spark counter.

The first alternative is suitable for spectrometry of alpha radiations but will not allow counting of gaseous emitters. The second alternative does not provide a spectrometric energy discrimination.

The current interest in the application of wire chambers goes back to 1968, following the work of Charpak et al. (1) who have shown that each wire in a MWPC can be used as a separate counter. The drift chamber was introduced in about 1971 (2, 3). The theory and practice of multiwire detection devices is given in a considerable detail in monographs of P. Rice-Evans (4) and F. Sauli (5).

It was decided to make the MPWC using a plane parallel geometry because in this geometry the overall dimensions of the detector are significantly smaller than in a cylindrical geometry. There is, however, no doubt that large cylindrical chambers are more sensitive, have superior energy resolution , even though they require elaborate procedures to change the sample.

2. DESIGN OF THE DETECTION CHAMBER

The design ultimately adopted in the reported work is that of a drift chamber (Figure 1). In this type of device the volume of the proper counter is contiguous to the so called drift space in which the interactions predominantly take place and the electrons and positive ions drift to their respective collecting electrodes. The guiding field in the drift region, does not initiate multiplication but assures an efficient collection of charges. Once in a volume of the counter proper the gas multiplication takes place and the waveform of the pulse on the anode wire reflects essentially the kinetics of charges within the proper counter volume rather than in the drift region.

A set of wires in the vicinity of sample which is in form of a deposit on a support acts as a Frisch grid. The Frisch grid makes the ionisation in the drift space and then in the active counter almost independent on the angle at which the alpha particle is emitted (as long as it is emitted into the upper half space!). This grid also shields the drift space from any charges, likely to be created by friction, that might have been carried on the sample support, which can be either metallic or dielectric.

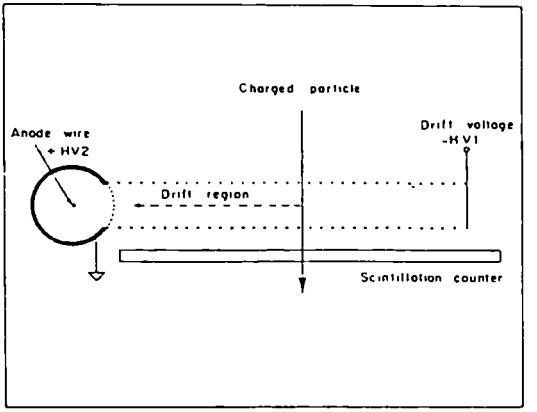

Figure 1 . [From Sauli (5)] Principle of operation of a single cell drift chamber. A set of field wires at suitable potentials generates in the drift space a region of uniform field. The electrons produced by an ionizing event migrate to one end of the cell, where avalanche multiplication occurs in a single wire proportional counter. (The scintillation counter shown is not used in the current application).

The electrode structure of the whole counter is shown in Fig. 2. There are essentially three separate counters sharing a large part of electrodes. Two counters are close to each other. They operate in parallel and they form the principal element for detection of alpha radioactivity. The third structure is an auxiliary counter. Its role is to detect alpha particles from a small calibration source, collimated into a pencil beam by an aluminium cover pierced with a narrow hole. This auxiliary counter which has the same electrodes as the principal counter and the same potentials applied to them and shares the same gas mixture is used to control the working point of the principal counter.

The alpha particle energies from ^{241}Am are 5.486 MeV and 5.443 MeV, which are resolved by the counter. The Americium alphas are detected by two window discriminators corresponding to both slopes of the ^{241}Am peak and two digital integrators. The imbalance signal can be used for variation of the EHT supplying the chamber. We shall indicate in the discussion that there are alternative simpler and more effective techniques of derivation of the correction signal for stabilization.

The radioactive contamination is deposited on a carrier and the sample can be changed without upsetting operation of the system for more than of a minute. The gaseous alpha emitter (e.g. radon) can be introduced into the same counter , without changing the electrode structure, if only detection with very low quality spectrometry is required. For this mode of operation signals from both parts of the principal counter must be linearly added. Electrode structures, with parallel wires forming structures resembling cylindrical counters are easy to make and in fact were made but could not be properly tested because of lack of available and reproducible gaseous alpha emitters.

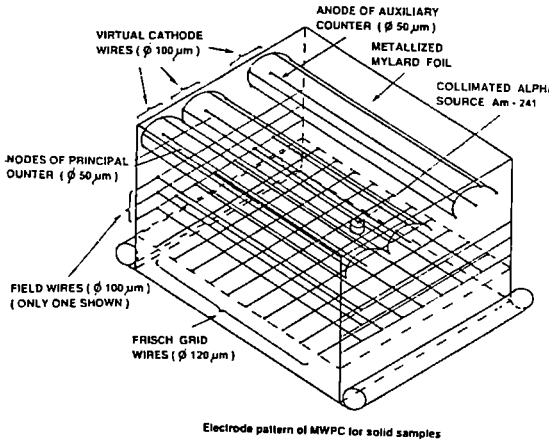

Fig. 2 - The electrode structure of the MWPC for solid samples.

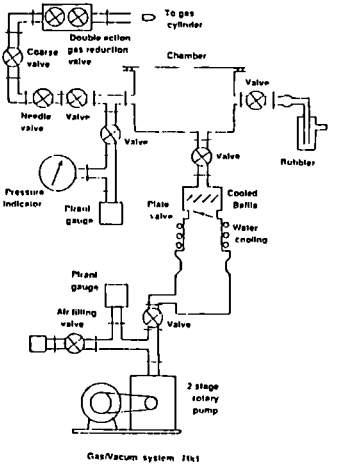

Fig. 3 - A Mk 1 gas filling system with a diffusion pump.

3. GAS MIXTURES AND FILLING SYSTEMS

The electrode system was placed inside a brass box fitted with feed through insulators and connected to the gaspumping and filling system. Originally a Mk1 system (Fig. 3) was used in which a rotary two staged pump has created an initial wacuum and a diffusion pump with cooled baffles was used to empty the chamber down to 10^{-4} - 10^{-3} Torr. The quality of vacuum and the amount of leak was determined from the observation of change of residual vacuum pressure with time. Only when that was found satisfactory, the vacuum system was cut off by a valve, to be filled with a counting gas from the gas cylinder. The gas pressure was kept at the atmospheric level by means of a bubbler filled with a silicon oil to a height of about 2cm. The needle valve was used to adjust the rate of flow to about 0.5 bubbles/second, bubbles being of about 3 mm in diameter.

This procedure, albeit leading to satisfactory results, was superfluous. A simpler gas system was assembled (Mk2), in which a single stage rotary pump is used to bring initially the pressure down to about 0.15 Torr, followed by flushing the box containing the MWPC with the counting gas (Fig. 4). This procedure is much faster and gives satisfactory results, though more wasteful on the counting gas.

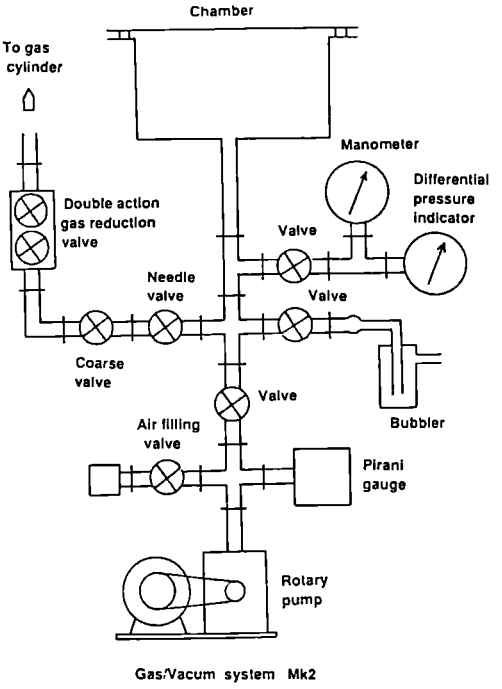

Fig. 4 . A Mk2 gas filling system using rotary pump and gas flushing.

The valves used originally were hand operated; they have been subsequently replaced by electromagnetically operated analogues with an exception of the needle valve which may not require adjusting. However, another needle valve parallel to the first will be installed in the near future, to control fast purging of the chamber after an exposure to air.

There is a variety of counting gases which can be used in the chamber of a type considered here, because the drift velocities are of little importance and the main factors are sufficiency and stability of gain. There are detailed references in the literature to the properties of gases for use in wire chambers (6) and to the gain factor attainable (7) . We have tried both Ar + CO_2< and Ar + hydrocarbon mixtures. While a mixture of 98 % of Ar and 2 % of CH_4 appears to have an easily attainable stable gas gain of about 10^4 the use of an commercial mixture of 90 % Ar and 10 % of CH_4 was almost as satisfactory. An advantage of in using commercial mixtures is that one does not need to check the composition with a gas chromatograph. The gases supplied for the use with the chamber were of Research Grade quality.

Pressure measurements in the system are made with a Bourdon type gauge which indicates the positive and negative pressures. Positive pressures may arise if a valve leading to the bubbler jams. A small

differential diaphragm manometer is used to indicate when the gas pressure in the chamber is precisely atmospheric. This is to protect the bubbler which can tolerate only very small differentials, otherwise the silicon oil in the bubbler will be blown out or sucked in.

4. CONSTRUCTION OF THE CHAMBER.

The internal structure of the chamber can be schematically seen in Fig. 2 . The structures supporting the electrode system are made from epoxy resin - glass fibre composition, which has been thermally treated to reduce the outgassing rate. Wires made of gold plated tungsten having sizes indicated in Fig. 2 were soldered under stress to the metallized pads on the frames. The wires were optically checked for defects. The individual frames are spaced with fiber resin spacers and assembled with screws for rigidity.

The assembled chamber is placed in a brass box with a lid made vacuum tight by means of a silicon rubber gasket.

The lid will be eventually operated by a motorized mechanism which will lift it to permit an automatic change of the sample strip. For this purpose two pairs of rollers are built into the housing of the chamber and another pair of rollers is attached to the chamber structure. The strip path through the chamber housing resembles figure 'U'. The vertical parts of the strip trajectory are shielded with a foil from the chamber structure so that only the horizontal part of the strip is exposed to the drift chamber.

5. SIGNAL PROCESSING ELECTRONICS.

The signals from the MWPC are processed essentially in the same way as would be any signals from a single proportional counter. The preamplifier either a hybrid RL-724 (REL LABS Inc., Fig. 5) or ORTEC model 113 is followed by a biased spectroscopy amplifier Canberra model 1467 which also acts as pile up rejector, then signals are fed to the multichannel analyzer EGG model ADCAM 100T. The hybrid preamplifier is mounted inside the housing (Fig. 6a). It is characterized by a very low noise level of about 300 eV. The circuit is provided with the internal protection diodes but, nevertheless, in period of adjustments an external discrete preamplifier with more robust protection elements have been employed (Fig. 6b). The anodes of the principal counter are joined together. As it was expected the gain vs. voltage characteristics of the both halves of the principal counter are identical having the anodes made from contiguous parts of wire from the same manufacturer's spool.

The formation of the analogue signal follows the usual process of converting the exponential detector pulse into a unipolar quasi gaussian shape through repeated process of integration and differentiation.

The signals from an auxiliary counter are processed in an identical way, then discriminated by two window discriminators and integrators. The unbalance of outputs of integrators produces a correction signal for the 5 kV electronically controllable EHT power source which is used to supply all the electrodes of the chamber.

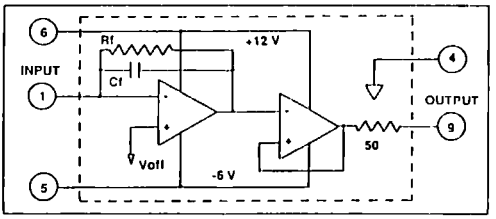

Fig. 5.The hybrid preamplifier.

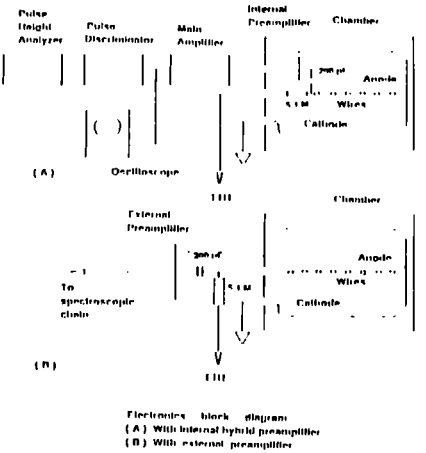

Fig. 6. Note, that functions of pileup pulse discriminator and a spectroscopy amplifier are carried by Canberra Model 1467 module.

The field wires are connected to the appropriate voltage divider chains.

The circuit solution of the automatic workpoint control, albeit functioning correctly, will eventually be simplified, and made more reliable by utilizing for the corrections an entirely digital solution. Unfortunately it is impossible to utilize for workpoint control the same memory of the analyzer which is used for the accumulation of spectra from specimens: The range of possible alpha particle energies straddles the ^{241}Am energy spectrum.

6. RESULTS

The MWPC was designed, constructed and tested. The tests were performed with activities of commercial uranium depleted in ^{235}U and with ^{232}Th. The specimens for counting were prepared by preparing the nitrates of known concentration and then depositing them on strips of impregnated filter paper, subsequently gently dried by infrared in vacuum. The two principal alpha energies for ^{232}Th and for ^{238}U (Fig. 7) separated correspondingly by 59 keV and 48 keV are not resolved. However, the twin peak of ^{241}Am having 43 keV separation is resolved, when a collimated test source is used (Figure 8).

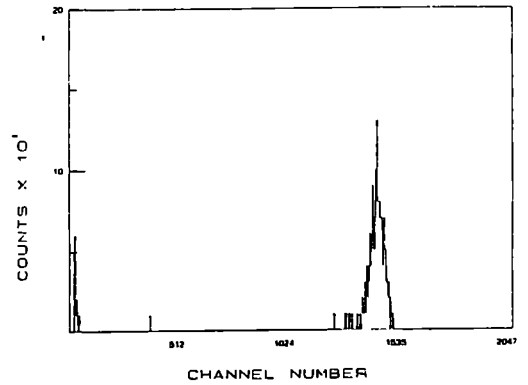

Fig. 7. An alpha particle energy spectrum from a solution of thorium nitrate deposited on impregnated filter paper.

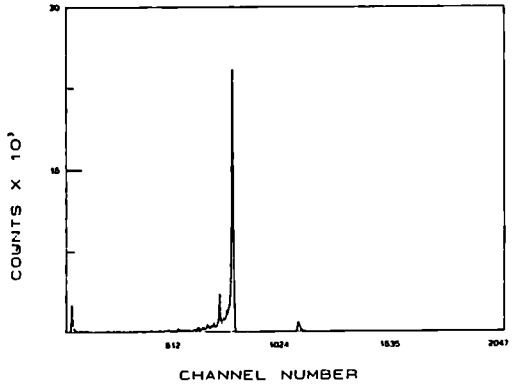

Fig. 8. An alpha particle energy spectrum from a ^{241}Am test source.

Preparing samples of nitrate deposits of uranium and thorium of known concentrations it was established that the MPWC has a linear response to the introduced activity of a solid sample and that the energy resolution is sufficient to distinguish between most of alpha emitters which can be encountered in laboratories and in nuclear emergencies, particularly if metal foil carriers are used which improve the resolution by reducing the thickness of the source. The spectra shown in Fig. 7 and Fig. 8 were not processed off line, but suitable codes are available and will be eventually appended to the MCA programme.

The long term stability was checked by an observation of the shift of the peak from the test source of ^{241}Am during recording of spectra. For an interval of 1 hr the drift did not exceeded 1 channel, which corresponded to about 7 keV. The workpoint stabilizer was disabled. However, after few hours of operation the shift was in excess of 20 keV, always towards the lower channel numbers which indicate that the quality of gas inside the chamber deteriorates owing to the outgassing of internal components or an air leak. With a workpoint stabilization it is possible to operate for 6 - 8 hours without a renewal of the gas filling. The drift of spectrometric electronic chain and of the multichannel analyzer was much smaller than the principal drift introduced by the chamber.

7. DISCUSSION

Before discussing the scientific progress achieved in the project it is important to point out that the original proposal called for a 2½ year period of research. Only two years were granted and in reality, for reason of delays independent on CEC and the staff of the project, the actual time spent on research until the reporting time is 1½ year.

The chamber meets the criteria of sensitivity and resolution for alpha particles, without the use of Frisch grid i.e .1.4% for an infinitely thin target. The detection efficiency is about 94%.

Under development is the implementation of the automatic operation of the chamber, using a dedicated PC XT. The control software which has been written is being tested, the communication software is in the process of debugging and a self diagnostic software is to be added to the programme.

An optional beta particle module has been assembled and tried on stand-alone basis. The work should proceed further to incorporate it in the common housing and operate it in the same conditions as the alpha particle segment. As this refinement is needed only for the detection of beta emitters in the presence of ^{222}Rn background before the work on this, optional, part of the project commences facility for handling small gaseous activity will be installed and registered.

The further development, improvement and testing of MWPC for radiation protection will continue in our laboratory outside the framework of the current CEC supported project, leading to eventual installation of few of these devices for regular monitoring.

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IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

V. Publications:

G. Izzo, A. Magrini. J. Holowacz, I. Vannucci, K. V. Ettinger and C. Franconi "Multiwire proportional chamber for environmental measurements" submitted to VI Congresso Naz. AIFB 24-28 June 1991 - Genoa Italy.

G. Izzo, A. Magrini, J. Holowacz, I. Vannucci, K. V. Ettinger and C. Franconi "Control and stabilization circuits for multiwire proportional chamber" submitted to VI Congresso Naz. AIFB 24-28 June 1991 Genoa Italy.

J. Holowacz, G. Izzo, K. V. Ettinger, A. Magrini, I. Vannucci, A. Canichella "Programming the autonomous operation of a multiwire proportional counter" submitted to VI Congresso Naz. AIFB 24-28 June 1991 - Genoa Italy

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-A-304-F

Inst.Nat.de Physique des Particules
et Physique Nucléaire (IN2P3)
20, rue Berbier du Mets
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Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 88286543

Title of the research contract:

Development of an universal personal dosimeter using semiconductor sensors for mixed radiation fields.

List of projects:

1. Development of an universal personal dosimeter using semiconductor sensors for mixed radiation fields.

Title of the project no.: BI6 - A - 304 F
Development of an universal personal dosimeter using semiconductor sensors for mixed fields.

Head(s) of project:

SIFFERT Paul
Centre de Recherches Nucléaires, Laboratoire PHASE
23, rue du Loess - BP 20
67037 STRASBOURG CEDEX (France)

Scientific staff:

SIFFERT Paul
JUNG Monique
TEISSIER Claude

I. Objectives of the project:

1. DEVELOPMENT OF AN ELECTRONIC PERSONAL DOSEMETER USING SEMICONDUCTORS FOR MIXED FIELD RADIATION MONOTORING.
2. COMPUTER SIMULATION OF THE OPTIMAL DESIGN OF THE FILTERS AND CONVERTERS ASSOCIATED WITH SILICON DETECTORS.
3. OPTIMALIZATION OF THE SILICON DETECTORS.

II. Objectives for the reporting period:

Develop a portable pocket sized system able to measure both the γ-ray and neutron doses for personal dosimetry.

III. Progress achieved:

I. Specific object of the proposal

Our aim was to calculate the response of some channels of an electronic pocket dosemeter able to measure X or y-rays, as well as fast, thermal and epithermal neutron doses. Here our subject was mainly concerned by thereoretical dose responses calculations of surface barrier silicon diodes placed behind fast neutrons or gamma converters : polyethylene or aluminium, the second which is used to enhance the detector efficiency was shielded by different thicknesses of Sn foils.

The polyethylene converter allows to detect fast neutrons towards the recoil protons of the (n, p) elastic scattering whereas the aluminium which provides electrons in the gamma energy region, associated with an Sn X-ray filter smoothes the strong energy dependance dose response of a semiconductor and makes it tissue- equivalent in a way comparable to that of other electronic dosemeters like proportional counters.

The calculations are done to provide the weigthing coefficients which have to be applied at the gamma and neutron channels for converting the current or counting readouts into integral or dose rate measurements.

II. Theoretical calculations

All our codes are implanted on the 3090 IBM of the CCIN2 P3, our laboratory being connected on its net. For the gamma ray interactions (X or y) we are using a set of codes, the first one being a version of MCGET (1) written in our laboratory for a few years. It treats X or y interactions (photoelectric, Compton and pair materialization) with different kinds of shielding materiel (Sn, Al...) or detectors (here a silicon diode). The story of each incomming gamma is followed through the different mediums and the secondary cascade electrons produced in, or crossing the detector are followed. Due to their energy lost, such electrons liberate along their trajectory free carriers which deplacement inside the sensor induce charges on the electrodes according to different processes depending on the characteristics of the regions where they are produced. For a low voltage working silicon, two regions contribute to the charge collection. In the first region (depleted one) the deplacement due to the electric field induces charges representing a quite complete collection. In the second non-depleted part, the carriers diffuse and the minorities reaching the junction will be added to the initial drift charges, their contribution being strongly depending on the signal integration time as well as on the minority lifetime, Tp.

Using the code MCGET we simulate the response of a gamma channel incorporating a small silicon diode of 7.6 mm^2 x 300 um working at a low bias of 3.6 V which sets the depleted region to 30 µm and is suitable for a pocket equipement. The simulations have been done for a fixed number of incident gamma rays between 20 KeV and 1.5 MeV and the outcomming signal treated into two ways : either in the current mode integrating during the time T all the charges delivered by

the detector, or in a digital counting mode setting an acceptance cut-off of 30 KeV (also 40 and 50 KeV) on each individual pulse.

Other codes afterwards treat the flux response curves converting the fixed incident number of gammas into an incident dose equivalence of 1 Sv following the ICRU recommandations (2). Such codes also allow to combine the dose response curves of several detectors (two or three) differently shielded (variation of the Sn thicknesses) setting a weigthing coefficient on each of them to minimize the fluctuations of their sum and make the device acceptable by international requirements.

To calculate the fast neutron dose response curves of a silicon we used an old code modified for ist application to small size devices (3) avoiding the event lost at the edge of the detector. This code treats the n,p elastic scattering inside an hydrogenic converter and follows the emitted proton loosing its energy inside the detector and liberating free carriers along its trajectory. The calculations have been done for several thicknesses of polyethylene placed in front of the silicon irradiated by a constant dose equivalence (1 Sv) fast neutron beam for energies between 1 MeV and 15 MeV. Here also both methods : current and counting, have been studied for a small layer of silicon (20 μm) - (4).

III. Results

a) X y ray channel

For X or y ray detection efficiency curves, the calculations have been done for energies between 20 KeV and 1.5 MeV, a fixed dose of 1 Sv entering the above silicon surface barrier sensor placed behind an 1,5 mm Al converter and eight different shielding thicknesses of Sn foils of 0.0 mm to 2.mm (see report Ref. 5). These different efficiency curves have been calculated for both detection modes : the digital counting one with three cut-offs (30 - 40 - 50 KeV) and the integral current mode.

The calculations have also be performed for several integration times : 1 us, 10 us and 1 ms, and for two lifetimes of the minorities : 100 us for the usual Si and 1 us for a dammaged one.

All the curves of the report are normalized to the 662 KeV Cs - 137 y ray. The values of the different countings or electron collections at 662 KeV can be found at the bottom of each figure, they vary in a range of around 1 - 10 counts per nSv for the digital counting, and 10^3 electrons per nSv. Some of such values can be found in the enclosed table. The number of electrons are converted into current (electrons per second) correlated to dose rate measurements (incomming y-ray per unit time) the detection threshold of the current mode is then fixed by the internal leakage current of the semiconductor (6). These thresholds are spread around several mSv cm^{-2}. s^{-1} wich makes such detection mode quite convenient for high dose rate controls.

In a similar way, it is possible to convert the pulse-height counting per Sv into countings per dose rates. One can then calculate the minimal detectable dose corresponding to 1 count per sec. given by the detector and obtain the detection threshold of the device which gives for such a digital counting thresholds of the order of a few $\mu Sv.\ cm^{-2}.\ h^{-1}$ (6) much lower than the previous ones which makes such a device well appropriate for personal survey.

On the other hand as far as for dosimetric purpose one does not only need devices as sensitive as possible, it becomes also necessary to obtain a dosemeter having a constant response to dose within an acceptable error in an X and y energy range as broad as possible. If one looks at the basic curves (report 1 ref. 5) clearly for a one channel gamma detection it is possible to smooth the responses aboved 100 KeV for Sn filters of 1.5 mm. Nevertheless such an energy is too high for most of radiation environnements (including the X - rays) and it becomes necessary to associate several filter thicknesses or to multiply the number of reading channels : two or three detectors association.

In report 2 (7) and 3 (8) calculations have been done for associations of the dose response curves given by two or three differently filtered detectors setting a weigthing coefficient on each of them which best values are found using a minimization method for the fluctuations around the mean value of the sum of the weigthed responses. The values of the weigthing coefficients are shown at the botton of each figure. One can also found there the efficiency values for Cs point, the lowest energy acceptable as well as the confidence level of the measurements.

Report 2 gives the results for the current mode detection for which one can expect for a three detector association (Sn : 0. , 0.5 and 1.5 mm) to get responses within $^{+}$ 17 % above 41 KeV.

For the pulse-height detection with a 30 KeV cut-off and a three detector association (Sn : 0., 0.5 and 1.5 mm) the lowest energy limit of 50 KeV is reached within $^{+}$ 15 % for an integration time of 10 μs (8).

b) Fast neutron channel

Fast neutron interactions inside several thicknesses of polyethylene have been simulated and the results obtained for the countings of the emitted recoil protons detected by the silicon are given below, they are in the range of a few counts per μSv entering the detector (4).

For fast netrons we also calculated a detection threshold for high intensities of dose rates-current measurement assuming a 5 nA leakage current for the silicon diode.

The efficiency curves are drawn for neutron energies between 1 MeV and 15 MeV and it seems that if one associates two converters (0.1 and 1 mm) one can obtain a flat response curve.

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V. Publications:

JUNG M., FASASI M., TEISSIER C., and SIFFERT P.
Use of semiconducteur detectors in personal dosimetry Bournemouth, UK. September 1988.

Report 1 contrat C.E.E. n° B16-A-304F Avril 1989
Dose response curves for differently shielded silicon and CdTe semiconductors.

JUNG M., TEISSIER C., and SIFFERT P.
Theoretical Cs-137 detection
7th. symposium on radiation measurements
AN. Arbor, May 1990.

Report 2 contrat C.E.E. n° B16-A-304F Mai 1989
Association of several silicon n-type sensors working in current mode detection.

Report 3 contrat C.E.E. n° B16-A-304F
Pulse height response curves for several associated silicon detectors.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: B16-A-305-D

Physikalisch Technische
Bundesanstalt
Bundesallee 100
D-3300 Braunschweig

Head(s) of research team(s) [name(s) and address(es)]:

Dr. H.M. Kramer
Physikalisch Technische
Bundesanstalt
Bundesallee 100
D-3300 Braunschweig

Telephone number: 0531-5926410

Title of the research contract:

Development of an universal personal dosimeter using semiconductor sensors for mixed radiation fields.

List of projects:

1. The investigation of the radiological properties of the dosemeter in photon and neutron reference fields.
2. The extension of the useful energy range for photons down to about 20 keV.

Title of the project no.: 1

The investigation of the radiological properties of the dosemeter in photon and neutron fields

Head(s) of project:

Dr. H. M. Kramer
Physikalisch-Technische Bundesanstalt, Braunschweig

Scientific staff:

Dipl.-Phys. D. Schäffler, Dr. H.M. Kramer,
Dr. H.-J. Selbach, Dr. L. Büermann

I. Objectives of the project:

Development of a semiconducting detector system to be used in a personal dosemeter and investigation of its dosimetric properties. Acquisition of a comprehensive set of data for systematic studies of the response and its energy dependence in project no. 2

II. Objectives for the reporting period:

Completion of the investigation of the relevant parameters determining the response and its energy dependence of the Si-diodes covered with compensating filters of different materials

III. Progress achieved:

Methodology

The dosimetric properties of the detectors were systematically modified by using filters of Al, Cu, Sn, Gd, Ce, Ta and Pb and selected combinations thereoff. The response of the detectors and its energy dependence were examined in the reference fields of the PTB. For X-radiations the heavily filtered radiation qualities according to ISO standard 4037 and γ-radiation from ^{137}Cs and ^{60}Co were used. Dosimetric measurements were made in terms of the quantity air kerma; the measurements were carried out with a primary standard or with a suitably calibrated reference dosemeter. As was outlined in the intermediate report, for each combination of radiation quality, diode type and filter a pulse height spectrum was recorded and the dose was measured, to which the diode was exposed. A pulse height spectrum represents the maximum amount of information which can be drawn from the signal generated by the radiation in the detector. A pulse height spectrum can be easily converted to other signals like for instance the total current. In a more elaborate evaluation each channel or groups of channels can be given certain weights in order to accomplish the conversion from the pulse height spectrum to the value of the dose. This method has the advantage that an analysis of the data is possible with respect to any objective even to one which was not foreseen at the time of the measurements. Altogether some 600 such spectra were recorded and stored on a computer for further evaluation.

Results

For reasons of completeness examples of pulse height spectra for an uncovered detector irradiated with X-radiation with tube voltages of 30 and 150 kV and with radiation from ^{137}Cs are shown in figure 1. The figure demonstrates the transition from the close resemblance of the pulse height to the photon fluence spectrum at a tube voltage of 30 kV, to a somewhat triangular pulse height spectrum with a high energy cut of at around 480 keV for the radiation of ^{137}Cs. This transition can be understood by examining the efficiency ϵ for the total absorption of the energy of a photon as a function of photon energy as shown in fig 2. ϵ falls off by about four orders of magnitude for photon energies between 50 and 500 keV. This implies that from a certain energy upward total absorption events are practically negligibly, and events of incomplete energy depositioning by Compton scattering are dominant.

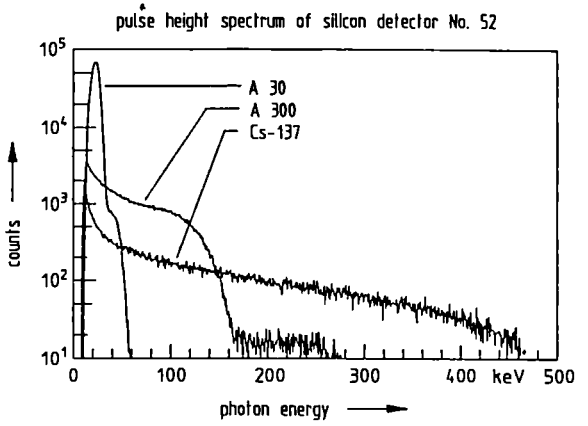

Figure 1

Pulse height spectra for X-radiation with 30 and 150 kV tube voltage and for Cs-137

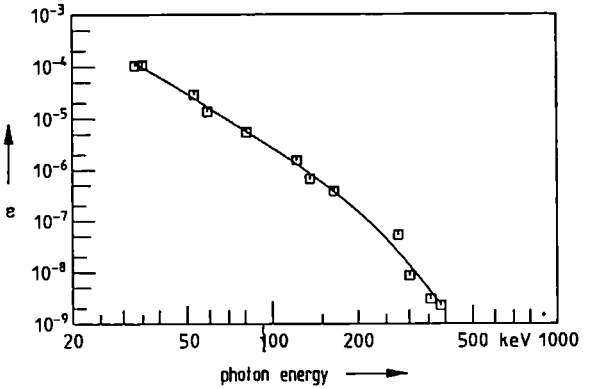

Figure 2

Efficiency ε for the full energy absorption as a function of photon energy

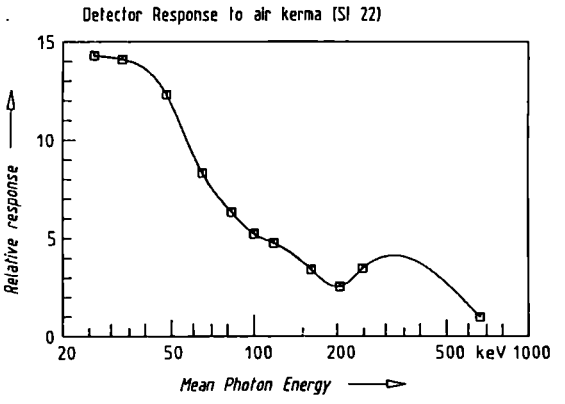

Figure 3

Energy dependence of response of a bare detector

Figure 3 gives the relative energy dependence of reponse in terms of the total number of pulses with respect to air kerma of a detector without any additional filtration. This arrangement is obviously not suitable as a dosemeter, because its response varies by a factor of 14 between 24 and 662 keV. Out of the great number of arrangements of only one detector with a filter two of the more promis-ing solutions are presented in figure 4. For a given detector the variation of response shows in terms of the total number of pulses with respect to air kerma as a function of photon energy. The difference between the continuous and the broken line in figure 4 is due to the orientation

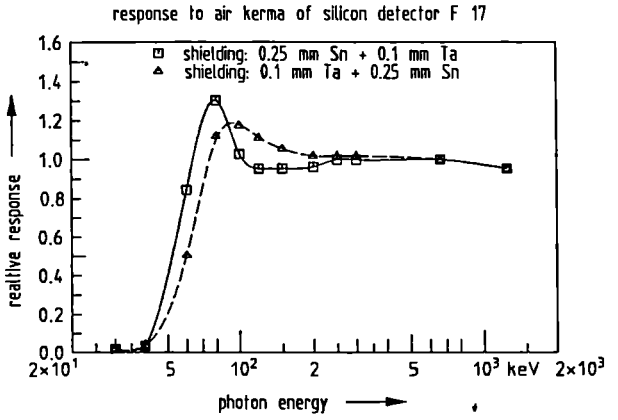

Figure 4

Energy dependence of response of a given detector with the same filter in two different orientations

of the Sn/Ta filter. In the case of the continuous curve the Ta-layer faces the radiation source and for the other curve the Sn-layer. This example demonstrates that it is not sufficient to consider the attenuating properties of the materials making up the filter. Photons scattered in the filter and, depending on energy, the spectral distribution of electrons emerging from the filter are as well relevant parameters. The arrangement represented by the continuous line in figure 4 has a useful energy range extending down to about 55 keV, if a maximum variation of response of ±30% with respect to that for the radiation of 137Cs is stipulated. The broken curve has the advantage of being flatter, however the low energy cut of is still somewhat higher.

Discussion

It should be realized that the curves in figures 3 and 4 would become flatter and that the useful energy range would extend down to lower energies if a phantom related dosimetric quantity had been used, like for instance ambient dose equivalent. The reason behind this effect is that the energy dependence of response of the bare detector has a qualitative resemblance with the energy dependence of the conversion factor from air kerma to ambient dose equivalent. This means that less strong modifications of the energy dependence of the bare detector are necessary for a measurement of ambient dose equivalent than for a measurement of air kerma.

On the basis of using only one detector and forming the sum of all pulses other filter combimations gave similar results to those shown in figure 4. However, no combination was found which resulted in a useful energy range extending down to substantially lower energies. By combining detectors with different filters the useful energy range could be substantially extended. The investigations showed, however, that a still greater potential for improvement was given by the algorithm for the conversion of the pulse height spectra to the dose. Therefore the main efforts were devoted to this issue. These investigations are described in part two of this report.

It was planned to examine the response of detector-converter arrangements also with respect to neutron fields. These investigations could not be conducted as the work in photon fields was considerably more extensive than foreseen. This extension is primarily due to the necessity to investigate the influence of additional parameters as presented in part two of this report, which were not included in the original concept.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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2. CENTRE NATIONAL de la RECHERCHE SCIENTIFIQUE
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3. CERN
   Service TIS - GENEYE (SWITZERLAND)

4. UNIVERSITY of AARHUS
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V. Publications:

D. Schäffler and H. M. Kramer, Untersuchungen von Silicium-Halbleiterdetektoren auf ihre Eignung als Detektor für Strahlenschutzdosimeter. Jahresbericht der Physikalisch-Technischen Bundesanstalt 1989, S. 196 (category 2)

Title of the project no.: 2

The extension of the useful energy range for photons down to about 20 keV

Head(s) of project:

Dr. H. M. Kramer
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Scientific staff:

Dipl.-Phys. D. Schäffler, Dr. H.M. Kramer,
Dr. H.-J. Selbach, Dr. L. Büermann

I. Objectives of the project:

Optimization of the dosimetric performance of a semi-conducting detector system to be used in a personal dosemeter. The signal from a configuration of one or more diodes each equipped with a suitable compensation filter as found in project 1 should be converted to the dose by means of various algorithms. An overall combination of detectors(s), filters and algorithms should be established allowing dose measurements in the energy range between about 20 keV and 1.3 MeV

II. Objectives for the reporting period:

Establish suitable combinations of compensating filters as investigated in project 1. Design and test of various algorithms for the conversion of pulses generated in the diode(s) by the radiation of the value of the dose

III. Progress achieved:

Methodology

The data base acquired in part one of this report was systematically analyzed in two different ways to find a system whose useful energy range would extend down as far as possible to low photon energies. The first method consisted of finding suitable combinations of two or three detectors each equipped with different filters and each given a relative weight which was obtained as the result of an optimization. The results of this approach have been presented in the intermediate report. This report will focus on the second approach which consisted of investigating various forms of algorithms for the conversion from the pulse height spectra of selected combinations of Si-diodes and filters to the value of the dose. This method represents a powerfull way of evaluation, which, of course, can not be used for Geiger Müller tubes as they do not have spectroscopic properties.

In the light of the availability of small and at the same time powerful integrated circuits specifically designed for one particular type of instrument the pulse height spectra were analysed by means what was termed a 'few channel analyzer'. For this approach the original pulse height spectra, with a total of 4096 channels each, were converted to pulse height spectra of two to eight channels. In the case of two channels the pulses were attributed to bins of energies up to and beyond a certain energy. Experimentally a value of 88 keV was found to be most suitable. Weighting factors for each bin were determined with which the total number of pulses was multiplied.

A different method was employed for the cases of four to eight channels. For each of the eleven radiation qualities used a few channel spectrum was obtained which led to the following over-determined system of liniear equations

$$D = \phi_{ij} \; W_j \; ,$$

were ϕ_{ij} is the number of pulses in bin j due to radiation quality i, D denotes the dosimetric quantity to be used and W_j is the weight attributed to bin j. By means of a linear regression the system of equations was solved resulting in a vector W_j. The advantage of this method lies in its great flexibility. However, this flexibilty also implies that there are so many degrees of freedom that it is almost impossible to vary simultaneously all of them in a systematic manner. These parameters are the number of bins, the location of their boundaries in terms of energy and the kind of filter in front of the detector, if a filter is

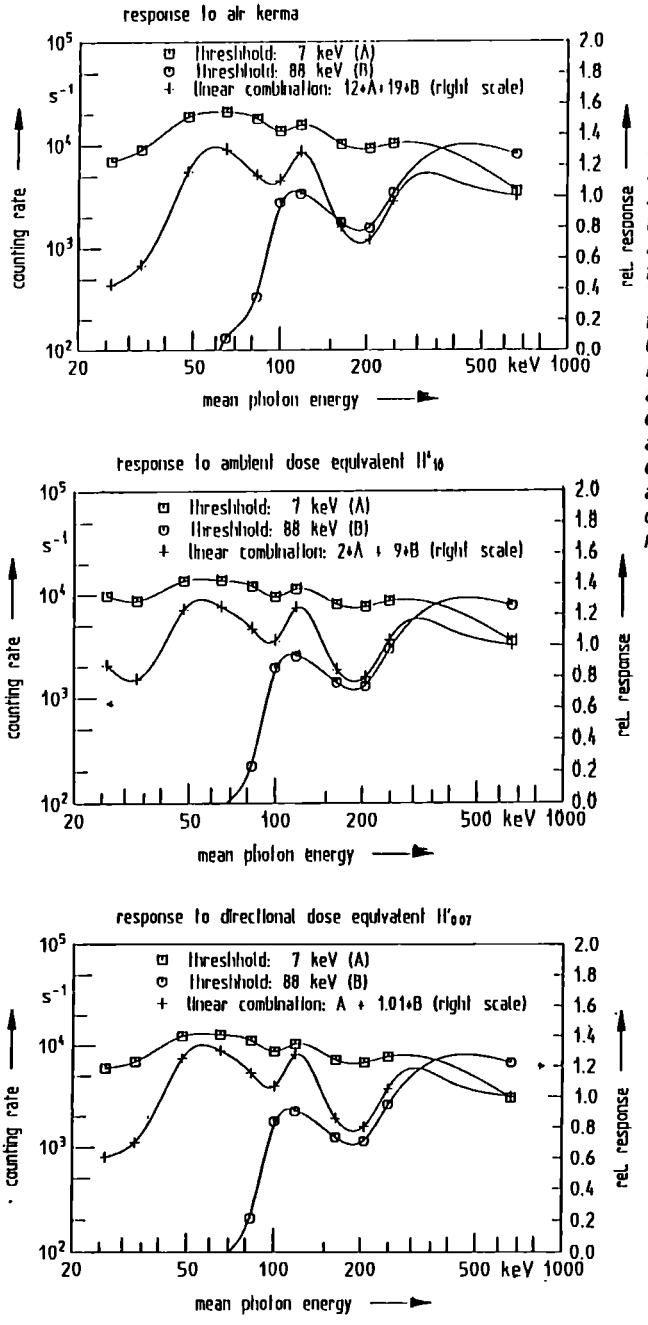

Figure 1

Results for the two-channel model, for explanation see text. The circular and square symbols refer to the left ordinate and the crosses to the right one. The filtration of the detector was 0.13 mm Pb. In a) b) and c) air kerma, ambient dose, equivalent and directional dose equivalent are the dosimetric quantities, respectively

used at all. Out of the great number of possible solutions only some were examined in detail. The energy range between 7 and 700 keV was subdivided into the desired number of bins in such a way that the width of each bin increased from one to the next higher one by aproximately the same factor.

Results

Figure 1 on the next page shows one of the better solutions which was obtained in the two-channel model. The figure strongly underlines the fact established in part one of this report already that it is more demanding to match the energy dependence with respect to air kerma than with respect to ambient dose equivalent. For the first quantity a lower limit of the useful energy range of about 37 keV is found while the the useful energy range for the latter quantity goes down to at least 24 keV.

The model using four to eight channels was applied to an unfiltered detector. If the number of channels was four, five or six the variation of the response with photon energy was stronger than the stipulated ±30% and hence these solutions were not acceptable. For the case of eight channels a satisfactory result is obtained as shown in figure 2. The energy dependence of response in terms of air kerma, H'(10) and H'(0.07) are represented by the square, circular and triangular symbols, respectively. For all the three quantities the useful energy range extends down to an energy of at least of 24 keV.

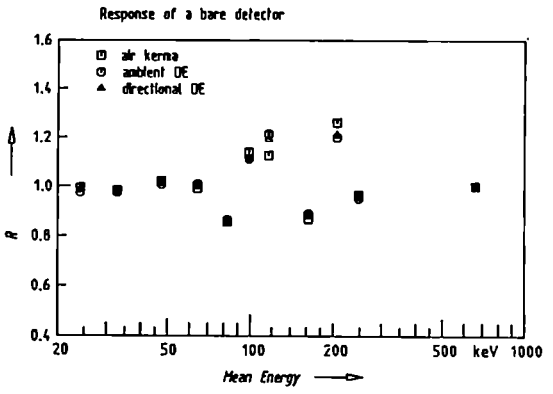

Figure 2

Energy dependence of response of a bare detector evaluated in the eight channel mode

Maximum deviations of the response from that to the radiation of ^{137}Cs of +26% and -15% are observed for all three quantities. It should be realized that the slope of all curves at low energies is practically negligibe, suggesting that the useful energy range will extend still further down.

The energy dependence becomes substantially flatter by using a simple filter in front of the detector. Figure 3 shows the example of a tantal filter of 70 µm thickness. Maximum deviations of the response from that to the radiation of ^{137}Cs of about ±13% are found and again the slope of all three curves at low energies is small.

Discussion

The 'few channel analyzer' approach has two great advantages. First, an exceptionally flat energy dependence over a large energy range can be realized irrespective of the quantity to be measured. Secondly, the choice of the quantity to be measured can be made even after the measurement itself has been completed, simply by attributing the appropriate set of weighting factors to the bins. At present no detector - suitable for an active individual monitor - is known which could match only one of the above two properties.

However, such a system has not been tested in practice and consequently no experience is availabe with respect to other properties. At present two points deserve a closer examination. One originates from the fact, that in fields of high dose rates two pulses can fall within the integration time of the analog-digital convertor resulting in one pulse of the size of the sum of both. This effect - known as pile-up is responsible for allocating one event to

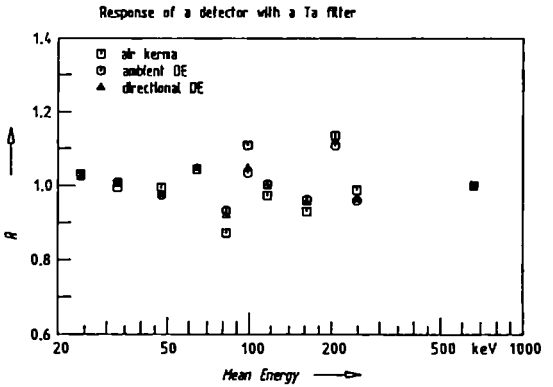

Figure 3

Energy dependence of response of a Ta-filtered detector evaluated in the eight channel mode

bin i instead of two events to bin i-1, which, of course, can result in an unacceptably large error of measurement. However, a closer examination shows that the pile-up effect does not present a serious problem, as one can determine a set of vectors W_j, each set being used in a certain dose rate range. Alternatively the weighting factors could be a function of the dose rate.

The second problem is a more difficult one. Depending on the number of channels some of the weighting factors have a negative sign, or are positive, but have a comaparatively low value. In the first case this could result in a negative and in the second case in a near zero response, if pulses fall predominantly just into one of the bins in question. In order to be sure that this entirely unacceptable property does not occur in reality it must be demonstrated for all possible spectral distributions of the radiation that the small or negative contributions are always compensated by larger positve contributions from the other bins. This does not mean asking for the impossible, because each input spectrum - and be it a monoenergetic one - produces a much wider pulse height spectrum due to incomplete energy depostion as outlined in part one.

As the configurational space of the solutions of the above system of linear equations also comprises all linear combinations of the radiation qualities out of which the equations are made up, it can be concluded in a general way that the system can cope perfectly with a wide variety of spectral distributions of the radiation for which it was not initially optimized. The above statement is equivalent to saying that the response of the instrument is constant, if it is exposed simultaneously or sequentially to a given arbitrary number of the eleven input spectra each responsible for a given abitrary dose. Due to their heavy filtration the input spectra are relatively narrow, at the same time they are also mutually overlapping due to their relatively close separation in tube voltage. This means that essentially all those spectra are covered by the solution which are wider than the input spectra. In pratical cases of radiation protection this will be almost always the case.

The general proof that the system behaves satisfactorily for **all** spectral distributions of the radiation can be given by means of a more theoretical treatment only. As the number of realizable spectral distributions - and in particular of monoenergetic distributions - is fairly limited the system must be simulated and optimized by Monte Carlo techniques. A concept has been developed on how the optimization can be carried out allowing a reliable estimate of the worst case error. Preliminary results of the investigations conducted along this line are encouraging.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. CENTRE de RECHERCHES NUCLEAIRES
   Laboratoire PHASE - STRASBOURG (FRANCE)

2. CENTRE NATIONAL de la RECHERCHE SCIENTIFIQUE
   Service Central de Sécurité - PARIS (FRANCE)

3. CERN
   Service TIS - GENEYE (SWITZERLAND)

4. UNIVERSITY of AARHUS
   Institute of Physics - AARHUS (DENMARK)

V. Publications:

D. Schäffler and H. M. Kramer, Untersuchungen von Silicium-Halbleiterdetektoren auf ihre Eignung als Detektor für Strahlenschutzdosimeter. Jahresbericht der Physikalisch-Technischen Bundesanstalt 1989, S. 196 (category 2)

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Department of Physics
Rome University "La Sapienza"
Ple. A. Moro 2
I-00185 Roma

Contract no.: BI6-A-306-I

Head(s) of research team(s) [name(s) and address(es)]:

Dr. C. Furetta
Dept.of Physics, Group FIME
Rome University "La Sapienza"
Ple. A. Moro 2
I-00185 Roma

Telephone number: 6-49913459

Title of the research contract:

Development of an universal personal dosimeter using semiconductor sensors for mixed radiation fields.

List of projects:

1. Development of an universal personal dosimeter using semiconductor sensors for mixed radiation fields.

Title of the project no.:

DEVELOPMENT OF AN UNIVERSAL PERSONAL DOSIMETER USING SEMICONDUCTOR DETECTOR SENSORS FOR MIXED RADIATION FIELDS

Head(s) of project:

Dr.C.Furetta, Physics Department Rome University La Sapienza P.le A.Moro 2, 00185 ROME. ITALY

Scientific staff:

Prof.C.Bacci, Prof.B.Rispoli Rome University

I. Objectives of the project:

The objective of the project is to develop a pocket sized personal dosimeter using semiconductor materials. This dosimeter will be able to give a reading as close as possible to the dose equivalence in radiation fields existing around reactors, accelerators, X and gamma sources. Furthermore, the pocket dosimeter will give a flat energy response according to the most recent ICRP recommendations.

II. Objectives for the reporting period:

The data contained in this report are concerning to the results of a serie of measurements on silicon detectors and mainly concern with theyr fast neutron response.

III. Progress achieved:

METHODOLOGY

The measurement program consists of several tests related to the fast neutron sensitivity of the silicon detectors:

- effect of radiators covering detectors during fast neutron exposure;
- neutron sensitivity as function of radiator thickness and shape;
- measurement of gamma sensitivity in order to be able to subtract the gamma radiation emitted from the neutron sources;
- response of Si-detectors as a function of bias voltage.

RESULTS

A Silicon detector with a large sensitive layer (900 micron) was chosen for finding the optimum radiator thickness for fast neutron irradiation.

The silicon detector was exposed to both Cf and PuBe neutron sources and the neutron spectra were obtained at a fixed distance from the sources and using different thickness of polythene radiator discs mounted in front of the detector window. The measurements show that the sensitivity varies strongly with radiator thickness.

The maximum neutron response for Cf neutrons occurs with about 1 mm polythene; with The PuBe neutron source the optimum sensitivity was achieved with 2 mm radiator thickness. The results are plotted in Fig.1

The influence of the bias voltage on neutron response was the objective of the present measurements.

The test was carried out using silicon detectors placed at a distance of 5 cm from the axis of a PuBe source and some spectra were obtained between 0 and 250 V. The detectors were exposed with a polythene radiator of 2 mm thickness. the results are plotted in the Fig.2

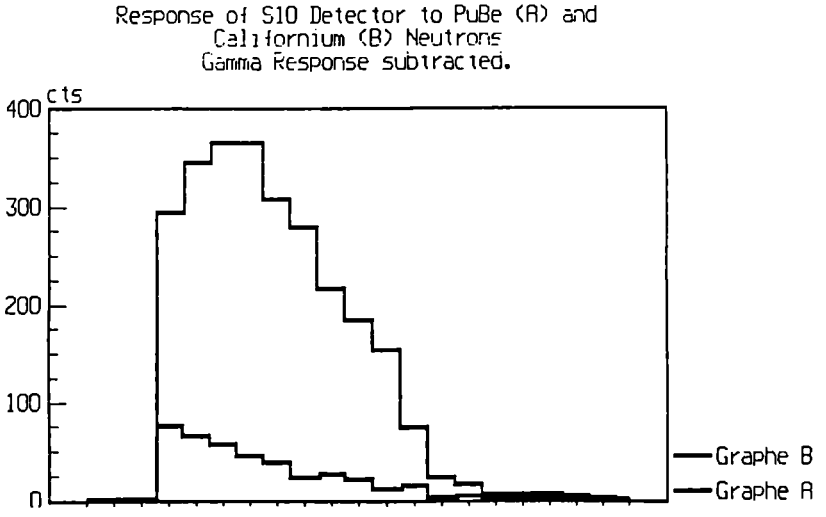

Fig.1 - Neutron response of detector covered by radiator

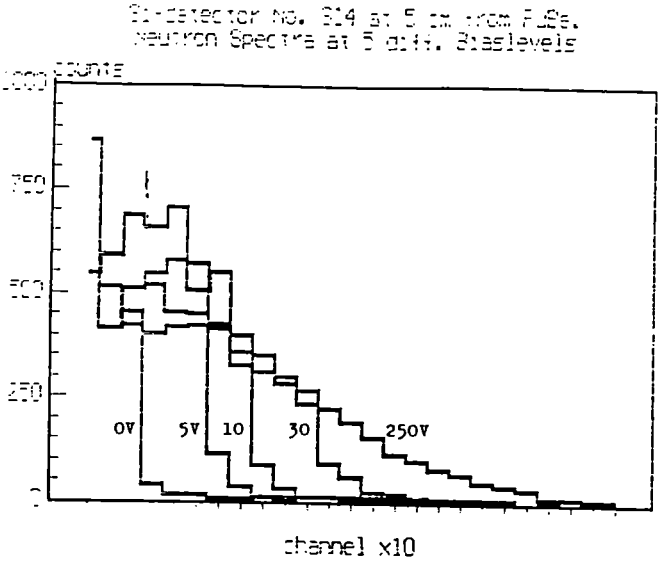

Fig.2 - Neutron response at different bias levels

CONCLUSIONS

Detection of fast neutrons by proton recoil is a useful application of Silicon detectors. The main problem arises in the discrimination against gamma radiation and to obtain a reasonable energy response and sensitivity. Using Cf (2.3 MeV) and PuBe (4.3 MeV) a factor of 2.5 in efficency can be observed.
The detectors can be used for neutron flux measurements as well as a simple spectrometer.
The response of the silicon detectors is comparable with both neutron film and large proton recoil counters.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-A-307-CH

European Organisation for
Nuclear Research (CERN)
CH-1211 Genève 23

Head(s) of research team(s) [name(s) and address(es)]:

Dr. K. Goebel
CERN
Div. TIS/RP
CH-1211 Genève 23

Telephone number: 022-832159

Title of the research contract:

Development of an universal personal dosimeter using semiconductor sensors for mixed radiation fields.

List of projects:

1. Development of an universal personal dosimeter using semiconductor sensors for mixed radiation fields.

Title of the project no.: Development of an universal personal dosemeter using semiconductor sensors for mixed radiation fields.
Project No. B16-A-307-CH

Head(s) of project: Dr. K.Goebel
CERN Div. TIS/RP
CH-1211 Geneve 23

Scientific staff: R.C.Raffnsoe
CERN Div. TIS/RP
CH-1211 Geneve 23

I. Objectives of the project:

The objective is to develop a pocket-sized personal dosemeter with dose-equivalent response. The instrument is intended to be used by persons working in areas near accelerators, reactors and other sources of radiation, which produces both gamma-radiation and neutrons of widely varying energies. The detector system in the dosemeter is composed of a set of solid-state detectors (Si, CdTe or GdO) with or without polythene radiators, each chosen to cover their part of the radiation spectrum.

II. Objectives for the reporting period:

The second and final part of the project consist in measurement of fast neutron response of the same batch of Si-detectors which were exposed to photons during the first period.The purpose was to choose optimum parameters for the amplifier system and to measure neutron response as function of bias voltage, detector characteristics and radiator thickness.Fast neutrons were obtained from sources of PuBe and Californium.

III. Progress achieved: The aim of this project is to develop a pocket sized dosemeter with dose-equivalent response for X- and Gamma-rays as well as for neutrons with energies from thermal to 15 Mev. A specific feature of the dosemeter is use of solid state detectors of different type and size, each optimized for measuring a particular radiation type and spectrum.

The project is carried out in a collaboration between different universities and research establishments in Europe. CERN's contribution is foreseen to be mainly tests and calibration of detectors with radioactive sources and generators available in a calibration laboratory and in mixed fields near high energy accelerators.

EXPERIMENTAL WORKS.

Several series of measurements on a batch of sample detectors (Si) were made between August 89 and April 90. Procedure and result are described in detail in two reports (Ref.1 & Ref.2). The purpose was to gain experience with measuring methods and to be able to choose important parameters like detector characteristics, thickness of polythene radiators and amplifier settings.

Design and expected performance was discussed at a few meetings between the participants and it was proposed to divide mixed fields into following components:

1. X- and Gamma-rays (50 keV - 2 MeV)
2. Thermal Neutrons
3. Epithermal Neutrons (up to 0.1 MeV)
4. Fast Neutrons (0.1 - 15 MeV)

The lower limit for photon detection was thoroughly discussed and it was agreed that the energy response should be within a tolerance of $\pm$ 20 %. This could be obtained with suitable filters or by electronic processing of the amplifier signal. The dose equivalent rate presented at the display of the dosemeter would be the sum of data measured with several sensors and it seems evident that a microprocessor becomes an essential part of the electronic system. An important proposal was also to include one or more miniature multichannel analysers (MCA) in the design.

It was also necessary to have a general idea of the dosemeter layout in order to prepare tests and calibration at CERN effeciently.

One possible solution is a system containing a number of measuring channels of almost identical design - all feeding data to a central processor. We estimate that 5 channels might be sufficient for the universal dosemeter, - two of these should be reserved for photons.

Each measuring channel in our hypothetical model consists of a detector with its associated filter or radiator, a charge-sensitive amplifier with gain and shaping optimized for its application - and a MCA, which sorts the output pulses - linearly or nonlinearly - in a limited number of analyser channels (24 - 32).

The MCA does not yet exist on the commercial market, but the design seems feasible and the device can be made in integrated circuit size. The input is an analog pulse ranging from 0 to 5V and of short duration (2 uS). Sorting takes place in a column of comparators and the output is an 8-bit byte informing the microprocessor where to add a count in the memory. The MCA channels are thus located in RAM and here might also be stored one specific calibration factor for each MCA channel converting counts to dose equivalent.

EXPERIMENTAL DETAILS.

The Si-detectors shipped to CERN were tested with conventional electronics (NIM amplifier and preamp.) and the nonexisting MCA was simulated with a PC-based card (ACCUSPEC) having 256 channels. This set-up corresponded thus to one measuring channel of the proposed dosemeter. The data from the MCA were reduced to 25 analyser channels by summing each 10 consecutive channels of the ACCUSPEC-spectra.

The detectors were mounted in small, cylindrical Al-housings supplied with SMA connectors. They were fixed to the pre-amplifier input with a small adaptor in order to reduce capacitance to a minimum. Radiation reached the sensitive part of the detector through a window on front of the housing. Due to the particular way of fixing the detector behind the window, the sensitive area was not circular and well defined. The size varied from detector to detector and the exact area had to be determined from measurements of mean diameter with a microscope, because it was found to have an important influ-

ence on the effeciency. Window areas varied between 8.04 and 16.26 squaremillimeter.

The response to ambient light was eliminated by covering the Al housing with a thin foil of pure Aluminium. (10.4 mg/cm^2). This foil served at the same time as support for polythene radiators during neutron measurements. The detectors were unfiltered during tests with X- and Gamma-rays.

The gain of the main amplifier was variable, but it was decided to make all tests with only two different gain settings. These were x4 and x64 and corresponded to charge-sensitivities of 25 and 400 V/pC, respectively.

Sampling times of the MCA were from 100 to 10000 Sec and the ADC had a fixed lower threshold corresponding to 3 % of maximum channel. Measurements in the first serie (Ref. 1) were made with a single channel analyser, only, but the results are in good correlation with those of the second serie.

PHOTON RESPONSE.

Photons interact by the photo-electric effect at low energies and by Compton scattering above 100 - 200 keV. This gives a non-linear energy response curve as shown in Ref. 1. It looks much like the curves obtained with GM tubes and it is required to employ a filter, which counteracts the excess sensitivity at low energies or to compensate by pulseheight analysis. The detector is basically measuring gamma-flux when it is employed in the pulse-mode and has a fixed discriminator level.

The enclosed table shows that the response to Americium 60 keV is 10 times higher than the response to Cesium (662 keV). The figures are in the order of 200 and 20 counts/mm^2/uSv. All doserate are given in the quantity photon dose equivalent (H_x), and calculated on basis of source values published in Ref. 3.

Ref. 2 mentions also that two measuring channels were allocated for photon dosimetry, one for the lower energies with amplifier gain x64 and one for Compton events with gain x4. The maximum chan. no. with counts is here seen to be ch. 16 for Cobalt (1.25 MeV) and channel 8 for Cesium (662 keV).

The table of detector characteristics shows that S5 to S9 had

different resistivity. All measurements with X- and Gamma-rays shows that the sensitivity increases almost linearly with that parameter. Measurements in Ref. 2 shows also that the photon sensitivity is independent of detector thickness from 200 to 1000 um.

We found an important correlation between window size (as determined with the microscope) and photon sensitivity both for low and high energies. This is the reason why the effeciency figures are quoted in cts/mm^2/uSv. The bias voltage was 45 V during most of the measurements as this is the maximum allowed voltage for some of the detectors. Increasing bias from 10 to 45 V approximately doubled the sensitivity, but there was no signs of saturation. The results shows that a measuring channel with Si-detector might be useful for measuring the gamma component in a mixed field, when a MCA is available. It might also be used for building a spectrometer for X-rays up to about 100 keV.

FAST NEUTRON RESPONSE.

Fast neutrons were foreseen to be detected by the n,p scattering process (proton recoil). This is done by mounting a polythene radiator in front of the detector window. This material is rich in Hydrogen and the recoiling proton looses its energy in the sensitive region of the Si-detector.

The lower limit for detection is at the energy, where charge produced by energetic photons is the same as that produced by a proton. Gamma discrimination is primordial because the dose per neutron is considerably higher than the dose per photon. The high energy limit, on the other hand, is influenced by a steep decrease in the n,p scatter cross section at energies above 1 MeV. It is 4.5 barns at 1 MeV, 1 barn at 10 MeV and 100 mb at 80 MeV. One uses the same detection principle as in other common neutron detectors like nuclear emulsions, proton recoil counters or hydrogen-filled ionisation chambers. A 10 MeV proton has a range of 32 cm in such a chamber filled to a pressure of 20 atm, but traverses only 800 um in Silicon. The amount of energy delivered by the neutron to a proton depends on the scatter angle and can be from 0 to 100% with equal probability.

The first measurements at CERN concerned effeciency as function

of polythene radiator thickness. Small discs of thickness between 12 um and 10 mm were in turn mounted in front of the detector window and exposed to fission neutrons from a Californium source and to PuBe neutrons. It was found that about 1 mm PE was sufficient to give maximum countrate and it increased the initial reading by roughly a factor of two. The neutron response of a Si-detector without PE radiator is largely due to (n,alpha) and (n,p) reactions in Silicium and in Aluminium of the housing and cover foil. This contributes with close to 50% of the total response obtained with optimum radiator thickness.

A single detector was used for measuring the response as function of bias voltage, when it was exposed to PuBe neutrons. The result was here the same as for gamma radiation with no signs of a plateau up to the highest permissible voltage (250 V). All the detectors were calibrated with both Californium (2.4 MeV) and PuBe neutrons (4.3 MeV) using optimum radiator thickness. The spectra are presented in tables 15 and 16 of Ref. 2 and shows clearly a difference between the two types of neutron spectra. An effeciency factor has been calculated by taking the sum of channels 21 - 256 and dividing with the dose equivalent (cts/mSv). It was later also normalized to unit detector area (cts/mm^2/mSv). The lower limit (ch. 21) was chosen to exclude all high energy gamma-rays - highest pulses from Cobalt (1.25 MeV) falls as beforementioned in channel 16.

The effeciencies for Californium and PuBe were different by more than a factor of two, which means there is no simple proportionality between the sum of counts in channels 21 to 256 and the dose equivalent. The reason is that many neutrons in the fission spectrum falls below the discriminator level and we must look for another detector, which will cover both the intermediate energy region and part of the fast neutrons up to 0.5 or 1 MeV. The response problem is not simple and might even be further complicated by new recommendations for weighting factors expected to be proposed by ICRP.

Energy less than 10 keV:	5
10 - 100 keV:	10
100 keV - 2 MeV:	20
Energy above 2 MeV:	10

THERMAL AND INTERMEDIATE ENERGY NEUTRONS.

It has been foreseen to include channels for measuring the dose equivalent from lower energy neutrons in the dosemeter. A new type of thermal neutron detector based on Gadolineum Oxide foil was tested preliminarily and the results were reported in a letter (Ref.4). A small sheet of GdO was sandwiched between two pieces of standard photographic film and exposed in a known field of thermal neutrons. This detector produced a significant image, but only one side of the foil seemed to emit light during irradiation. Next part of the test consisted in mounting the GdO foil directly on a photomultiplier (150AVP) and in contact with a photodiode (HAMAMATSU). None of these two experiments did, however, produce an acceptable response to thermal neutrons. The reason may be a poor matching between emission of light from the foil and response curve of the detectors.

CONCLUSION.

Si-detectors operated in pulse-mode and exposed to gamma-rays and fast neutrons produces a countrate, which is proportional to the flux. The height of the pulses contains, however, also qualitative information about the radiation energy and this can be used in practice for radiation dosimetry, if some form of pulseheight analysis can be made by the measuring system. Our measurements shows that the principle will be usable for estimation of dose equivalent in the proposed universal dosemeter, when some problems with the neutron response have been solved.

R,C.Raffnsoe

Table 17. Effeciency of Silicon Detectors to different types of Radiation.

Radiation Type	Condition, Setting Dose, Unit	S5	S6	S8	S9	S10	S11	S12	S13	S14
AM-241 X-Rays 60 keV AM-2619 Distance: 1 m 311.9 uSv/h	Coarse Gain: x64 Channels: 12 - 25 Sampling Time: 200 S Dose: 17.3 uSv Unit: cts/mm2/uSv	46.5	136	229	389	204	228	204	216	208
CS-137 Gamma 662 keV CS-2045 Distance: 2 m 27.475 mSv/h	Coarse Gain: x64 Channels: 10 - 256 Sampling Time: 200 S Dose: 1526 uSv Unit: cts/mm2/uSv	7.5	15.1	22.6	30.1	19.6	21.7	19.0	21.7	19.0
Cf Neutrons 2.348 MeV CF-1569 Distance:10cm 13.7 mSv/h	Coarse Gain: x4 Channels: 21 - 256 Sampling Time: 1000 S Radiator: 1 mm PE Unit: cts/mm2/mSv	8.5	9.2	8.5	6.4	9.2	11.0	9.6	10.5	9.7
PuBe Neutrons 4.3 MeV PuBe-1120 Distance:20cm 29.47 mSv/h	Coarse Gain: x4 Channels: 21 - 256 Sampling Time: 1000 S Radiator: 1.88 mm PE Unit: cts/mm2/mSv	18.8	24.9	25.6	47.1	26.8	28.3	25.7	29.6	24.9

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

CRN/PHASE	Strasbourg	Dr. Siffert
Univ. Aarhus	Aarhus (DK)	Dr. E.Uggerhoj
Univ. Rome	Rome (I)	Dr. C.Furetta
PTB	Braunschweig (D)	Dr. Kramer

V. Publications:

Ref. 1 RP-CD/89-02 Internal Report
PHOTON SENSITIVITY OF SOLID STATE DETECTORS.
H.C.Raffnsoe, C.Furetta, C.Bacci
3. October 1989

Ref. 2 TIS-RP-CD/90-01 Internal Report
FAST NEUTRON SENSITIVITY OF SILICON DETECTORS.
H.C.Raffnsoe - 16. August 1990

Ref. 3 RP-CD/89-04 Internal Report
YEARLY SOURCE COMPARISON 1989
H.C.Raffnsoe - December 1989

Ref. 4 Letter to Dr. Siffert
12. June 1990

Progress Report

Contract : Bi7-020 **Sector: A12**
Title : Study and development of an individual electronic neutron dosemeter.

1	Decossas and Vareille	Univ. Limoges
2	Tommasino	ENEA
3	Zamani-Valasiadou	Univ. Thessaloniki
4	Barthe	CEA - FAR
5	Fernandez Moreno	Universidad Autonoma de Barcelona

I. Summary of Project and Global Objectives

This research proposal concerns the study and development of an individual electronic dosemeter working as a real time dosemeter. The aim of this contract is to obtain a macroelectronic dosemeter which will be compared with optimized H.E.C.E., E.C.E. and C.E. track etch dosemeters.

LEPOFI (LIMOGES University) and SIDR (C.E.A. Fontenay aux Roses) work on the electronic system, the principle of which is to detect the secondary particles from a polyethylene converter using a PIPS (passived implanted Silicon) detector and to convert the pulses in term of dose equivalent as fast as they are registered. Differential method is used in order to eliminate background contribution.

ENEA (ROMA), NPD (TESSALONIKI) and SFR (BARCELONA) are concerned in a first time with dosemeters based on track etching.

Two works are proposed :

- calculations which take into account incidence angle and energy of neutrons, γ - n discrimination, background
- experiments, with a first step which will permit to optimize the dosemeter device of each staff and a second step for realization of joint irradiations which can lead to intercomparison taking into account calculations.

We have to point out that contract began the 1st November 1990, in consequence only six months of work are reported here.

Head of Project 1 : Dr. Decossas and Dr. Vareille

II Objectives for the reporting period (November 1990 - April 1991)

Experiments on our own electronic dosemeter were realized in order to test and calibrate the device. The characteristics of the two diodes have been measured with different values of bias voltage and the adjustment of electronic system of treatment has been realized. Some works on calculation of neutron and γ contribution began through definition of a theoritical model which allows the study of parameters modifying the response. A Monte Carlo computer code is in progress.

III Objectives for next period (one year)

We have to optimize the computer code which will be used to determine neutron response taking into account :

- energy and incidence angle of neutrons, on the converter,
- contribution of direct interactions of neutrons with the detector,
- electronic background
- γ contribution due to the interactions with the whole sensor

Joint irradiations in the contract framework will take place in November or December 1991 in order to compare experimental and theoritical results and, on the other hand, electronic sensor to SSNTD based dosemeters. Neutron fluxes used will be monoenergetic in the range [144 keV - 14 MeV] with different dose equivalent (0,5 to 2mSv) and 0°, 30°, 60°, 75° incidence angle.

IV Progress achieved including publications (November 1990 - December 1991)

1 - Presentation of the device (1) (2)

Differential method is used in the configuration of the figure n° 1. One diode is covered by polyethylene converter implanted with boron and the other one is free of converter.

Briefly we can consider the sensor response as :

$$R_T = S_n H_n + S_{ni} H_n + S_\gamma H_\gamma + B$$

Where R_T is the total response,S_n the response per unit of neutron dose equivalent due to converter from which protons, α and Li are emitted.

S_{ni} the response per unit of neutron dose equivalent due to neutron interactions on Si and other materials around the detector.

S_γ the response per unit of γ dose equivalent due to the dosemeter taken as a whole.

B : background

Each contribution may be modified by bias voltage, geometry of sensor and other around materials, thickness and implantation of converter.

Our aim is to determine through experiments and calculations each component of R_T, the value which leads to dose equivalent is :

$$R_{T0} = R_{T1} - R_{T2}$$

Where R_{T1} corresponds to path n° 1 and R_{T2} to path n°2.

2 - Diode test and calibration of the device

We realized :

* comparison of the two diodes through :
  - measurements of the reverse current (figure n°2)
  - measurements of junction - capacity as a function of bias voltage (table n°1)
* adjustements and linearity tests which lead to optimization of the two paths.

The polyethylene thickness and boron implantation characteristics have been well defined.

In spite of a small dissymetry between the two diodes (two new diodes have been ordered) which can be reduced through electronic adjustements, the device is now suitable for the next joint irradiations in the framework of the contract.

Bias voltage (V)	Diode 1	Diode 2
0	4679	4652
1.5	2700	2740
3	1150	1497
4.5	871	921.4
6	75908	795.1
7.5	684.1	712.3
9	627.8	652.1
10.5	583.7	605
12	548.1	566.6
13.5	518.2	534
15	493	506.6

Table n° 1 Capacitance (pF) as a function of bias voltage

3 - Calculations

It is difficult to present theoritical results because of the short delay since November 1991. Nevertheless we can point out the principles of calculation method and the first choices concerning the methods used. Monte Carlo method is used in the two cases : neutron and γ responses.

*** neutron contribution**

During these six months we work on the bases of calculations, modelization... We decide to choose the following neutron energy steps :

- thermal neutrons (0,02 eV - 0,5 eV) : one channel
- intermediate neutrons (0,5 eV - 50 keV) : two channels, 0,5 eV to 1 keV and 1 keV to 50 keV
- fast neutrons (50 keV - 20 MeV) : 50 channels, with steps of 50 keV [50 keV to 1 MeV], 250 keV (1 MeV - 5 MeV), 500 keV (5 MeV to 10 MeV) and 2 MeV (10 MeV to 20 MeV).

The calculation program is writen and tests are in progress.

Neutron response was obtained from previous caculations already done in our laboratory on polyethylene converters, but we have to determine the electronic sensor response to protons, α and Li emerging from the converter and to direct interactions of neutrons on diodes and other materials which are around the diodes.

*** γ contribution**

For the first modelization which is a almost achieved we consider γ interactions with an infinite plane sensor. A better model is in preparation which takes into account the real geometry of the dosemeter.

EGS4 code is used [Walter R. NELSON, Hideo HIRAGAMA and David W.O. ROGERS - Stanford University] and we consider the following physical processes :

- for photons : pair production, Compton diffusion, Ragleigh diffusion, photoelectric effect.
- for electrons : Bremsstrahlung, Bhabha diffusion, Moller diffusion, annihilation, Moliere diffusion, ionization energy loss.

This first period of contract permited us to get accustomed with the computer code.

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(1) - B. BARELAUD, thesis, Limoges (1989)
(2) - B. BARELAUD, J.L. DECOSSAS, L. MAKOVICKA, J.C. VAREILLE, Radioprotection, 26, 2, (1991).

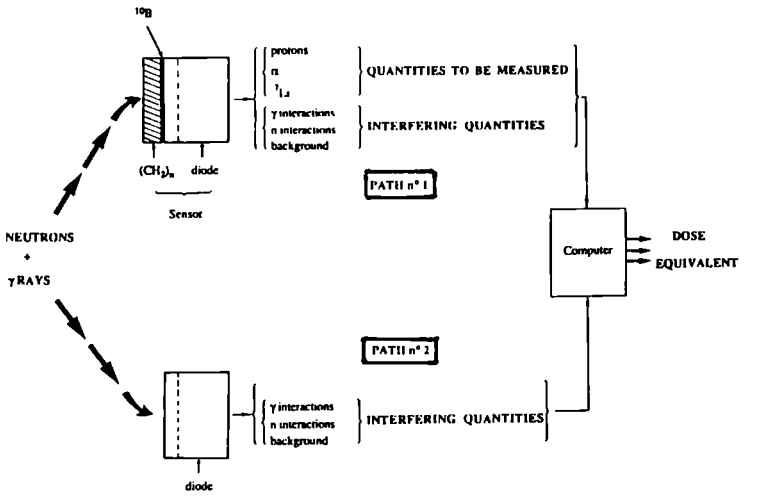

Figure n° 1 : Configuration of the electronic system

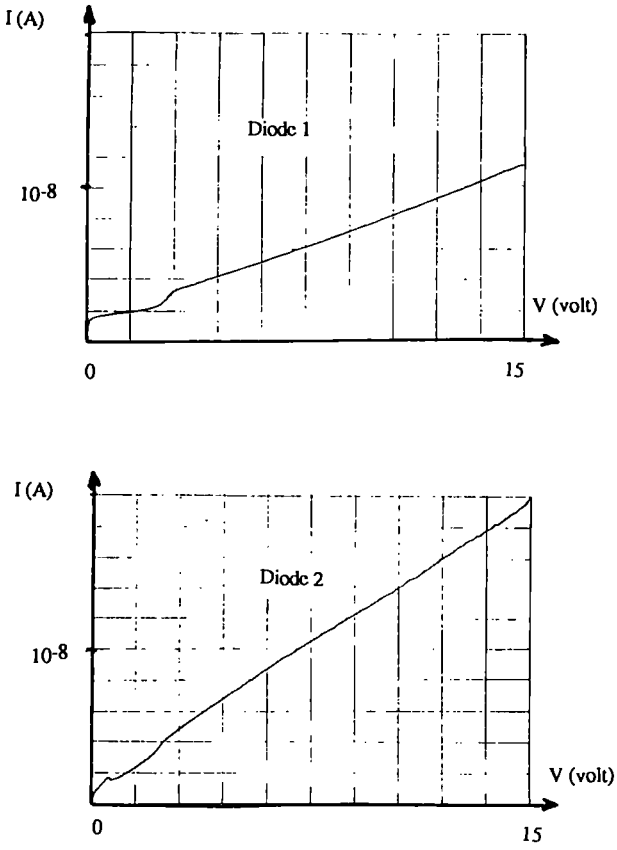

Figure n° 2 :Reverse current of the diodes as a function of the bias voltage

Head of Project 2: Dr. Tommasino

II Objectives for the reporting period

Electrochemical etching of thin films of polycarbonate detectors for the registration respectively of fission-fragments, alpha particles and neutron-induced recoil tracks.

Realization of the apparatus and optimization of both the etching processes and the thin films of aluminized polycarbonate detectors.

III Objectives for next period

The major objective for the next contract period is to extend the investigations to thin films of cellulose derivatives with special regard to LR-115 and cellulose triacetate detectors.

Particular efforts will also be made to improve the apparatus for the thin films electrochemical etching.

IV Progress achieved including publications

The new electrochemical etching - NECE of thin films was first developed in the early 80's (Tommasino et al., 1981a).
No many efforts have bee made in the past for the further development and exploitation of this new electrochemical etching.
In the past decade, most of the research and development in the field of etched-track radiation dosimetry has been dealing with CR-39 detectors, which detectors are not easily available with thickness suitable for the NECE.
In spite of the great many efforts made in the past, the background of CR-39 remains unpredictable (Lembo, 1989).
The applications of CR-39 in personal neutron dosimetry are far from being satisfactory, specially if the new ICRP recommendation will decrease the permitted dose levels and increase the Quality Factors for neutrons.
At present, a possible alternative solution to the complex problem of personal fast neutron dosimetry seems to be provided by the thin-film electrochemical etching of neutron-induced recoil-tracks in large areas of polycarbonate and cellulose nitrate, followed by spark counter registration (Cross and Tommasino, 1970).
In this first contract period, only polycarbonate thin films have been investigated.
For the thin-film electrochemical etching, a two-cells apparatus has been set up which is of the same type of that required for conventional electrochemical etching. In the NECE, however, one cell is filled with the etchant electrolyte, while the other with a dielectric fluid. In these investigations

diesel oil has been used (Tommasino et al.,1981a, 1981b; Krause et al.,1984). The plastic detectors used are aluminized on one surface. DC and/or AC voltage is applied between the thin Al electrode and the electrolyte etchant. The best non-shorting characteristics are obtained with the Al electrode at the positive polarity. In this case, any electrolyte current or even any initial avalanche, localized at the track, leads to Al-oxide formation, which increases the local resistance and stops the current. When compared with the results obtained under DC conditions an intermediate type of self-healing process occurs with AC voltage, since the non-destructive cathodic half-cycle (cathodic dissolution) is repaired during the remaining half-cycle of anodic current (anodic oxidation).

Fig. 1 shows the number of spots produced by normally incident fission fragments from a Cf-252 source on a 10 microns polycarbonate foil electrochemically etched at 25°C with 30% KOH in water. While the frequency was about 2kHz, the overall AC voltage applied to the detector was about 25 Volt, when measured from the zero level to the peak value. However this waveform was superimposed on a DC voltage (so that it resulted entirely positive) and the positive polarity applied at the thin Al electrode.
From Fig. 1 it appears clear that number of the aluminum spots reaches a plateau after only 60 minutes of NECE at 25°c. The aluminum spot-formation is very rapid since the electrolyte foil-breakthrough starts at submicroscopic track-induced channels. Under these NECE conditions it is possible to see the Al spots by naked eye, while it is difficult to see the fission tracks even under high microscope-magnification. Furthermore the magnitude of the etched-removal layer is less than one micron. By contrast, the observation of the aluminum spots by echant penetration with no electric field (Fleischer et al., 1966; Dajkò and Somogyi, 1984) or by spark counting etched-through tracks requires etching times up to one order of magnitude longer and etched-removal layers of several microns. Attempt to use the alcohol-based KOH solution to electrochemically etch alpha tracks in 30-micron-polycarbonate films have shown limited success, because of the poor self-healing properties of this etching. In this case a two-steps etching is required, in which the tracks are formed by the alcohol solution and the non-shorting electrical perforations are produced by 30% KOH water solution. At present, efforts are being made to electrochemically etch alpha-particle tracks in 25 microns-thick cellulose nitrate films from Kodak Pathè.

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Dajkò and Somogyi G. (1984) Study of Spot Development around Track and Electric-Tree-Induced Perforations through an Aluminized Track Detector. Nuclear Tracks and Radiation Measurement 8, 125-128.

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Lembo B. (1989) "Results of a Survey of Backgrounds of CR- 39 Track Neutron Dosimeters" organized by EURADOS-CENDOS in 1988. ENEA/PAS-FIBI-DOSI (89)-1.

Tommasino L., Zapparoli G. and F. Caiazzo (1981a). A New Method for Electrochemical Etching: I Results with DC Voltage. Proc. 11th Int. Conf. Solid State Nuclear Track Detectors, Bristol, Pergamon Press, 211-214.

Tommasino L., Zapparoli G. and Caiazzo F.(1981b). A New Method for Electrochemical Etching: II Results with DC Voltage Proc. 11th Int. Conf. Solid State Nuclear Track Detectors, Bristol, Pergamon Press, 215-219.

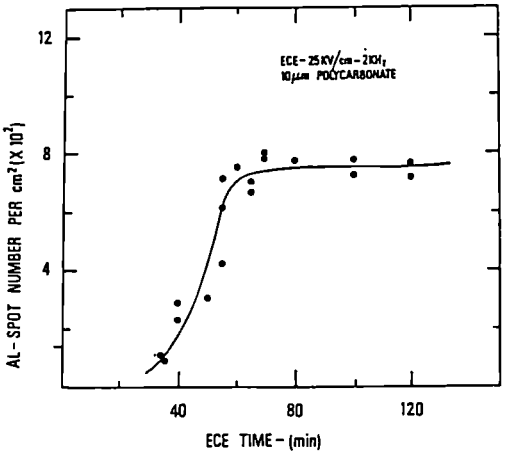

Fig. 1

Pubblications for the reporting period:

L. Tommasino, G. Torri and M. Notaro (1990)

Unique Characteristics of Thin Films Electrochemical Etching. Paper presented at the 15th Int. Conf. on SSNTD, Marburg - Germany. Journal Nuclear Tracks and Rad. Measurements. In Press.

Head of Project 3: Dr. Zamani-Valasiadou

II Objectives for the reporting period

Development of a fast neutron detecting system consisting from CR-39 track detector and thick PE radiator. Alpha particles from ^{6}LiF converter are counted at the same time with proton recoils. Study of PE radiator thickness in order to obtain high response at 2.5 MeV neutrons. Choise of optimum etching time for counting alpha particles and proton recoils at the same etching condition.

III Objectives for next period

Calculation of alpha particle response as function of neutron energy. Combination with data of proton recoil experiment. Study of PE radiator thickness as function of neutron energy.

IV Progress achieved including publications

1. Description of the detecting system.

CR-39 (Pershore Mouldings) of 500 μm was used as SSNTD. On the one surface of the detector ^{6}LiF is evaporated in order to obtain a thin film of about 1 μm in thickness . The system was irradiated inside of PE radiators of various thicknesses (fig.1). The PE radiator is also used as neutron moderator. For comparison of the results to that of proton recoils CR-39 samples were irradiated in the same field of neutrons.

2. Theoretical study of the system.

a) Proton recoils:

The total number of proton recoils registered on CR-39 detector depends on radiator thickness. The minimal radiator thikness for which saturation is obtained define the protonic equillibrium of the system. However, the effect is connected to the range of the most energetic protons for each given neutron energy. For 2.5 MeV neutrons protonic equilibrium is started from 100 μm and continue up to a radiator thickness from which attenuation of neutrons take place. So we can use thick PE radiators under protonic equilibrium conditions.

b) Alpha particles:

$^{6}Li(n,\alpha)t$ reaction can be used for neutron detection in fast neutron fields. Thick PE radiators shifts the shape of neutron spectrum to lower energies giving a considerable number of neutrons in the epithermal region. The reaction cross section in this energy region is close to the elastic neutron scattering cross section at 2.5 MeV. For higher neutron energies a number of alpha particles, contributes also to the total number of counted tracks from the same reaction with lower cross sections.

The energy of alpha particles is related with their emission angle. Figure 2 shows alpha particle energies as function of the emission angle (relative to the neutron beam).In the insert the angular distribution of alphas for 2.5 MeV neutrons is given by Monte Carlo calculation. The distribution is wide and peaked at 60°. For this angle the alpha particle energy is about 3.5 MeV,(fig.2). For smaller angles their energy is higher (4.5 MeV at 0°) . The range of alpha particles of such energies in LiF is of the order 7 μm. For epithermal neutrons the outgoing alpha particles have energies around 2 MeV and their ranges are of the order of 3 μm.

From paragraphs a) and b) is concluded that we expect to detect a high track number attributed to proton recoils as well as to alpha particles.

3.Results and discussion

The samples were irradiated with 2.5 MeV neutrons from the D-D neutron generator of the Nuclear Physics Laboratory in the University of Thessaloniki. Irradiations were performed at neutron fluxes corresponding to equivalent doses of about 5 mSv. Chemical development of CR-39 samples was applied in 20% NaOH solution at 70°C.

The PE radiator is an important parameter in our experiment because of the requirement to working under proton equilibrium. In figure 3 we give the number of track counted as a function of PE radiator thickness. Curve (a) corresponds to proton recoils only while curve (b) represents alpha particles plus proton recoils. The maximum track number is obtained for 0.5-1 cm PE thickness. The decreasing number of tracks for thicker radiators could be explained by attenuation of neutron beam as can be seen from curve (a). This means clearly that protonic equillibrium is destroyed although alpha particle number is relatively constant in the region of radiator thickness used. An other reason of the reduction in proton recoils number is that increasing the PE radiator thickness an important number of neutrons is scattered to various angles relative to the neutron beam escaping the detetion system. The diffusion of the beam inside the radiator changes also proton recoils spectrum. It is so necessary to study the depth from the detector surface at which protons can be counted at the same time with alpha particles.

In figure 4 the number of tracks counted are presented as function of the removed thickness layer from the detector surface. The curve corresponds to 0.5 cm PE radiator thickness. A stabilisation in track number is reached at about 12 μm indicating that protons are developed. However, the greater part of alpha partiles is completely etched before protons, due to the higher dE/dx of alpha particles than protons.

It can also be seen that counting both protons and alpha particles the response of the system is higher than that with only proton recoils.This response is also higher from systems based in PE radiator with combinations of chemical-electrochemical development.

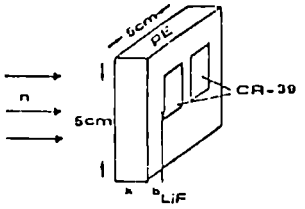

Fig. 1. Experimental arrangement of the detectors for irradiations.

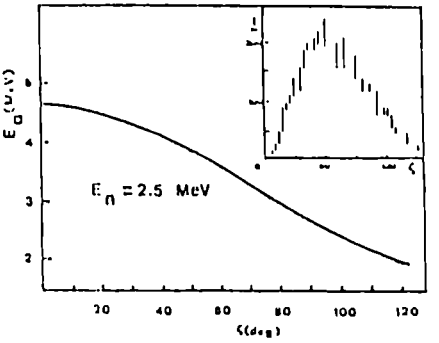

Fig. 2. Alpha particle energy of the $^6Li(n,\alpha)^3H$ reaction as a function of their emission angle. Inset: Angular distribution of alpha particles.

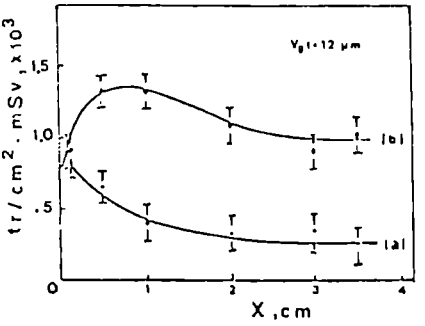

Fig. 3. Response, of proton recoils curve (a) and of total tracks counted curve (b), as a function of PE radiator thickness.

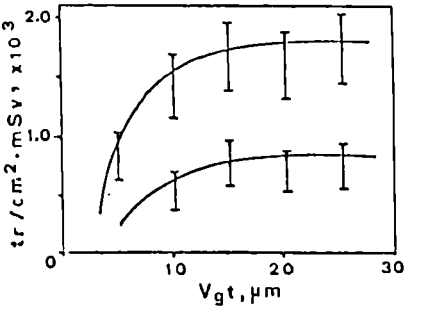

Fig. 4. Response, of proton recoils curve (a) and of total tracks counted curve (b), as a function of the removed thickness layer of the detector. Thick LiF converter and 0.5 cm PE thickness is used.

Head of the project 4: Dr. J. BARTHE

II Objectives for the reporting period

The main objectives, envisaged for the reporting period, are in the first step, design and realization of the dosimetric assembly and, in a second step, electric studies and first use in a reference neutron and gamma field.

III Objectives for the next period

The objectives, planned for the next period of the contract, concern mainly the study of dosimetric response. Characterization envisaged are measurements of angle and energy responses in reference radiation fields. At the beginning these studies will be carried out in neutron source field, following by the use of monoenergetic radiation beams. In the last step, the use of realistic beams such as simulated nuclear power plant neutron gamma fields is foreseen.

IV Progress achieved including publications

1. Detection principle.

Neutrons are detected indirectly through an elastic (n,p) interaction with a hydrogeneous material e.g. polyethylene or polypropylene. The protons emitted by the converter are detected by a semiconducting diode. The system is provided with two diodes: the first diode in contact with the converter detects, not only protons, but also all particles interacting in the depleted zone: p^+, n°, gammas, etc...; the second diode is not provided with converter and only detects "parasitic" particles interacting directly with the diode (essentially neutrons and gammas). The difference between the responses of the two diodes is therefore mainly due to (n,p) interactions in the converter. Thermal neutrons are detected through (n,α) reactions on boron-10 implanted on the converter face in contact with the diode.

The main advantage of this method developed by Barelaud (1) et al. is that the proton fluence is sensibly proportional to the equivalent dose over a range of neutron energies extending from thermal energy up to a few MeV. In order to optimize gamma rejection, the depleted zone of the diodes is made as small as possible, compatibly with the mean free paths and LET's of the particles to be detected.

A synoptic schema of the experimental set-up is shown in figure 1. This equipment constits of the detecting unit, two diodes and a two way multichanel analyzer. A PC microcomputer control is employed.

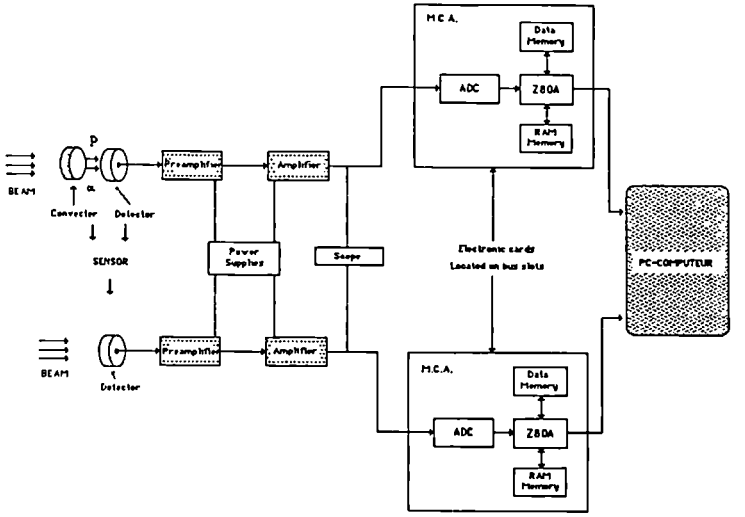

Figure 1: Synoptic schema of the experimental set-up.

2. Electrical characteristics.

Some of the essential characteristics, necessary to obtain a significant result when substracting the background noise from the converter signal are:

a) maintenance of a single depth over the depleted zone in order to assure a minimum energy detection threshold.
b) same mean noise for the two diodes.
c) minimization of detection power by minimizing the depth of the depleted zone.

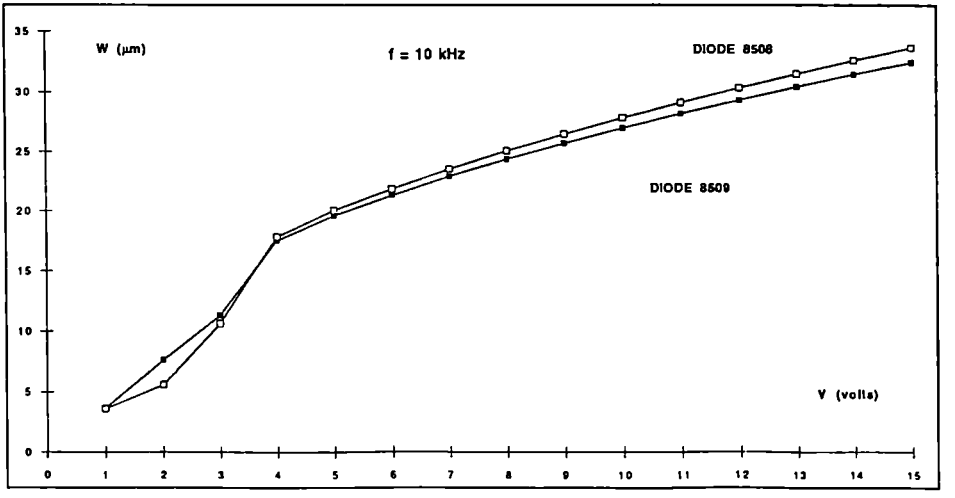

Figure 2: Depth of depleted zone versus applied voltage.

Electrical measurements relate principally to measuring the thickness of the depleted zone. This thickness is determined by measuring the capacitance of the junction using a polarization type impedance bridge.

Figure 2 shows the way in which the thickness of the depleted zone varies as a function of the polarizing voltage. A good correspondance is observed when a polarizing potential exceeding 5 volts is applied. In this case, the junction is blocked. The two diodes exhibit a sensibly equivalent mean noise.

5. Response to radiation fields.

Some special tests have been conducted in specific radiation fields. The aim of the work is to distinguish between the spectra of different incident particles.

Figure 3 shows the spectra obtained for α, ß and gamma particles and also the background noise. The maximum amplitude is normalized to the same value.

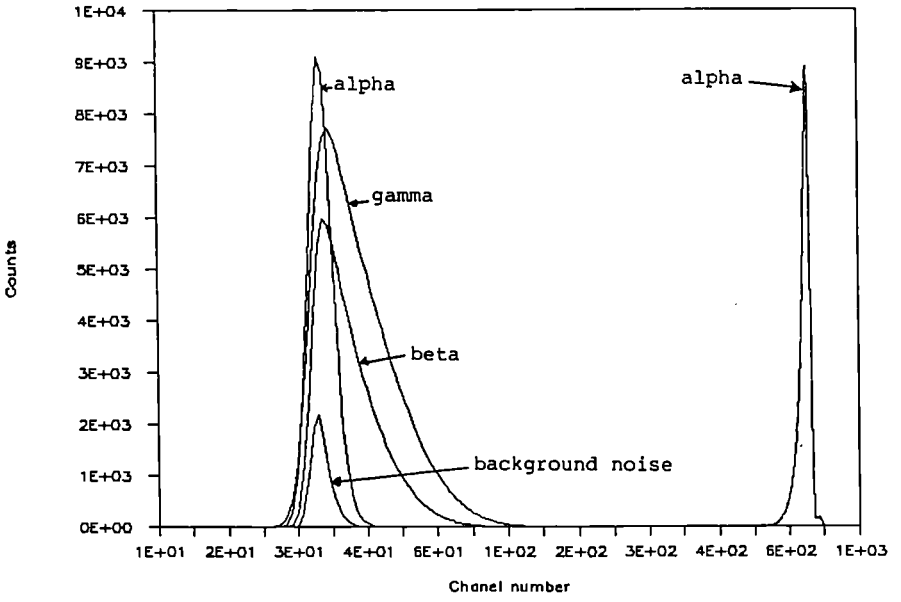

Figure 3: Spectra obtained for different particles.

The diode responses with (a) and without (b) converter are shown in figure 4. The curve (c) represents the difference between counts obtained with the two diodes. This spectrum (c) corresponds to the component due to protons generated in the converter.

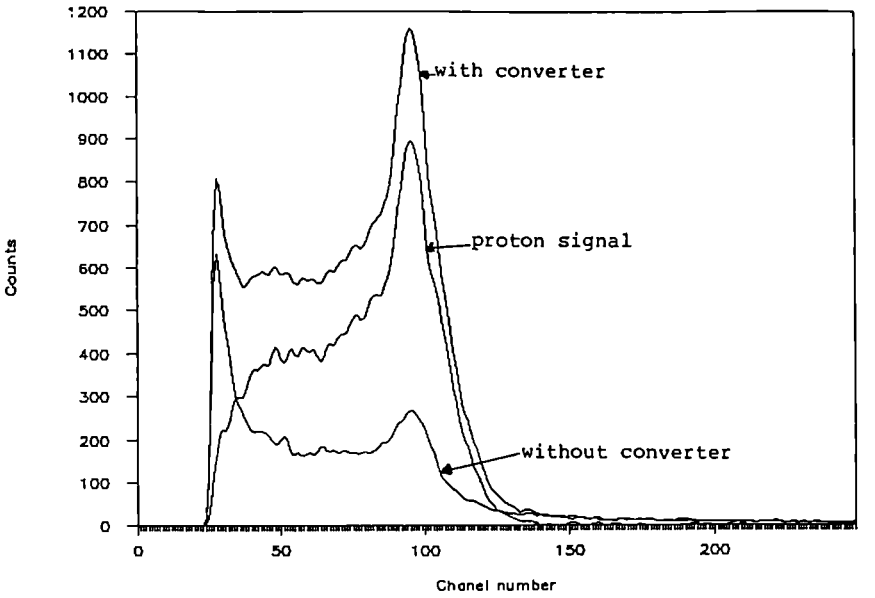

Figure 4: Spectra obtained with an Am-Be neutron source.

6. Conclusion

Preliminary tests confirm the reliability of the dosimetry principle employed. The most problematic aspects of the work undertaken are neutron-gamma rejection, high sensitivity and energy response compatible with radiation protection purposes.

(1) B. BARELAUD:
"*Conception et réalisation d'un capteur pour les neutrons thermiques et rapides*"
Thèse n° 7-1989, Université de Limoges, Avril FRANCE (1989).

References

No references in 1990.

Head of project 5: Prof. Francisco Fernández Moreno

II Objectives for the reporting period

Development of the ECE dosemeter for automated measurements.

III Objectives for next period

- Simultaneous experiments in neutron fields
- Intercomparisons with the other groups

IV Progress achieved including publications

Neutron response calculation

A Monte Carlo method has been used to simulate the passage of a normally incident fast neutron beam through an hydrogenous material. As a first approximation it has been considered that only elastic (n,p) scattering is relevant in the studied energy range. It has also been considered that a given neutron cannot interact more than once in the radiator, as neutron mean free paths for the energies involved are far larger than radiator thicknesses.

The interaction probability of neutrons with radiator H nuclei is calculated from the neutron mean free path, inferred from the elastic (n,p) cross section. It is, then, possible to calculate the number of protons produced in the radiator. The distance between the neutron entry point in the radiator and the interaction point is randomly obtained, and the kinetic energy and emergency angle of every proton produced is calculated assuming that proton emergency is isotropic in the center of mass reference system. The proton range-energy tables obtained from Steward program (Steward, 1968) are used. All these considerations allow the calculation of the number of protons that reach the detector, as well as their angle and energy, with fluctuations smaller than 1.5% if a fluence of 10^7 neutrons$\cdot$cm^{-2} is used. Our computer program takes into consideration not only the protons originated in the radiator, but also the ones produced in the CR-39 layer removed by the etching process.

Experimental detection efficiencies for proton registration as a function of angle and energy have been used in order to calculate the effective number of recorded protons. Conversion to tracks$\cdot$cm$^{-2}\cdot\mu$Sv^{-1} is performed by means of the quality factors given in ICRP 21.

In order to calculate the response for neutron sources and normal incidence, the energy spectrum of the source has been divided into thin energy bands, so obtaining the probability of emission from the source of a neutron of a given energy. The number of neutrons of each energy used in the calculation is modulated by this probability.

A semiempirical function of the form

$$\varepsilon = \epsilon^{(\theta-\mu)/\tau} \tag{1}$$

where ε is the registration efficiency, θ is the proton incidence angle (in degrees) in the detector, μ is a function of the proton energy (in MeV) and τ is a constant, has been used to fit registration efficiency data from Fernandez *et al.* (1987) and Cross *et al.* (1986, 1987). The following values for the parameters have been obtained:

$$\tau = 5.1464$$

$$\mu = \begin{cases} -3372.86E^2 + 1116.88E - 31.53, & \text{if } E < 0.1979 \text{ MeV}; \\ -12.82E^3 + 48.55E^2 - 72.48E + 69.94 & \text{if } E \geq 0.1979 \text{ MeV}. \end{cases} \tag{2}$$

Fig. 1 shows the proton detection efficiency (tracks·cm^{-2} per fluence unity) as a function of their incidence angle and energy for CR-39, using the values (2) in function (1). Protons having energies above 2.5 MeV have a low detection efficiency at all incidence angles due to CR-39 registration threshold, while detection efficiency tends swiftly to 0 for protons with energies below 100 keV.

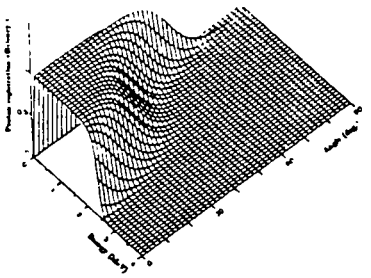

Figure 1.– CR-39 fitted proton detection efficiency as a function of incidence energy and angle (data from Fernández *et al.* (1987) and Cross *et al.* (1986, 1987).

Experimental procedure

Experimental studies have been carried out using CR-39 plates, 250 μm thick and 32 hours curing time, manufactured by Pershore Mouldings Ltd. in 1988. Samples

of (2 x 2) cm^2 have been irradiated in the IPSN-DPT-SIDR CEN Fontenay-aux-Roses (France) to normally incident neutrons flowing from Am-Li and Pu-Be sources, in free-in-air geometry and to various doses. A 1 mm polyethylene radiator (protonic equilibrium for the energies involved) has been placed beside the CR-39 plates, so that neutrons fall in its surface.

After irradiation samples have been electrochemically etched in cylindric methacrilate cells, equiped with electrodes whose distance to the sample can be varied. Etching has been carried out with a 6.5 N KOH aqueous solution at 60°C, using the following steps: a) 30 kV/cm RMS at 50 Hz during 2.5 hours; b) 30 kV/cm RMS at 2 kHz during 1 hour; and c) 15 minutes post-etching. The etched area is about 1.5 cm in diameter, and track density, measured in a centered region of the samples of 1.2 cm in diameter to avoid boundary effects, is analyzed by means of an optical microscope under the magnification of x50.

Fig. 2 shows the dosemeter (protonic equilibrium thickness polyethylene radiator + CR-39 detector) response ($tracks \cdot cm^{-2} \cdot \mu Sv^{-1}$) curve as a function of neutron incidence energy obtained from our Monte Carlo method considering different thicknesses (2, 3.25 and 5 μm) of the CR-39 layer removed by the etching process. The value of CR-39 layer removed has a very important influence on the response curve for low energies of neutrons. In our case the value which best fit the experimental data was 3.25 μm.

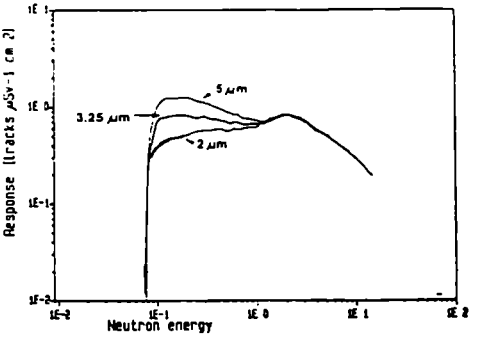

Figure 2.- Energy response of electrochemically etched neutron dosemeters for different thicknesses of the layer removed by the etching process.

Shown in table 1 are the response calculated from our program for Am-Li and Pu-Be sources for 3.25 μm of CR-39 layer removed as well as the measured ones. Background level is found to be 106 ± 40 $tracks \cdot cm^{-2}$, about 10% of the number of recorded protons. There is a good agreement between the calculated and measured values, within experimental errors, so that it can be concluded that our Monte Carlo method can be applied to reproduce the response of a neutron dosemeter if the correct experimental conditions are taken into account.

Table 1.– Measured and calculated response (tracks·cm^{-2} · μSv^{-1}) of the dosemeter to non-monoenergetic neutron sources (Am-Li and Pu-Be).

Source	Experimental value	Calculated value
Am-Li	0.78 ± 0.06	0.71 ± 0.02
Pu-Be	0.50 ± 0.04	0.51 ± 0.01

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Fernández, F., C. Baixeras, M. Zamani, D. López, S. Jockiv, M. Debeauvais, and J. Ralarosy (1988). *Radiat. Prot. Dosim.*, 23, 175–178.

Steward, P.G. (1968). *Ph.D. Thesis.* Lawrence Radiation Laboratory, University of California at Berkeley.

Publication

Fernández, F., C. Domingo, C. Baixeras, E. Luguera, M. Zamani and M. Debeauvais (1990). *Fast neutron dosimetry with CR-39 using electrochemical etching. Nucl. Tracks and Radiat. Meas.*, accepted for publication.

Progress Report

Contract: Bi7-025 **Sector: A12**
Title: Use of the variance-covariance method in radiation protection.

1 Kellerer. Univ. München
2 Jessen Univ. Aarhus Hospital
3 Lindborg Nat. Inst. of Rad. Protection

I. Summary of Project and Global Objectives

The demand for increasing accuracy in the dosimetry of ionizing radiations requires more quantitative assessments of radiation quality. The use of tissue equivalent counters is restricted to radiation fields of low dose rate and it is time consuming if the single-event spectrum is determined in conventional pulse height technique. The application of the variance method on the other hand, where multiple events are observed rather than single events, is restricted to the special circumstances of sources with constant dose rate. Thus there is a broad field for the application of the variance-covariance method which permits the determination of dose averaged lineal energy in time varying radiation fields.

To assess the potential of the method the efforts of the groups cooperating in the further development of the variance-covariance method are, presently, focused on the rather difficult case of pulsed radiations at high dose rates, as they occur in x-ray diagnostics and in radiation therapy with linear accelerators. In these applications the possibilities of the variance-covariance method are particularly impressive.

Head of Project 1: Prof.Dr. Kellerer.

II Objectives for the reporting period

The scientific objective for the reporting period have been measurements with the existing experimental equipment in its present configuration in pulsed diagnostic and therapeutical radiation fields. Further aims were the improvement of the equipment to simulate smaller diameters, to increase the stability of gas flow and gas pressure, to increase sensitivity and accuracy of signal processing, and to evaluate the inherent possibilities of the method for noise rejection.

III Objectives for next period

During the forthcoming period the improved system will be thoroughly tested, and measurements will be performed in therapeutic photon and electron fields. To cope with the high dose rates at the usual distances from the isocentre of the field special small tissue equivalent ionization chambers will be developed. To proceed towards a portable system, applicable in radiation protection, detectors will have to be developed that are independent of a gas flow system. This will require optimization of the detector materials and suitable modified housing of the detectors.

IV Progress achieved including publications

Measurements in the field of a diagnostic x-ray tube:

An example of the results is given in fig.1, where $\bar{y}_D$ is given for the field of a diagnostic x-ray tube. The measurements have been performed with a two-pulse generator i.e. the high voltage has the shape of a rectified sinusoidal with two pulses per 20ms. The beam was filtered by 1mm of aluminum.

The twin detector consists of two cylindrical proportional counters. The detector walls are made of A-150 plastic and have a thickness equivalent to 13mm of tissue. The currents from the detectors are integrated on capacitors. The voltage at the capacitors is repeatedly and simultaneously digitized, and the results are stored in a PC. If the voltage reaches 10V the capacitors are discharged by electric-relais contacts, and a new measurement cycle commences. The measuring cycle and the image pulse of the x-ray tube are triggered by the PC and are thus synchronized.

Special attention has been given to the calibration of the proportional counters which was performed with a Am-241 α source. This source is enclosed in a gas tight capsule which can be attached to small ports in the detector walls. The α particles enter the detector as well collimated beam

perpendicular to the central wire. The energy distribution of the α particles entering the sensitive volume of the detectors depends on the gas pressure - methane based tissue equivalent gas is used - within the system. For the pressures actually used the distribution is determined in a separate chamber which can be connected to the gas-flow system and which is equipped with a semiconductor spectrometer. When the α-source is used with the proportional counter the energy deposition due to single α particles in the counter is deduced from the voltage increment at the integration capacitor.

As a side product of the calibration measurements the Townsend-coefficients for methane based tissue equivalent gas have been determined for a broad range of reduced field strengths. The results are in line with the data given in the literature - there are only few for this gas mixture - including the deviation from the Townsend theory at low gas pressures which, according to Sègur , is due to the increased path length of the secondary electrons at non-equilibrium conditions.

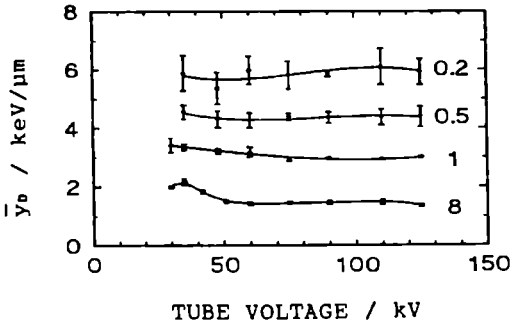


Fig.1. Measurements of $\bar{y}_D$ as a function of tube voltage in the field of a diagnostic x-ray tube. The diameters of the simulated volumes are 0.2, 0.5, 1 and 8µm.

In further measurements variations of $\bar{y}_D$ during the 10ms time interval of the high voltage pulse of the x-ray tube have been determined. In this case the data are collected only during a narrow time segement of the pulses of typically 1ms. At the maximum of the pulse the value of $\bar{y}_D$ is minimal; and at 50kV and a simulated diameter of 8µm it is less by 40% than the higher values of $\bar{y}_D$ at the beginning and at the end of the pulse. This example shows in a particularly impressive way the potential of the method.

Suppression of noise:

In a theoretical analysis the inherent possibilities of the variance-covariance method for suppression of noise and electric pick-up have been examined; several types of disturbances have been considered. One example is given in fig.2. Noise of variable amplitude is superimposed to an experimental set of data. The disturbance has sinusoidal shape and is added with equal amplitude and phase to the signals of each detector. The example demonstrates the stability of the variance-covariance method against this type

of interference. If the mean signal amplitude is exactly equal in both channels, there is no influence of the disturbance on the result. In practice, however, there will always be a slight difference which will cause deviations of the results in opposite directions. Fig.2 shows that averaging of the results of both channels cancels the disturbance in good approximation, and even if the amplitude of the disturbance exceeds the mean signal amplitude.

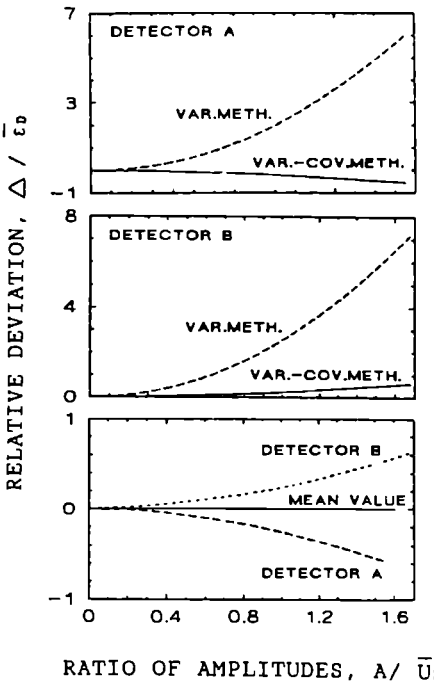


Fig.2. Influence of disturbances; relative deviation as a function of the ratio, of the disturbance amplitude to the average signal amplitude.

Measurements in therapeutic photon and electron beams:

Preliminary measurements have been performed in the photon and electron fields of a 20MV linear accelerator. In both cases it was necessary to reduce the beam current of the accelerator and this caused untypical instabilities of the accelerator. To cope with the high dose rate two improvements of the instrumentation are necessary. Increased sampling frequency will reduce the dose per sampling interval and smaller detectors will reduce the current delivered.

Improvements of the instrumentation:

Several improvements have been implemented in the experimental system. The signal processing has been improved by the implementation of faster and more accurate ADCs. The higher accuracy together with the use of better amplifiers will facilitate measurements with small ionization chambers in high dose rate fields. The simple mechanical device for pressure stabilisation in the gas-flow system has been replaced by a electronic pressure control, and in addition the gas flow is now adjusted by a mass-flow control. These improvements will make it possible to simulate smaller volumes. Testing of the new system and of small spherical ionization chambers is currently performed.

Publications:

J.Chen, J.Breckow, H.Roos and A.M.Kellerer. Further development of the variance-covariance method. Radiat. prot. dosim. Vol.31. 171-174 1990.

Head of Project 2: Dr. Jessen

II Objectives for the reporting period

To modify and improve existing equipment used for variance-covariance measurements in pulsed therapeutic radiation beams of high dose rate. To prepare measurements in radiological X-ray beams in order to extend the use of the variance-covariance method. Development of suitable and reliable detectors and the necessary electronics applicable for measurements in both pulsed and continuous radiation beams. Adjust and extend computer programs for data processing. To identify the areas for collaboration with the other participants in the project.

III Objectives for next period

Testing and optimisation of the constructed cylindrical detector pair. Measurements in different diagnostic X-ray beams qualities of the dose averaged lineal energy $\bar{y}_D$. The measurements will be compared with traditional determination of half value layers in the same beam qualities. Further optimisation of the measuring technique and simplification of the equipment for practical applications will be considered and suggestions formulated for further research work together with the other participants.

IV Progress achieved including publications

The variance-covariance method is a measuring technique designed especially for measurements of the dose averaged lineal energy $\bar{y}_D$ in fluctuating radiation beams. The advantage of this technique is due to the employment of two detectors instead of only one.

$\bar{y}_D$ is a parameter which is very relevant for evaluation of biological effects of ionizing radiation. The fact that the variance-covariance technique makes it possible to measure also under high dose rate implies, that the method is suitable as the basis for developing a measuring method in radiation protection and optimisation of doses in diagnostic radiology - it is not necessary to interfere with the radiation source to perform the measurements.

The first experience of our group with the variance-covariance method was obtained with a system designed for measurements in pulsed therapeutic x-ray and electron fields (H. B. Honoré et al, 1990).

The present work is concentrated on transforming this system into a system appropriate for measurements in continuous Co^{60}-beam, which is regarded as an intermediate step before measuring in diagnostic x-rays.

For the measurements in pulsed beams two Far West Technology proportional counters with an internal alfa source were employed. The entire system was based on analysing the voltage pulses from these detectors.

The situation in a continuous beam is somewhat different. The measurements must be based on integration of the signal from the detectors. After several investigations, and discussions with the other two groups in this coordinated project, it has been concluded that it is not possible to integrate the signal created in the Far West detectors without radical changes in their construction or a need for highly specialized electronics, because they are constructed for pulse measurements. Therefore the Far West Technology detectors have been found to be unsuitable for such measurements.

As a consequence of this it has been decided to apply new detectors. To achieve a more satisfactory calibration in the new system ionization chambers and charge integration have been chosen.

To make a succesful charge integration in the variance-covariance technique, integration of the charge from both chambers in separate integrators in simultaneous intervals must be possible. Two Keithley 617 programmable electrometers together with a computer controlled circuit with relay switches designed in our department fulfil this demand. A two channel electrometer was for a long time under consideration and testing but did not allow for simultaneous integration of the two channels.

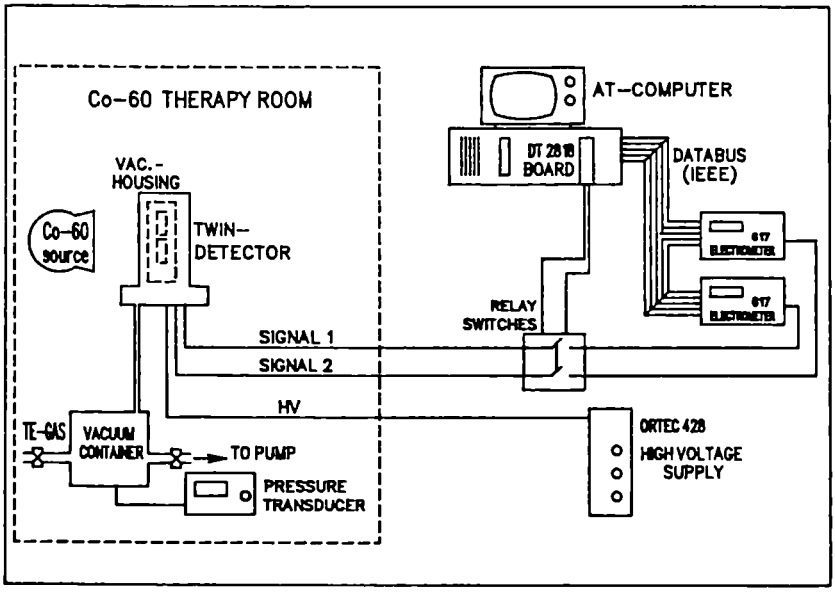


Fig. 1. The experimental arrangement for measurements in the Co^{60}-beam.

The electrometers are connected to the computer via a bus and new software has been developed to control the integration and handle the collected data.

A prototype twin chamber developed and kindly lent to us by the coordinator group in Munich has been used for a general testing of the system. After this testing it has been decided to change the integration method to avoid interferens on the signals from the relay switches. This can be done without major changes of the system.

A new pair of cylindrical detectors has been designed and manufactured together with a vacuum housing which can be used for several chamber designs. The housing has proved to be vacuum tight and the testing of the chambers has started.

More free parameters of the cylindrical shape for optimisation of performance after construction make this shape preferable to the spherical. The lack of rotational symmetry is not regarded as a problem as the chambers are aligned according to beam direction.

Measurement of signal versus voltage and optimisation of free parameters in the chamber design as for example the physical size of the active volumen, the thickness of the central wire and guard electrode parameters will be a first task. Revision of software and designed electronics will be done in parallel.

The system will be optimised in a Co^{60}-beam; when this is achieved measurements in diagnostic x-rays will follow.

H.B.Honoré, K.A.Jessen and H.H.Nielsen. Variance-Covariance Measurements of the Dose Mean Lineal Energy in Beams for Radiotherapy. Rad.Prot.Dosim., 31, 453-455 (1990).

Head of Project 3: Dr. Lindborg

II Objectives for the reporting period

The objectives were:
- To construct ionization chambers suitable for the measurements of absorbed dose in water for x-ray beams with HVT between 0.1 and 2.5 mm Cu at various gas pressure. Detectors made of both tissue-equivalent plastic and air-equivalent plastic shall be used.
- To investigate the photon spectral distribution of the x-ray beams.
- To have electrometers, both with capacitors and resistors in the feedback circuit, designed and tested.
- To write a computer program allowing automatic variance-covariance measurements.
- To install a Monte-Carlo code capable to simulate the measurements.

III Objectives for next period

The objects will be to complete the measurements with the ionization chambers in a water phantom and at different gas filling pressure. From the measurement results the absorbed dose will be calculated both starting with an air kerma calibration free in air in a ^{60}Co-gamma beam and applying Bragg-Gray principles and an air kerma calibration free in air in the x-ray beam and applying the IAEA-code.
The two different electrometer types will be compared concerning their electronic noise characteristics.
Monte-Carlo calculations shall be completed.

IV Progress achieved including publications

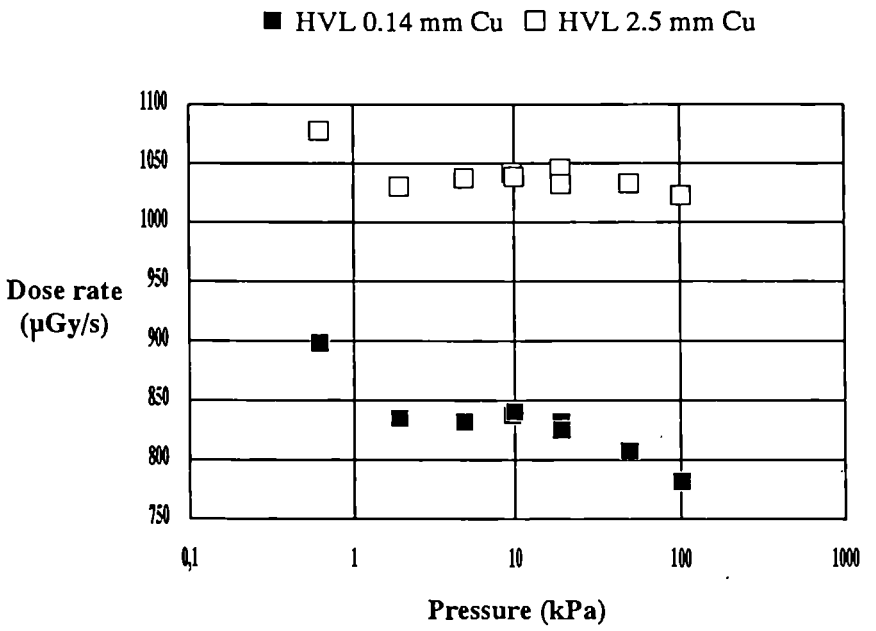
Four cylindrical ionization chambers have been constructed. The wall and the central electrode were made of A-150 plastic for two detectors and of C-552 for the two other. The central diameter is 6 mm and the length for two detectors is 6 mm and for the two other 12 mm. The detectors have a guard ring made of the same material as the wall. The insulators are made of rexolite. The detectors made of C-552 will be used with air inside the detector and the detectors made of A-150 will be used with methane based TE-gas. The detectors were constructed to fullfill the requirements given in the dosimetry protocol issued by the IAEA (1987).

Vacuum tight shields of a thickness which ensures a proper build-up in a ^{60}Co gamma beam are used. With the shield and the existing vacuum system it is possible to do measurements in the pressure range between 0.6 kPa and 101 kPa, which corresponds to a mean chord length range between 0.04 and 7 um. At a pressure of 0.6 kPa the pressure is stable within 2 % during a working day.

Four different x-ray radiation beams with HVL between 0.14 and 2.5 mm Cu and high voltages between 100 and 250 kV have been investigated. The photon spectral distributions have been investigated with a compton spectrometer were the 90 degree scattered radiations from a small scatterer were measured with a hyper pure Ge-detector. From the measurements the spectra at the position of the scatterer was reconstructed.The measured spectras were in good agreement with expected spectra.

Figure 1 shows preliminary results for the long air equivalent (C-552) ionization chamber. The measurements were made at 5 cm depth in water. The figure shows the absorbed dose as determined with different gas pressures in the air cavity of the chamber. The average values of positive and negative polarity are shown. The dose was calculated as if the Bragg-Gray conditions were fulfilled. The results for 250 kV (HVL = 2.5 mm Cu) is independent of the pressure. This is interpreted as that Bragg-Gray conditions are fullfilled already at normal atmospheric pressure. For 100 kV (HVL = 0.14 mm Cu) there is an initial increase with decreasing pressure. The Bragg-Gray conditions are supposed to be fullfilled in the plateau region. At both energies there is an increase at 1 kPa which has not yet been explored. One reason might be secondary electron emission from the wall or a polarity effect.

Figure 1



The same measurement data was used to calculate the absorbed dose to water according to the IAEA-code. At 250 kV the two dose values agree within the experimental uncertainty of +- 3 %. At 100 kV the agreement in the preliminary data is less good.

Since the used values of S/ϱ and μ/ϱ are calculated from spectral measurements in air and the dose measurements are made in a water phantom, correct values have not been used. Therefore variance-covariance measurements were planned to determine y_D and use this as an

indication of the radiation quality. Two types of electrometers will be used one with a resistor in the feedback circuit and the other with a capacitor. As the ionization current from the constructed chambers somtimes is very low (down to 20 fA for the lowest used pressure) it is necessary to place the preamplifier part of the electrometer close to the detector to reduce electrical noise. As the preamplifier has to be shielded from ionization radiation to avoid ionization current in the preamplifier it it easier to make that part of the electrometer small and have the rest of the electrometer distanced from the radiation field. This arrengement led to self-oscillation for all electrometers. Reconstruction of the electrometers with feedback resistors solved the problem and those electrometers are now possible to use for the whole current range. The electrometers have been calibrated during a period of 1 year. The standard deviation is less than +- 0.2 %. The noise currents have also been measured and they are less than 2 fA for a measuring time of 1 s. For the electrometer with feedback capacitor a reconstruction only partly solve the problem. It is now possible tho use them with capacitors down to 1 nF. This is too large to give good precision in the measurements of ionization currents below 100 fA.

For the variance-covariance method a computer program have been written, which allows automatic measurements with both types of electrometers.

A Monte-Carlo code, ITS, have been installed during the period for simulation of the measurements, and to determine the S/ϱ and μ/ϱ The number of interaction is however very low specially for the cases when simulation of the detector with reduced pressure is made. Therefore a technique to separate the calculation in two parts is tested. First the photon spectra is calculated in a plane so distanced from the sensitive volume of the detector that no secondary electrons will reach the cavity of the detector. From that spectra the dose in the detector will be calculated. Until now only the first part of the calculation have been made.

Contract: Bi7-027 Sector: A12
Title: The measurement of environmental gamma doses

1 Bøtter-Jensen Risø National Laboratory
2 Lauterbach PTB
3 Delgado Martínez CIEMAT

I. Summary of Project and Global Objectives

The aim of the project is mainly to determine and analyse the response of different active doserate meters and passive integrating TL dosemeters for measuring the environmental background photon radiation and to establish reference calibration procedures to enable an international intercomparison of measuring results. To ensure that measurements can be made with sufficient accuracy, it is thus necessary to determine the detector responses to cosmic and terrestrial radiations and to take into account the inherent instrument/dosemeter background and linearity at low dose rates.

Instrument/dosemeter calibration methods include free-field and shadow-shield calibrations using certificated gamma sources where ground albedo, air build-up and room scatter components for a variety of source-detector geometries and surroundings are calculated by the Monte Carlo code MCNP. Field measurements are performed to determine the detector/dosemeter responses to terrestrial and cosmic radiation.

The determination of instrument/dosemeter linearity, angle- and temperature dependence and inherent background are performed in the new low-level measurement laboratory at 925 m depth in the Asse saltmine facility where the radiation background is less than 1 $nGy \cdot h^{-1}$. The photon energy responses are determined using different gamma sources in addition to 4 and 6 MeV photons provided by accelerator installations.

Environmental monitoring with integrating TL dosemeters are improved by introducing a new-developed evaluation method based on numerical analysis of the glow curves which especially has proved to be important for assessing the individual dosemeter background from the initiald dose readout.

Emphasis is laid on long term air-kerma rate measurement studies around the Hinkley Point Nuclear Power Station (UK) to assess how different detector types respond to small variations of the background radiations due to the release of Ar-41 plumes and the direct 6 MeV N-16 radiation. The integrated air-kerma values from each of the radiation components are evaluated from the measurements and compared with those from energy discriminating TL dosemeters placed on each active detector.

Head of Project 1: Dr. Bøtter-Jensen

II Objectives for the reporting period

a) Monte Carlo calculations of different free-field and shadow-shield calibration geometries for a certificated Cs-137 source.
b) Long term measurement studies at Hinkley Point Nuclear Power Station of the variation of the ambient radiation due to Ar-41 plumes and the N-16 6 MeV radiation.
c) Free-field and shadow-shield calibration studies using certificated Cs-137, Co-60 and Ra-226 sources and determination of detector responses from terrestrial and cosmic radiations.

III Objectives for next period

- Refinement of Monte Carlo calculations by further including Co-60 and Ra-226 certificated sources and field geometries where surrounding buildings and detector energy responses etc. are taken into account.
- Detailed analysis of free-field and shadow-shield calibrations.
- Detailed analysis of the Hinkley Point measurements using precise calibration data and detector response information obtained from free-field and 6 MeV (accelerator) calibrations.
- Implementation of CIEMAT glow curve analysis in Risø TL environmental monitoring procedures (non-linear heating).

IV Progress achieved including publications

A. Studies of calibration methods and field measurements

Instrument/dosemeter calibration studies included free-field and shadow-shield geometries where ground albedo, air build-up and room scatter components for a variety of source-detector geometries were determined using Monte Carlo calculations (MCNP code). Field experiments included the determination of the detector responses to terrestrial and cosmic radiations.

A.1. Monte Carlo calculations of a free-field set up with a certificated ^{137}Cs source using the MCNP code

The MCNP code has been used to calculate the air kerma rate from a certificated ^{137}Cs source for different source-detector distances in a free-field geometry (open field). The elemental compositions of soil, air and source material are needed for these kinds of calculations, which can be extended to more closed and complex geometries with surrounding buildings, trees etc.

At the detector point the air kerma rate, $\dot{K}$, has four components: the uncollided kerma rate, $\dot{K}_{uncol}$, from photons emerging from the source; the scattered kerma rate from photons scattered in the source material, $\dot{K}_{source}$; the scattered kerma rate from the ground, $\dot{K}_{ground}$, originating from photons leaving the ground surface into the detector point after one or more

scattering reactions in the air/ground media; and finally the scattered kerma rate from air, $\dot{K}_{air}$, originating from photons leaving the air into the detector point after one or more scattering reactions in the air/ground media.

The MCNP code can be used to separate these components:

$$\dot{K} = \dot{K}_{uncol} + \dot{K}_{source} + \dot{K}_{ground} + \dot{K}_{air}$$

In Figure A.1.1 the ratios of each of the components to the uncollided kerma rate are shown as a function of the distance, D, between the source and the detector point and with the source and detector elevated 1 meter above ground.

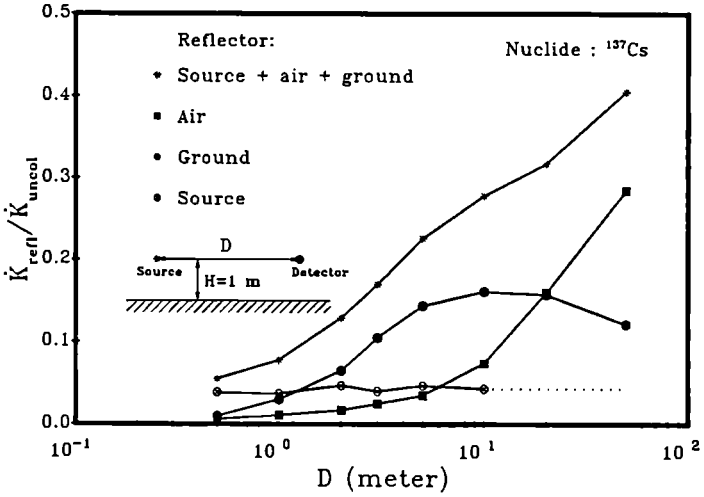


Figure A.1.1. Relative free field kerma rate components in air from a ^{137}Cs source.

The energy distribution of the scattered air kerma rate at the detector point is shown in Figure A.1.2. The source/detector distance, D, is here 3 meter and both source and detector are elevated 1 meter above ground.

The measured air kerma rate, $\dot{K}$, can be expressed by the uncollided air kerma rate, $\dot{K}_{uncol}$, as:

$$\dot{K} = \dot{K}_{uncol} + \dot{K}_{total} = 1.17 \cdot \dot{K}_{uncol}$$

which implies that uncollided photons contributes with approximately 85% of the measured kerma rate, $\dot{K}$, and scattered photons with the remaining 15% for which the energy distribution is shown in Figure A.1.2. These values agree very well with experimental results obtained during an earlier European intercalibration study carried out at Risø (1).

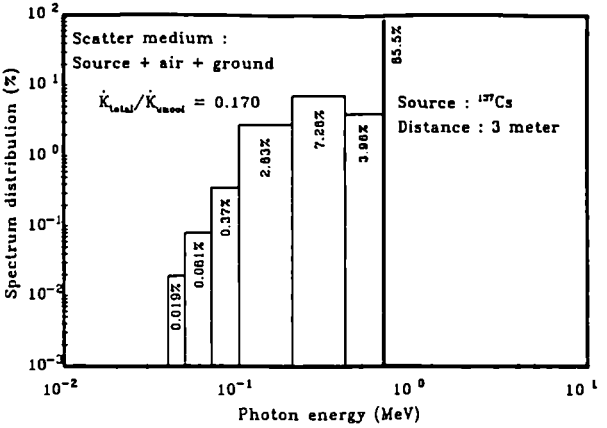


Figure A.1.2. Energy distribution of the relative free field kerma rate in air from a ^{137}Cs source with photon interactions in the source material.

A.2. Monte Carlo calculations of a shadow-shield geometry with a certificated ^{137}Cs source using the MCNP code

The MCNP code has also been used to calculate the dose rate to a high pressure ionisation chamber in a shadow shield geometry. The elemental composition of the room walls, floor and ceiling were used in the calculations of the scatter contributions from these surfaces.

The relative dose rate to the ionisation chamber, normalised to the total dose rate without the 10 cm lead shadow shield, has been calculated to be:

Dose rate without shadow shield:	100%
Dose rate with shadow shield:	
Scattering medium:	
Wall + ceiling + floor + air	27.8%
Source material	3.8%
Shadow shield	0.03%
Total	31.6%

It appears from the calculations that the scatter contribution is around 30% of which the source material is contributing about 4%. Only 0.03% scatter is due to the shield and this contribution can thus be considered negligible.

B. Long term measurements at the Hinkley Point Nuclear Power Station (UK).

Continuous measurements of the air-kerma rate at the Hinkley Point Station (UK) were successfully completed partly under a Risø subcontract (Dr. I.M.G. Thompson) using 4 different designs of active monitoring systems over the period 6th November, 1990

to 7th March, 1991. The 4 monitoring systems used different types of detectors. The two systems from the Physikalisch Technische Bundesanstalt (PTB) used a proportional counter and a plastic scintillation detector and recorded the photon dose equivalent rate every 10 minutes. Both the monitors supplied by Risø National Laboratory, which used a high pressure ionisation chamber (Reuter Stokes RSS-111), and the Geiger-Müller detector system from the GEC, UK recorded the air-kerma rate every 5 minutes.

The detectors were located approximately 200 metres S.W. of Hinkley Point, A Power Station which operates two Magnox reactors having a combined electrical output of 490 megawatts. Over a shorter 2 month period, 11th January to 7th March, 1991, TLD dosemeters supplied by CIEMAT, Spain were also exposed at the same position as the active detectors.

At this location there are two contributions to the photon air-kerma rate from the power station. The 1.29 MeV photons from the Ar-41 discharge plume, which is present when the wind direction is from the station and the direct radiation due predominately to the 6.13 MeV (approximately 68%) gamma ray emissions of the 7.13 second half-life N-16 isotope. This is produced in the CO_2 coolant gas via the O-16 (n,p) N-16 reaction.

On the last day of the experiment the photon spectrum of this radiation was confirmed by measurements made with a portable intrinsic Ge spectrometry system of the PTB.

In addition to the air-kerma rate measurements, coincident records of the wind speed, wind direction, and reactor operation history were obtained.

A preliminary estimation of the integrated air-kerma over the two month period that the TLD's were exposed was 520 μGy from the Geiger-Müller measurements and 490 μGy from the high pressure ionisation chamber measurements and this compares with the mean dose from 8 TLD's of 425 μGy (reported in sec 3 CIEMAT, of this report). Variations in air-kerma rate over a ten day period as measured by the high pressure ionisation chamber are shown in Figure B1. Preliminary analysis of the results over this period show that of a total dose of 45.2 μGy the Ar-41 contributed 2.6 μGy (5.8%) compared to 30.2 μGy (67%) from the N-16 and 12.3 μGy (27.2%) from natural radiation.

A preliminary comparison of the results from the four active detectors shows that though they all very closely followed the variations in air-kerma rate with time, they did not all give agreement on the magnitude of the different components. In particular the scintillator detector measured lower values of the air-kerma rate compared to the other three detectors.

Future work to be undertaken within this contract will involve a detailed analysis of the results using more precise calibration data and response information for the passive and active detectors. The calibration data and response of the detectors to

cosmic radiation and inherent activity will be determined at both Risø and the PTB. In particular the 6 MeV photon response of the detectors will be assessed at the PTB.

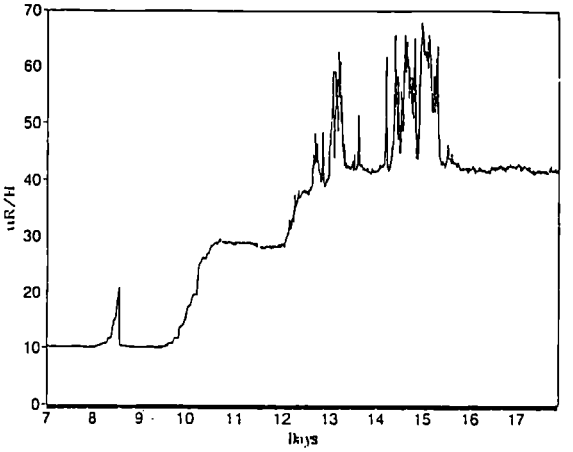


Fig. B1. High pressure ionisation chamber measurements at the Hinkley Point Station over a 10 day period. The stepwise increases of the 6 MeV photons (two reactor units), and the superimposed contributions from Ar-41 plumes are clearly seen.

C. Free-field and shadow-shield calibration experiments

Free-field and shadow-shield calibrations and field measurements to determine different detector responses from terrestrial and cosmic radiations were carried out at the Risø facilities (1) during 12-17 May 1991. Four groups of active detector types were studied namely 1) High pressure ionisation chambers, 2) Plastic scintillators, 3) A new proportional counter type, and 4) a GM counter. In addition TL dosemeters were included as reference due to their well known energy response. The free-field calibrations were carried out in two different geometries 1 m above flat ground 1) far from buildings etc., and 2) close to Risø laboratories where the scatter contributions from buildings, trees etc. were taken into account. The cosmic radiation component was measured onboard an old boat (low contaminated) during a cruise on Roskilde Fjord near Risø. The data obtained from these measurements are now being analysed. A preliminary evaluation shows that the calibration measurements compare very well with the Monte Carlo calculated values and the response to cosmic rays for a high pressure ionisation chamber is about 36 $nGy \cdot h^{-1}$.

Reference: (1) Intercomparison of environmental gamma dose rate meters, EUR reports 11665 and 12731 (1990).

Publications: The Monte Carlo calculated free field and shadow shield calibration geometries and the Hinkley Point measurements are being prepared as two articles for publication in the Radiation Protection Dosimetry Journal.

Head of Project 2: Dr. Lauterbach

II Objectives for the reporting period

a) Completing of the ultra low background facility in the Asse salt mine (Untergrundlaboratorium für Dosimetrie und Spektrometrie (UDO)).
b) Measurement of the inherent background of the proportional counter FHZ 600A in the UDO facility.
c) Long term measurements of small variations of the environmental radiation at Hinkley Point Nuclear Power Plant in the UK.
d) Free-field and shadow shield calibrations and determination of detector responses for terestrial and cosmic radiations at Risø.

III Objectives for next period

a) Determination of dose rate meter responses for 6 MeV photons from an accelarator facility at the PTB.
b) Measurements in the UDO facility:
   - Inherent background of the dose rate meters
   - Linearity at low dose rates
   - Energy dependence, angel dependence of the detector responses and calibration at low dose rates.
c) Detailed analysis of the Hinkley Point and Risö measurements on the basis of the detector calibrations.
d) Preparation of a publication and of the final report.

IV Progress achieved including publications

a) Establishment of an ultra low background laboratory in the Asse salt mine.

In the Asse salt mine at a depth of 925 m a laboratory with an ultra low background was established and completed. The official opening of this facility was on 30th of April 1991 (s. Fig. 1).
The radiation level in this laboratory is below one hundredth of the normal natural background level. The measured dose rate is in the order of 1 nSv/h which was determined by a special scintillation dose rate meter (s. section 4.1 (1)).

To obtain the site for this laboratory a new gallery was driven in pure old rock salt with a very low content of the natural K-40. The specific activity of this surrounding rock salt is 2 Bq/kg up to 3 Bq/kg.

The contribution of the cosmic radiation to the background radiation level in a depth of 925 m is negligible. The air-conditioning plant is equipped with an activated carbon filter for reducing the radon concentration of the air inside this facility.

b) Determination of the inherent background of the proportional counter FHZ 600A.

The inherent background of the proportional counter FHZ 600A was determined in the Asse laboratory "UDO". This instrument was operated in the period from 26.4.91 - 6.5.91 in this laboratory. Taking into account a background level of 0,9 nSv/h in this facility the inherent background of the proportional counter amounts to 14 nSv/h.

c) Long term measurements in the vicinity of the Hinkley Point Nuclear Power Plant, UK.

Two recently developed commercially available dose rate meters for environmental radiation measurements have been positioned in the near vicinity (200 m from the reactor buildings) of the Hinkley Point Nuclear Power Station at the Bristol Channel in Great Britain. The instruments have been operated continuously from the 6th of November 1990 till the 7th of March 1991 with automatic recording mean values every 10 minutes.

The instruments used by the PTB were a proportional counter FHZ 600A equipped with the special countrate meter of the manufacturer and a "Funksonde" a special kind of a scintillation dose rate meter. The time dependent dose rate over the whole measuring time (Fig. 2) shows mainly two different structures. Constant dose rates of different heights are caused by the natural background and the high energy N-16 radiation at different power steps of the reactors. The peak shaped variations riding on the constant dose rates have their origin in the changes of the wind direction blowing the Ar-41 plume above the dose rate meters. The dose rate value during the 7th of January represents the natural background level since the reactors were shut down completely. With these knowledges the dose rate readings of the various components of the radiation field become separable and the relative responses of the different dose rate meter types can be compared for the various components.

The table shows the integrated dose value readings of the proportional counter for the total measuring time and for the period during which also the thermoluminescence dose meters have been exposed:

Exposure period	Total in mSv	Background in mSv	Total-Background in mSv
06.11.1991 - 07.03.1991	1,40	0,40	1,00
11.01.1991 - 07.03.1991	0,8	0,18	0,62

During the period of the reported measurements temporarily arose technical problems with the "Funksonde". Therefore sometimes the measured values dropped out. Additionally this dose rate meter seems to be not accurately calibrated in some dose rate ranges. Values for this instrument can only be evaluated after its calibration during the next period.

Spectrometric measurements with an intrinsic germanium detector show besides the gamma radiation from the natural radionuclides the 1293,6 keV photons of Ar-41 from the plume as the most prominent peak (Fig. 3). The 6,13 MeV gamma radiation from the N-16 with its single and double escape peak is also clearly seen (Fig. 4).

d1) Free field measurements within the area of the Risoe National Laboratory.

In the course of the Risoe 1991 experiments (13.5.91 - 17.05.91) free field calibration measurements were performed at a flat ground in the area of the Risoe National Laboratory surrounded by buildings. The detectors of the used instruments were set up at distances of 3 m, 5 m and 10 m from the different sources which were installed on a source holder at a height of 1 m above ground.

d2) Free field measurements outside the area of the Risoe National laboratory.

In addition free field calibration measurements were also performed at a flat ground far from buildings e.t.c.. The same source set-up was used at this calibration site as in the area of the Risoe National Laboratory.

d3) Shadow shield calibration measurements at Risø National Laboratory.

Shadow shield calibration measurements were performed in the calibration facility described in (1) with a certificated Cs - 137 source. Measurements with the proportional counter have shown a strong dependence of the results on the orientation of the detector in the radiation field. If the cylindrical detector was installed with its longer axis in direction to the source no reliable values have been obtained. The reason is the dependence of the response on the photon energy and the angle of incidence of the photons.

If the longer axis of this detector is placed perpendicular to the incidence direction of the photons the dependence of the response on the photon energy and the angle of incidence is much smaller. The results of these measurements were affected only by the unsuited shield, the dimensions of which were to large for this detector.

d4) Determination of detector responses from cosmic radiation (Risø).

The responses from cosmic radiation were determined on a cruise with an old motor ship on the Roskilde Fjord. All detectors were placed on the upper deck of this ship for a time period of 3 hours.

No influence of the water depth was recognized.
The preliminary value for the response of the proportional counter is 0,072 uSv/h.
Subtracting the inherent background with 0,014 uSv/h yields a value of 0,058 uSv/h which corresponds very well with the value of 0,057 uSv/h given by the manufacturer.

Reference: (1) Intercomparison of environmental gamma dose rate meters, Part I (EUR report 11665)

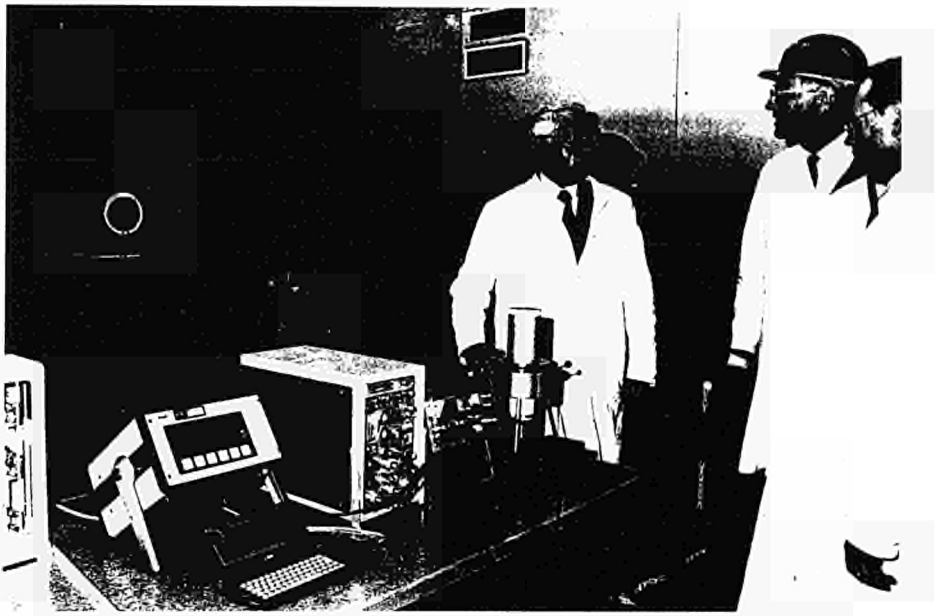


Fig. 1. Demonstration of measurements in the UDO Laboratory.

Fig. 1. Demonstration of measurements in the UDO Laboratory.

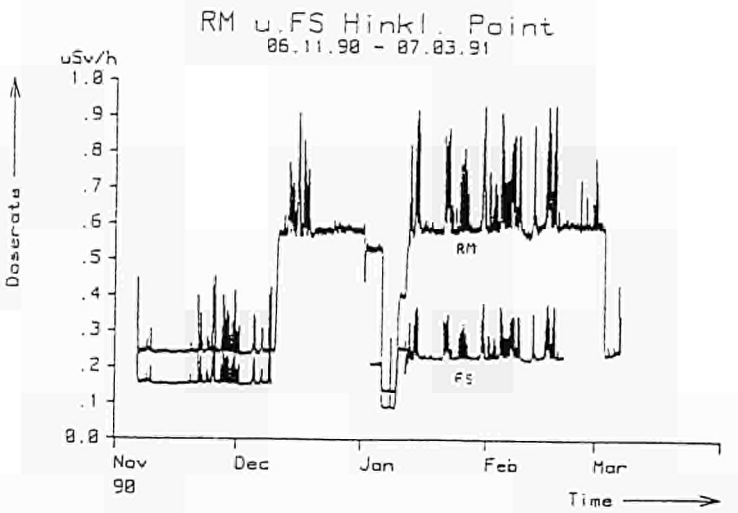


Fig. 2. Dose rate measurements during the whole monitoring period for the proportional counter (RM) and the "Funksonde" (FS).

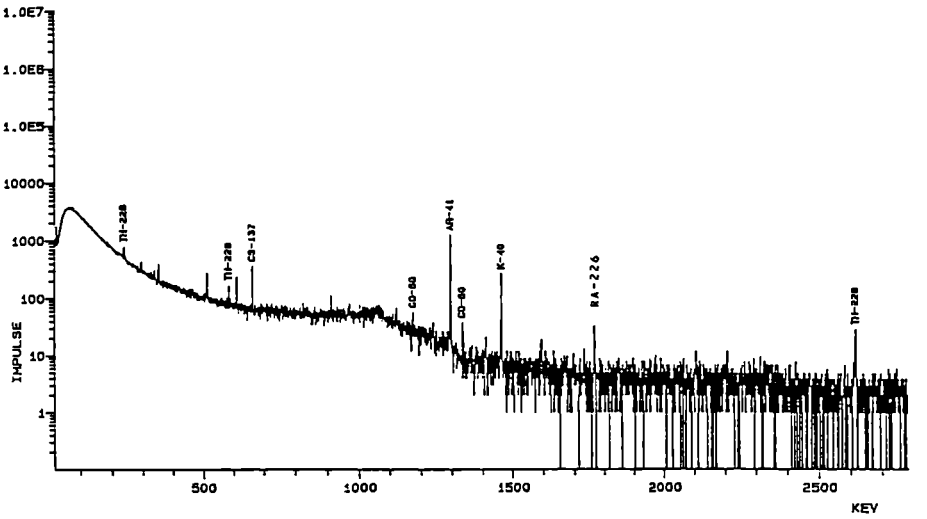


Fig. 3. Low energy part of the Hinkley Point gamma ray spectrum showing the Ar-41 radiation.

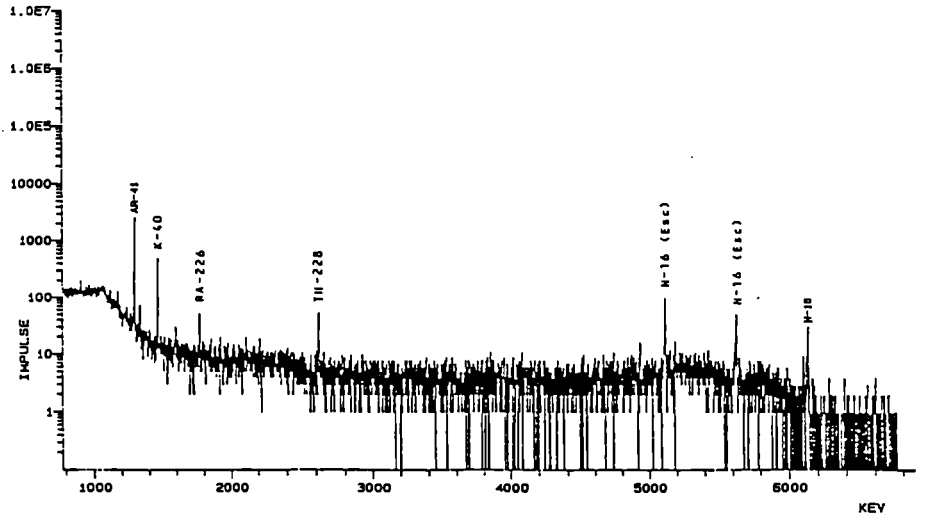


Fig. 4. High energy part of the Hinkley Point gamma spectrum showing the N-16 radiation peaks.

Head of Project 3: Dr. Delgado Martínez

II. Objectives for the reporting period

a) Application of the new CIEMAT TL evaluation procedure for field measurements. Determination of the TLD-100 operational performance for very low dose measurements (10-100 μGy).
b) Study of the modifications experienced by TLD-100 glow peaks in severe ambient conditions. Temperature effects during long exposure intervals.
c) Estimation of dose using TL dosemeters exposed over a 2 month period at Hinkley Point Power Station (joint project with RISØ and PTB).

III. Objectives for the next period

- Refinement of the computer programmes for TL evaluation in the light of the experience gained by their use in field conditions.
- Development of a new computer programme for non linear heating methods (Risø hot nitrogen system).
- Completion of the study of temperature effects in LiF TLD.
- Determination of self-dose characteristics of different TLD materials, using the Asse mine facilities and the new glow curve based TL analysis method. Study of the influence of non radiation induced TL peaks.
- Support with TL measurements to be carried out as part of the common research project with RISØ and PTB.

IV. Progress achieved including publications

a) The first operative version of the CIEMAT Simplified Glow Curve Analysis programme (SGCA) for LiF TL dosemeters has been employed for the TL evaluation of detectors exposed at different monitoring sites in the CIEMAT area. At first, dosemeters specially issued for the test of the new evaluation method were employed. After the integrity of the method was established a new improved computer programme has been incorporated into the environmental monitoring procedure employed by CIEMAT using LiF TL dosemeters. These studies have resulted in improvements in the reliability and operational quality in the lower dose range for TLD-100 (10-100 μGy) extending the conventional lower detection limit.

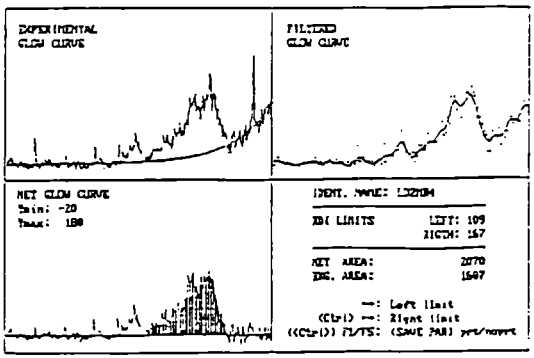


Fig.1. SGCA data output

Figure 1 shows an example of a monitor display for the SGCA programme including the experimental glow curve, the filtered one and the net TL curve with the region of dosimetric interest shaded. The last box includes the relevant dosimetric data such as the TL and background areas and the limits of the region of dosimetric interest.

A paper describing the fundaments of the new TL evaluation method and the advantages derived from its use has been published:

- "A simple method for glow curve analysis improving TLD-100 performance in the dose region below 100 μGy". A. Delgado and J.M. Gómez Ros. Radiation Protection Dosimetry 34, 357-360 (1990).

Further refinements are being developed to improve the method and to extend it to a range of readers and heating profiles.

b) The study of the individual evolution of the TLD-100 glow peaks during prolonged exposures at normal and elevated ambient temperatures (20-70°C) has been completed. From this study it has been concluded that Randall - Wilkins fading (spontaneous leakage of trapped charges) cannot explain variations in the TL yield during ambient exposures. These variations are due to changes in the trap structure and to their distribution which occur within the material. It should be noted that for one of the dosimetric peaks, peak IV, the trapped charges have the effect of stabilizing the trap itself, so that filled peak IV traps are more stable than empty ones.

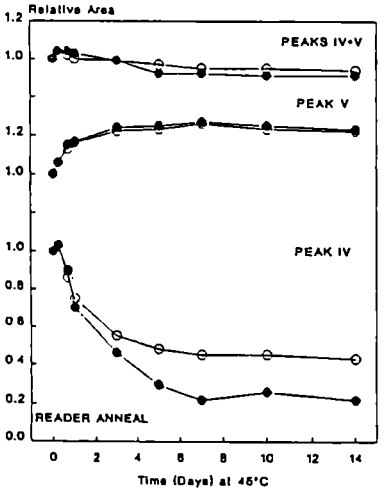


Fig.2. Glow Peak evolutions during storage at 45°C

Figure 2 shows the evolution of the TLD-100 peaks IV and V (and their sum) with the time of storage at 45°C. Black dots represent the evolution during storage of unirradiated dosemeters and white dots of the irradiated dosemeters. The unstable character of peak IV should be noted, as should the fact that this peak is more intense when storage follows irradiation than in reverse.

During 1990 two papers on this subject have been published and one more has been accepted for publication:

- "Evolution of TLD-100 glow peaks IV and V at elevated ambient temperatures". A. Delgado and J.M. Gómez Ros. Journal of Physics D: Applied Physics 23, 571 (1990).

- "Modifications induced in the TLD-100 trap distribution during exposures at different ambient temperatures". A. Delgado and J.M. Gómez Ros. Radiation Protection Dosimetry 34, 233 (1990).

- "High ambient temperature effects in LiF TLD-100". A. Delgado, J.M. Gómez Ros and J.L. Muñiz. Accepted for publication in Journal of Physics D: Applied Physics.

c) CIEMAT provided TL dosemeters which were exposed along with active doserate meters from UK, RISØ and PTB, at a location near to the Hinkley Point Nuclear Power Station. The TLD's were exposed during two months (January and Frebuary 1991) and evaluated at CIEMAT after completion of the integration period. Transit control dosemeters were included together with the set of field dosemeters.

Two types of dosemeters were used. A non-discriminating LiF TLD-100 dosemeter for dose measurements and a discriminating dosemeter based on the Panasonic UD-802 combining $Li_2B_7O_4$:Cu and $CaSO_4$:Tm detectors under different plastic and lead filters.

The TL evaluation, using for the TLD-100 detectors the SGCA programme, produced consistent satisfactory results, the measurements all agreeing within 3%. The discriminating dosemeters showed the existence of high energy photon radiation. The energy was estimated by the dose calculation algorithm always being in the high energy limit of its range.

The dosimetric TL results contained in Table I have been sent to RISØ to be compared with the results obtained by the active detectors.

TABLE I

TL results (transit dose corrected)

	LiF TLD-100	$CaSO_4$:Tm	Discriminating dosemeter	
	K_{air} (μGy)	K_{air} (μGy)	K_{air} (μGy)	Range energy (keV)
Site 1	417 ± 15	443 ± 16	591 ± 30	800 - 1300
Site 2	451 ± 10	416 ± 15	537 ± 27	800 - 1300
Site 3	423 ± 25	407 ± 14	581 ± 29	800 - 1300
Site 4	419 ± 4	380 ± 13	538 ± 27	800 - 1300
Site 5	434 ± 24	410 ± 14	570 ± 29	800 - 1300
Site 6	405 ± 9	389 ± 14	473 ± 24	800 - 1300
Mean Sites 1-6	425 ± 14	408 ± 20	548 ± 39	800 - 1300

Contract: Bi7-028 **Sector:** A12

Title: Dosimetry of beta and low-energy photon radiation using extrapolation chambers and thin solid state dosimeters.

1	Christensen	Risø National Laboratory
2	Chartier	CEA - FAR
3	Herbaut	CEA - Grenoble
4	Francis	NRPB

I. Summary of Project and Global Objectives

Ultimate objectives of the project are to develop standardised calibration facilities and measurement procedures for the dosimetry of weakly penetrating radiations with a view to establishing a more consistent and reproducible dosimetric practice throughout the EEC countries. These include:

- Study and refine extrapolation chamber measurement technique for beta dosimetry. Investigations will be conducted with a view to establishing a common regimen for the extrapolation chamber measurement of absorbed dose rate to tissue at different depths due to beta radiation incident at different angles. Sources will be exchanged between the participating laboratories with a view to comparing extrapolation chambers of different construction and to evolve a standardised measurement and evaluation procedure.
- Realisation of practical beta calibration fields that conform to ISO series 2 specification, characterisation of such fields in terms of ICRU operational quantities and dissemination of information gathered among Member States.
- Characterisation of beta radiation fields in terms of directional dose equivalent rate, $\dot{H}'(d,\alpha)$ by studying depth-dose profiles for several source to phantom distances and angles of radiation incidence.
- Development of individual dosemeters for monitoring weakly penetrating radiation. Highly sensitive TL materials and TSEE detectors will be studied with a view to employing them for individual monitoring of weakly penetrating radiation.
- Development of low-energy photon dosimetry. A facility for generating well characterised low-energy (below 15 keV) X rays will be established. This will be used for studying and characterising solid state dosemeters.

Head of Project 1: Dr. Christensen

II Objectives for the reporting period

Establishment of a calibration facility for beta radiations for irradiation at different incident angles and at different distances from the source. Establishment of an automised, computer controlled extrapolation chamber measurement method for determination of absorbed dose rate to tissue. Study of the homogeneity of beta radiation fields using TLD. Characterisation of the beta radiation from an extended $^{106}Ru/^{106}Rh$ source in terms of $\dot{H}'(d,\alpha^{\circ})$ for different distances from the source. Study of thermoluminescent materials for application as thin detectors for individual dosimetry of weakly penetrating radiations.

III Objectives for next period

Study of the extrapolation chamber measurement method for dosimetry of beta radiation from an extended ^{14}C source. The possibility of using ultra-thin foils as entrance windows of the chambers will be studied. Characterisation of the beta radiation field from ^{14}C in terms of $\dot{H}'(d,\alpha)$ at different distances from the source. Continuation of the study of TL materials for application as thin detectors for individual monitoring for weakly penetrating radiation. Standardisation of measurement methods with extrapolation chambers by participation in comparative measurements among the participants of this programme.

IV Progress achieved including publications

IV.1. Experimental set-up

The set-up established for the beta irradiations and dose rate measurements consists of a rotating table carrying the extrapolation chamber and a source holder which by means of two horizontal slides can be positioned at any distance up to 50 cm from the centre of the rotating table. The source holder allows different source sizes to be used. A $^{106}Ru/^{106}Rh$ source with an active area of diameter 42 mm and a total diameter of 58 mm (Amersham) was acquired. To facilitate the handling of the source it has been fixed into a 60 mm diameter, 1 mm deep depression in a 100 mm x 100 mm x 20 mm perspex block.

The extrapolation chamber used for the dose rate measurements contains a, 30 mm diameter, graphite-coated perspex electrode and a, 2.60 $mg.cm^{-2}$, graphite-coated hostaphan entrance window (PTW type 23382). The chamber has been provided with a step motor and a distance sensor (Heidenhain type MT 12) enabling the chamber depths to be set automatically. The operation of the extrapolation chamber, as well as the registration of the measured data are made automatically by the aid of a personal computer (Olivetti type M 240). A computer program has been developed for evaluating the experimental data, according to methods described in ref. [1] and [2].

Two types of automised computer controlled TLD read-out systems are available for the study and development of TL detectors: One using a linear planchet heating method (model Risø TL-DA8)[3] and

the other one using hot nitrogen gas as the heating medium (Alnor model DOSACUS). In particular the Risø model is suited for analyses of TL materials due to a flexible computer software built-in in this reader.

IV. 2 Experimental results.

IV. 2.1. Homogeneity of beta radiation fields

The homogeneity of the beta radiation field of a 40 mm x 40 mm ^{147}Pm source and that of a 42 mm diameter $^{106}Ru/^{106}Rh$ source was studied by irradiating TL detectors placed on a perspex plate in depressions equally distributed over an area of 100 mm x 95 mm. For the Pm source the variation in dose rate over a 30 mm diameter area parallel with the source plan and with centrum in the centre line from the source was measured to 3% at a distance from the source of 150 mm, 2% at a distance of 20 cm and 1% at 27.5 cm. The corresponding values for a 50 mm diameter area were 10%, 7% and 3%, respectively. The values for the $^{106}Ru/^{106}Rh$ source for a 90 mm diameter area were 9% for a distance of 200 mm and 2% for a distance of 400 mm from the source.

IV. 2.2. Characterisation of the beta radiation field of a $^{106}Ru/^{106}Rh$ extended area source.

In addition to beta rays of maximum energy 3.5 MeV the $^{106}Ru/^{106}Rh$ source also emits photons, essentially of energies 0.512 MeV and 0.662 MeV [4]. The dose rate due to the photon radiation, measured behind a 2 cm shield of perspex and corrected for the attenuation caused by this shield, is varying from 0.10 mGy h^{-1} for a distance of 20 cm from the source to 0.05 mGy h^{-1} at a distance of 40 cm. The corresponding values due to the beta radiation were measured to 130.4 mGy h^{-1} and 33.97 mGy h^{-1}, respectively. These values refer to a source activity of approximately 1.8×10^8Bq. The dose contribution from the photon radiation has been subtracted in all the dose rate data reported below. Values of absorbed dose rate to tissue, $\dot{D}_t(d;\alpha^o)$ for different tissue depths, d, and incident angles of radiation, α, were measured for different distances from the source. The value $\dot{D}_t(d,\alpha^o)$ can be regarded as a good approximation of the directional dose equivalent rate, $\dot{H}'(d,\alpha^o)$ [5]. Of particular interest for individual monitoring is the values $\dot{D}_t(0.07;\alpha^o)$ which can be expressed in terms of $\dot{D}_t(0.07;0^o)$ and the conversion factors $F(0.07,\alpha^o) = \dot{D}_t(0.07;\alpha^o)/\dot{D}_t(0.07;0^o)$. Table 1 presents results from some of these measurements.

Table 1. Values of $\dot{D}_t(0.023;0^o)$ and conversion factors $F(0.023;\alpha^o) = \dot{D}_t(0.023;\alpha^o)/\dot{D}_t(0.023;0^o)$ measured at different distances from a 1.8×10^8 Bq, 4.2 mm diameter $^{106}Ru/^{106}Rh$ source.

Distance from source (cm)	$\dot{D}_t(0.023,0^o)$ (mGy·h^{-1})	$F(0.023, \alpha^o)$ 0°	45°	60°
20	130.4	1.00	1.13	1.21
30	55.7	1.00	1.18	1.25
40	33.97	1.00	1.04	1.05

In the case of accidental exposure of the skin knowledge of the depth-dose profile is particularly important for irradiation with high-energy beta rays where a significant increase of the dose with depth may be observed [6]. At a distance of 30 cm from the $^{106}Ru/^{106}Rh$ source the increase of the depth dose for normal radiation incidence amounted to less than 10% which is lower than that measured for a $^{90}Sr/^{90}Y$ point source [5].

IV. 2.3. Study and development of thin TL detectors.

The study has been concentrated on the LiF:Mg,Cu,P phosphor that is an interesing TL material for individual monitoring for several reasons: high sensitivity, excellent tissue-equivalence for photon exposure [7], low intrinsic background, and low sensitivity to fast neutrons. A detector made of a thin layer of LiF:Mg,Cu,P fixed onto a polyimid tape (commercially available from SDD Lab., Beijing, China) was studied for practical use. The detector is a promising dosemeter for accurate determination of absorbed dose to the skin due to weakly penetrating radiations. A disadvantage of the detector is that the maximum heating temperature is limited to 240°C implying that a relatively long heating period is required before reuse. Improvement of the detector would therefore be achieved by modifying the phosphor to allow for a higher heating temperature.

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4. ISO Standard 6980. Reference beta radiations for calibrating dosemeters and doserate meters and for determining their response as a function of beta radiation energy. 1. revision 1990.

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Head of Project 2: Dr. Chartier

II - Objectives for the reporting period

The general objective of the DPHD/S-DOS is to contribute to the realisation of calibration facilities required for the study and the calibration of radiation protection dosemeters and dose rate meters for the weakly penetrating radiations : beta radiation and low-energy photons. For the 1st year, the activity has been concentrated on the realisation of a computer controlled measurement chain, the main element of which being an extrapolation chamber. That equipment has been involved in the characterisation of radiation fields produced by intense and extended area beta sources, named Series II sources (ISO Standard 6980) : homogeneity, doserate range, conversion factors. Most of measurements deal with ^{204}Tl sources (AMERSHAM TEC type) with and without flattening filters.

III - Objectives for the next period

The work will concentrate on 2 main topics. Firstly, dosimetric measurements and the characterisation of radiation fields will be devoted to those of an extended area source of ^{147}Pm. Secondly, the dosimetry of low energy photons, by means of an extrapolation chamber, will be developed in 2 steps. An irradiation facility, producing 15 keV monoenergetic photons will be used for the determination of conversion coefficients air kerma → H' (0.07). In the mean time, a modification of the facility will be prepared to perform similar measurements with lower energy photons (down to 5 keV). Irradiations of solid state dosemeters will be scheduled in collaboration with the partners of the Joint Contract.

IV - Progress achieved including publications

IV.1 - Experimental set-up

The experimental set-up is composed of 2 main parts: the extrapolation chamber (PTW ref. 23382; "Boehm type") and the equipment for the measurement of the ionisation current, the basic element consisting of an electrometer Keithley 642. In order to work in satisfactory conditions, the extrapolation chamber has been installed, in an independent cell (2.1 m x 2 m x 3.5 m) in which the temperature stability is better than ± 0.5°C over a period of 24 hours and the air displacement is suppressed.

The movement of the collection electrode is motor-driven through connecting gears, and a Heidenhain linear sensor (type MT 12) measures the chamber depth.

The chamber has been modified to be automatically operated through a COMPAQ 386 microcomputer, thanks to a software able to define or change the following parameters : chamber depths, polarisation voltage, number of measurements in series, input of T, p and H from the probes, input of the charge capacity value, calculation of the average value of the current (with statistical uncertainties), etc... By this way, many actions of the operator have been suppressed during the measurements (extrapolation curve, depth-dose curve). In the next software release, the files (kI; l) where l stands for the chamber depth, will be directly edited and then processed in FORTRAN programs. Presently, those files (kI; l) have to be prepared manually.

The AMERSHAM radioactive sources, TEC type for ^{204}Tl, PHC type for ^{147}Pm (active diameter 42 mm) have been fitted in containers fixed on a jig. They have been especially realised to be safely operated. When the cover has to be dissociated from the container, it is simultaneously screwed onto a movable frame and becomes the motor driven shutter, remote controlled from the console.

The different elements of the set-up have been aligned with an optical instrument and control gauges according to an horizontal axis. After some minor changes, the irradiation of dosemeters can be performed on an horizontal calibration phantom.

IV.2 - Characterisation of radiation fields

The radiation fields are those provided by a ^{204}Tl extended area sources (Activity $\simeq$ 25 mCi).

IV.2.1 - Homogeneity of the radiation fields

For calibration purpose, the dosemeters have to be irradiated by uniform radiation fields. The absorption of the beta radiation between the source and the calibration plane may strongly vary according to the length of the considered path and the energy of the beta radiation, and consequently, the dose distribution in a cross section of the "beam".

The ISO Standard 6980 gives a specification of ($\pm$ 5 %) inhomogeneity for the ^{204}Tl.

Therefore, the dose homogeneity has been checked at 3 distances: 15, 20 et 30 cm by a densitometric method, after having exposed unpacked photographic films (Kodak Industrex type). In table 1, the results can be compared with those obtained at 30 an, with flattening filters (as recommended for Series I sources).

Calibration distance (cm)	15	20	30	30 with filters
"Beam" diameter (cm)	7,5	10	16,4	$\simeq$ 20

Table 1: Homogeneity of the radiation fields

From the results presented in table 1, the ISO specifications are fulfilled for fairly large field diameters, but it seems risky to use the total diameter, the 10 % decrease of the doserate being located on the outer ring of the "beam". It could be necessary to revise the ISO Standard values.

Furthermore, the flattening filters, recommended for the Series I sources, give also satisfactory results with extended area sources.

IV.2.2 - Available dose rates $\dot{D}_t(0.07; \alpha^o)$

The main advantage in the use of extended area sources without flattening filters lies in the availability of intense doserates. For the same distances: 15, 20 and 30 cm, measurements of $\dot{D}_t(0.07; 0^o)$ have been performed. For the extrapolation curves, the "polynomial" method (degree 2) has been applied for chamber depths ranging from 250 µm to 2500 µm [1]. For the depth-dose curves, from which the values $\dot{D}_t(0.07; 0^o)$ derive, the least squares fitting to a degree 3 polynomial (for layer thicknesses of hostaphan between 2.4 and 76 $mg.cm^{-2}$) has given the values presented in table 2.

Calibration distance (cm)	15	20	30	30 with filters
$\dot{D}_t(0.07; 0^o)$ ($mGy.h^{-1}$)	366	207	84	54
Uncertainties (%)	2.6	2.1	1.9	2.3

Table 2: Dose rates at typical calibration distances

Comments:

a) Those numerical values show that high dose rates are available, even with the flattening filters, for Series II sources, and that most of usual needs for calibration of detectors used in radiation protection can be solved. Nevertheless, these topics have to be discussed further by participants of the contract.

b) Uncertainties have been evaluated according to the approach recommended by the Comité International des Poids et Mesures (Rec. 1-CI 1981) using numerical values of type B uncertainties given in [2].

IV.2.3 - Conversion factors

The conversion factors are numerical data required for the determination of the angular response of radiation protection. The following expression gives the definition:

$$F(0.07\,;\,\alpha^{o}) = \frac{\dot{D}_t(0.07\,;\,\alpha^{o})}{\dot{D}_t(0.07\,;\,0^{o})}$$

where:

$\dot{H}'(0.07;\ \alpha^{o})$ = directional dose equivalent at a depth of 0.07 mm, for the angle α°

$\dot{D}_t(0.07;\ 0^{o})$ = absorbed dose to tissue at a depth of 0.07 mm, for normal incidence.

For the practical calibration of dosemeters, it has largely been agreed that, instead of the ICRU sphere, a practical phantom (a plexiglas slab) is appropriate. The relation becomes:

$$F(0.07;\ \alpha^{o}) = \frac{\dot{H}_{s,c}(0.07;\ \alpha^{o})}{\dot{H}_{s,c}(0.07;\ 0^{o})}$$

in which:

$\dot{H}_{s,c}(0.07;\ \alpha^{o})$ is the calibration quantity defined in the slab phantom and closely related to $\dot{H}'(0.07;\ \alpha^{o})$.

For the ^{204}Tl, measurements of $F(0.07, \alpha^{o})$ have been performed for the experimental conditions as follows:

a) calibration distances : 15, 20 and 30 cm (without filters)
b) calibration distances : 30 cm (with flattening filters)
c) angle of incidence irradiation : 0°, 15°, 30°, 45°.

The results are given in table 3.

	F(0.07; α°)			
Incidence angle (degrees)	^{204}Tl 15 cm	^{204}Tl 20 cm	^{204}Tl 30 cm	^{204}Tl 30 cm with filters
0	1.00 ± 3.6 %	1.00 ± 3.0 %	1.00 ± 2.7 %	1.00 ± 3.3 %
15	1.02 ± 3.4 %	1.01 ± 3.1 %	0.98 ± 2.7 %	0.99 ± 3.2 %
30	1.03 ± 3.4 %	1.00 ± 3.6 %	0.97 ± 2.9 %	0.96 ± 3.2 %
45	1.04 ± 3.4 %	0.97 ± 3.4 %	0.93 ± 2.9 %	0.91 ± 3.2 %

Table 3 : Conversion factors

1°) Without filters, the conversion factors $F(0.07;\ \alpha^{\circ})$ for ^{204}Tl depend, in addition, on the calibration distance. Such a behaviour may be due to the change of the angular distribution of the radiation field with the distance from the source.

2°) With filters, the values in the last column agree quite well with those already published [3] with point beta sources (Series I).

3°) Those measurements represent the first step of an intercomparison exercise between the participants of the joint CEC contract and the evaluation of results will be achieved at a next coordinating meeting.

IV.2.4 - Remark on the future program with ^{147}Pm

The joint program of the EURADOS Committee involved in Skin Dosimetry brings specific elements for the next intercomparison circuit with a ^{147}Pm source. In particular, the methodology for evaluation of data relative to the depth dose curves has been largely studied and conclusions have been presented in a paper at the Dublin Workshop (May 1991).

V - Publications

1 - T.M. FRANCIS, J. BOEHM, J.L. CHARTIER, P. CHRISTENSEN
Experience gained on extrapolation measurement techniques from an intercomparison exercise conducted with a Promethium. 147 source - Workshop on Skin Dosimetry - Dublin, 13-15 May 1991.

2 - J.L CHARTIER, D. CUTARELLA, C. ITIE
Characterisation of the radiation fields of beta secondary standards with extended area sources (ISO 6980 - Series II). Workshop on Skin Dosimetry - Dublin, 13-15 May 1991.

VI - Bibliography

1 - T.M. FRANCIS, J. BOEHM, J.L. CHARTIER, P. CHRISTENSEN
Experience gained on extrapolation measurement techniques with a promethium. 147 source - Dublin Workshop (May 1991).

2 - J. BOEHM - PTB Bericht 13 (April 1986).

3 - J. BOEHM - Proceedings of the International Beta Dosimetry Symposium NUREG/CP - 0050 - Washington D.C. (February 1983).

Head of Project 3: Dr. Herbaut

II Objectives for the reporting period

- Realization of calibration set-up for beta radiation from extended area beta sources and Büchler facility for irradiation at different incident angles of the radiation and at different distances from the sources.
- Establiment of computer-controlled automatized extrapolation chamber measurement set-up.
- Study of the influence of the size of the collecting electrode.
-Determination of $\mathring{D}_t$ (d, α) for different values of d and α and different distances from the sources, (42mm diameter ^{147}Pm).
-Study of energy and angular responses to beta radiation of thin and ultra-thin thermoluminescent detectors and TSEE detectors.

III Objectives for next period

- Acquisition of 42 mm diameter $^{90}Sr+^{90}Y$ source.
- Continuation of measurements of $\mathring{D}_t$ (d, α) with two different designs of extrapolation chamber using different $^{90}Sr+^{90}Y$ sources (Büchler and 42 mm)
-Comparative measurements between the participating laboratories of dose rates from a 42 mm diameter ^{147}Pm-source-for different distances and angles.
-Continuation of study of energy and angular responses to beta radiation and low energy photon beams of TL and TSEE detectors.

IV Progress achieved including publications

IV.1. Design and realization of calibration devices

The CEA irradiation facility for beta extended sources is now-operational. A 42 mm diameter ^{147}Pm source(518 MBq) has been bought and calibrated without flattening filter by LMRI.
Différent rotating devices have been designed and built for the Büchler secondary standard facility and for the CEA one, for extrapolation chambers and TL dosemeters.

IV.2. Extrapolation chamber

IV.2.1. General description and characteristics

The PTW type 23391 extrapolation chamber consists of two parallel plate electrodes, one of them, the collecting electrode is movable with respect to the other (entrance window) in order that the thickness of the detector volume approaches zero. The thickness of the cavity can vary between the minimum thickness (0,6mm) to 25 mm with a variable spacing of 0,001 mm increments. The collecting electrodes (10 mm, 15 mm, 20 mm, 30 mm, 40 mm in diameter) and the guard ring are made of A 150 tissue equivalent plastic material. The entrance window is made of polythène 7,05 mg.cm^{-2}thick, corresponding to the thickness recommended for the determination of the dose of the skin.
the cavity of the chamber is filled with air.
The electric field applied to PTW chamber is equal to 50 V.mm^{-1}.
The PTW chamber is connected to a Keithley electrometer (model 642 with a measuring limit of 10^{-17}A).
An on-line HP VECTRA ES 12 computer carries out the data acquisition (including ambient parameters), the data processing and the computation of the dose with its uncertainty.
The main characteristics (saturation effects, colelcting electrodes areas,...) have been studied and reported in the progress report concerning the BI7-021 contract.

IV.2.2. Characterization of beta rays beams in term of $\overset{\circ}{D}_t(0,07, \alpha)$

The determination of the absorbed dose rate in tissue D_t at the specified depth of tissue is obtained from the dose rate measured in the gas of the cavity $\overset{\circ}{D}_g$ at the same depth of tissue, by applying the relation ships 1 and 2 and taking into account the necessary corrective terms /1/.

$$D_t = \overset{\circ}{D}_g \cdot S^t_g \qquad (1)$$

$$\overset{\circ}{D}_g = \frac{\overline{W}_g}{e} \frac{1}{a\rho_g} \left[\frac{\Delta I}{\Delta y}\right] y \rightarrow 0 \qquad (2)$$

where

S^t_g = ratio of average mass collision stopping powers for tissue and gas ;

$\overline{W}_g$ = average energy required to produce an ion pair for the beta rays in the gas.

e = elementary charge ;

ρ_g = gas density (air);

a = effective collector area of the measuring volume ;

$\left[\frac{\Delta I}{\Delta y}\right]_{BG}$ = increment of the ionisation current ΔI per increment of the chamber depth Δy, obtained for Bragg-Gray conditions. $\left[\Delta I/\Delta y\right]_{BG}$ is calculated from the slope [dI/dy] of the function I(y).

Measurements have been done in different radiation fields, described in table 1, delivered by beta irradiation facilities (Büchler or CEA), with the PTW chamber (the thickness of the entrance window is equivalent to 7 $mg.cm^{-2}$ of tissue).

Table 1 - Beam characteristics

Radionucléide Emax	source diameter (mm)	Filter yes or no	Irradiation facility	Calibration distance (cm)	angle α
^{147}Pm E_{max}= 0,225 MeV	42	no	CEA	20	
^{204}Tl E_{max}= 0,763 MeV	10	yes	Büchler	30	0° 15° 30°
^{90}Sr + ^{90}Y E_{max}=2,27 MeV	10	no	Büchler	30	45° 60°

The α maximum value has been 60° because of the entrance ring diameter of the chamber.
To determine the value of $\frac{\Delta I}{\Delta y}$ for y = 0 (relation 2)we use a $\overline{y_i}$, ($\frac{\Delta I}{\Delta y}$) $_i$ linear regression /2/ :

$$\left(\frac{\Delta I}{\Delta y}\right)_i = \frac{I_{i+1} - I_i}{y_{i+1} - y_i} \qquad (3)$$

$$\overline{y_i} = \frac{y_{i+1} + y_i}{2} \qquad (4)$$

i = increment number

The computations of the results are in progress .

IV.3. Thermoluminescent dosemeters

The study of dosimetric characteristics of LiF graphite loaded PTFE discs (VINTEN) is in progress with a VINTEN-RIALTO reader. In a first time, experiments are done in photons beams ; after,these dosemeters will be used in the beta rays beams described in Table 1.

IV.4. References

/1/ BÖHM, J - PTB - DOS 13 (1986)
/2/ CHRISTENSEN P, BÖHM,J . FRANCIS, T.M.
Report EUR 11363 (1987)

Head of Project 4: Mr T M Francis

II Objectives for the reporting period

Standardisation of measurement procedures with extrapolation chamber between participating laboratories in this programme. Acquisition of an extended area $^{90}Sr/^{90}Y$ source that will comply with ISO Series 2 specification. Design and construction of a holder for that source. Commencement of measurement programme with a view to characterise the beta field from that source in terms of directional dose equivalent rate, $\dot{H}'(d,\alpha°)$.

III Objectives for next period

Determination of conversion factors from $H'(0.07,0°)$ to $H'(0.07,\alpha°)$ for several values of α and calibration distances and residual maximum beta energy of the spectrum from this source at different calibration distances. Exchange of data acquired as a result of first year's measurements with other participants of the project. An intercomparison programme of measurements with this source is also proposed.

IV Progress achieved including publications

An extended area $^{90}Sr/^{90}Y$ (E_{max}=2.27 MeV) source was acquired. The source has the activity incorporated in a rolled silver foil, the face thickness of the inactive silver layer covering the sources being 50 ± 10 mg cm^{-2}; the uncertainty refers to one standard deviation. The source (overall thickness ≈ 0.3 mm) is square in shape (overall dimension 60 mm x 60 mm) with the activity confined to a central area of 40 mm x 40 mm. A special holder was designed for the source. The holder is made from a block of Dural measuring 75 mm x 75 mm x 28 mm thick with a centrally placed recess 60 mm x 60 mm x 18 mm deep. A block of low atomic number material (phenolic laminated 'Tufnol' BS 2572: 1976) measuring 60 mm x 60 mm x 17.7 mm thick was placed in the recess of Dural block over which the source foil was positioned. It is held in position on the backing material by means of a 2.25 mm thick sheet of tufnol with a 40 mm x 40 mm window through which the active area of the source is exposed. The holder has a threaded stud on one of its sides into which a rod can be screwed to enable positioning the source in front of extrapolation chamber. Figure 1 shows a cross-section of the source holder.

The results of an intercomparison carried out between three of the laboratories participating in this project and PTB, Braunschweig were used for the standardisation of measurement procedures and evaluation techniques with extrapolation chambers. An extended area ^{147}Pm source of similar construction as the source described above was used for this intercomparison. A protocol based on the principles agreed among all participants was established for the measurements to be carried out in this programme.

Towards the latter part of this reporting period, measurements of $\dot{H}'(d,\alpha°)$ with the $^{90}Sr/^{90}Y$ source had commenced. Measurements at a source to chamber distance of 30 cm and at α = 0° have been completed for

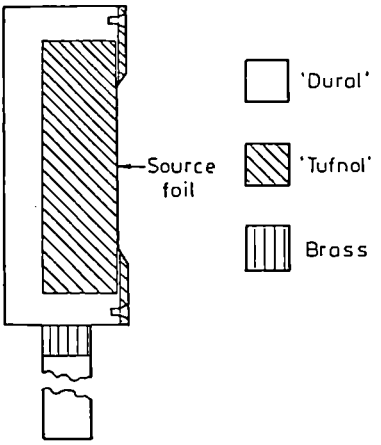

Figure 1. Cross section of source holder.

several tissue depths. The analyses of results of these and the remainder of measurements targeted for this period are in progress.

Publications

1. Francis, T.M., Böhm, J., Chartier J.-L. and Christensen, P. Experience gained on extrapolation chamber measurement techniques from an intercomparison exercise conducted with a ^{147}Pm source. Presented at the Workshop on Skin Dosimetry, Dublin 13 -15 May 1991.

V - Bibliography

1. Francis, T.M., Böhm, J., Chartier J.-L. and Christensen, P. Experience gained on extrapolation chamber measurement techniques from an intercomparison exercise conducted with a ^{147}Pm source. Presented at the Workshop on Skin Dosimetry, Dublin 13 -15 May 1991.

2. Francis, T.M. The development of a reference instrument for the direct determination of dose equivalent from beta radiation at various depths in tissue. Radiat. Prot. Dosim., 12, 219-222 (1985).

3. Böhm, J. The National Primary Standard of the PTB for Realising the Unit of the Absorbed Dose Rate to Tissue for Beta Radiation. PTB-Report PTB-Dos-13 (1986).

4. Christensen, P., Böhm, J. and Francis, T.M. Measurement of Absorbed Dose to Tissue in a Slab Phantom for Beta Radiation Incident at Various Angles. Fifth Information Seminar on the Radiation Protection Dosemeter Intercomparison Programme, Bologna., Report EUR 11363, C.E.C. Luxembourg (1988).

Progress Report

Contract: Bi7-030 Sector: A12

Title: "Development and Application of Different Types of Dose Equivalent Meters in Neutron and Mixed Radiation Fields".

1	Grillmaier	Univ. Saarlandes
2	Brede	PTB
3	Zoetelief	TNO-ITRI
4	Schmitz	KFA Jülich GmbH
5	Segur	ADPA

I. Summary of Project and Global Objectives

The partner laboratories will develop different types of detectors for the measurement of dose equivalent in neutron-photon fields. The methods used include low pressure tissue-equivalent proportional counters and high pressure ionisation chambers as aera monitors, and a semiconductor type detector as individual dosemeter. The research is aimed at the implementation of these techniques for various tasks in operational radiation protection. The technical development of these instruments will be combined with the experimental and theoretical investigations required to improve their performance.

Measurements will be performed in reference photon and neutron fieds in order to compare the dose equivalent response of the dosemeters. Neutron fields in the energy range from thermal up to 20 MeV will be produced among the partner laboratories. Neutron energies above 20 MeV will be investigated in collaboration with high energy accelerator facilities (Paul Scherrer Institute, Ch; UCL, Louvain-la-Neuve, Belg.). The determination of basic physical characteristics of the beams will be an important contribution, in particular the knowledge of photon and neutron fluences will enable the comparison of experimental and theoretical data and improve the analysis of variations in the reading of the dosemeters with regard to different operational dose equivalent quantities. The consequences of radiation quality studies, in particular the concept of biological response function, will be investigated taking into account new recommendations by the ICRP.

The dosemeters developed will also be tested in environments of practical relevance for radiation protection enabling the assessment of the effective capabilities of the instruments in work conditions.

The basic characteristics of the detectors (swarm parameters, gas gain, recombination, dead layer) will be investigated on theoretical and experimental basis in collaboration with Committee 10 of EURADOS on Basic Physical Data and Gas Ionisation Devices by combining in particular electrical discharge modelling calculations and experimental work with gas detectors. These studies will improve the knowledge of the detector properties and extend their range of applicability for dosimetry and microdosimetry research, with also practical consequences for the optimisation of operational dose equivalent meters.

Combined experiments using different techniques for the measurement of neutron fluence and neutron kerma in various materials will be performed in the energy range from 14 to 70 MeV. Interaction quantities of relevance for neutron dosimetry (fluence-to-kerma conversion factors, fractional kerma, average W) will be provided.

Head of Project 1: Dr. Grillmaier

II Objectives for the reporting period

To improve the dose equivalent response of low pressure tissue-equivalent proportional counters (TEPCs) for low and intermediate energy neutrons by testing TEPCs with various sizes, filling gases, simulated diameters and wall thicknesses in reference fields. To assess the influence of ambient temperature on the stability of gas gain for TEPCs to improve their practical performance for radiation protection work.

To continue the expertise of radiation fields and environments of relevance for radiation protection dosimetry using various types of TEPC, in particular the portable survey meter HANDI (Homburg Area Neutron Dosimeter).

To perform experiments at the PSI aimed at the determination of kerma factors in carbon for neutrons from 20 to 40 MeV by using PCs to measure absolute kerma.

III Objectives for next period

To complete the optimisation of the TEPC dose equivalent response for low and intermediate energy neutrons by combining results from experimental investigations with computer simulations to search for the appropriate choice of different parameters (gas pressure, gas composition, wall thickness). To investigate the consequance of varying the detector characteristics for its calibration.

To complete and evaluate the measurements performed at different research reactors and radiation protection environments.

To perform an experiment at the PSI aimed at extending measurements of kerma factors in carbon for neutrons up to 70 MeV.

IV Progress achieved including publications

The development of the ambient dose equivalent meter HANDI based on a low pressure tissue equivalent proportional counter (TEPC) has been continued in collaboration with the Bundes Minister für Umwelt Naturschutz und Reaktorsicherheit. An operational version of the HANDI system was achieved to be tested by different institutes in order to assess their capabilities for radiation protection dosimetry in different environments. The work within the contract was concentrated on improving the performance of the TEPC, in particular with regard to its response for neutrons with low and intermediate energies and to the gas gain stability as a function of temperature.

The reading of a TEPC for neutrons with energies below several hundred keV is considerably lower than the ambient dose equivalent due namely to the combined effects of short range particles and neutron transport. Several experiments were performed during the previous periods in reference fields to assess quantitatively the influence of these effects and to vary several parameters with the aim to optimize the TEPC response over the largest neutron energy range possible. First results showed that the solution depends on a compromise for the choice of the wall thickness, the gas pressure and the gas composition which leads to a higher response at neutron energies below 500 keV and preserves the response already adequate at higher energies as well as the photon-neutron discrimination capability of the TEPC. An additional experiment was performed in the 144 keV filtered neutron beam of the Research and Measurement Reactor (FRMB) at the PTB to investigate more precisely the influence of counter size and wall thickness using different counting gases, in particular compounds with variable amounts of ^{3}He to increase the effect of thermal neutrons produced in the counter wall.

The results collected so far are summarized in Table I for 24 and 144 keV neutrons. They show that, accounting for the calibration method used, the TEPC dose equivalent response can be improved at these energies to +/-40 % by reducing the simulated diameter to 1 μm, using rather thin walls and adding only several percents of ^{3}He. Adding ^{3}He does not influence the determination of the photon dose component and has a negligible effect on the response of the TEPC to fast neutrons. Also shown is the main

Table 1. Optimisation of the dose equivalent response of a TEPC (1/2") to low and intermediate energy neutrons evaluated from various sets of experimental data. Two calibration methods are considered.

Neutron energy	$\overline{R_H}$ absorbed dose calibration [^{60}Co source]				$\overline{R_H}$ H*(10) calibration [2] [^{252}Cf(D_2O) source]
	2.5 mm wall propane TE d=2 µm	2.5 mm wall propane TE d=1 µm	2.5 mm wall propane TE + 2 % ^{3}He d=1 µm	8.5 mm wall propane TE + 2 % ^{3}He d=1 µm	8.5 mm wall propane TE + 2 % ^{3}He d=1 µm
24 keV	0.21 [1]	0.29 [1]	0.43 [4]	1.03 [4]	1.35
144 keV	0.43 [2]	0.54 [3]	0.59 [3][4]	0.49 [3][4]	0.65

[1] after Schuhmacher et al, 1990, Rad. Prot. Dosim. 31, 383-7
[2] after Menzel et al, 1989, Rad. Prot. Dosim. 29, 55-68
[3] present work
[4] after Pihet et al, 1989, Rad. Prot. Dosim. 29, 113-8

difficulty to improve the response for neutrons with energies around 100 keV except when using thick walls which unavoidably deteriorates the detector properties. Furthermore using propane TE gas with 1 % of ^{3}He and for equal wall thicknesses of about 3 cm, the dose equivalent reading of 2" TEPCs was observed lower by about 40 % compared with that of 1/2" TEPCs. Using thin walls however no significant difference could be detected. Due to the multiple parameters involved, further progress requires to combine the present results with computer calculations to search for the optimum detector characteristics. This work is planned in collaboration with the PTB. It is hoped to achieve a dosimeter with a response within +/- 30 % for the whole energy range, a major step for radiation protection dosimetry in mixed fields as an alternative to conventional detectors using thick moderators (Figure 1).

In practical environments, it may be required to use TEPCs in ambient temperatures varying from about 15 to 40 °C. The temperature dependence of gas amplification was therefore investigated by testing the variation of the alpha source calibration peak for TEPCs maintained in a thermostatically controlled chamber. For various TEPCs filled with the propane TE gas mixture a decrease in gas gain of about 0.7 %/°C was observed for increasing temperature from 10 and 50 °C. Furthermore, no significant variation was observed for counters filled with pure isobutane leading to associate the effect of temperature with the CO_2 content. The assumption of multi-body attachement processes was invoked. However a counter with metal walls and filled with propane based TE gas also did not show significant variation leading to interprate the effect as rather due to the interaction of A-150 plastic wall and CO_2.

In addition to the higher stability with temperature, the replacement of the propane TE mixture as counting gas by isobutane offers better gas gain properties enabling to operate the detector at lower high voltage and to further reduce the gas pressure. Reducing the simulated diameter down to several hundred nanometers indeed would substantially improve the TEPC response as shown above. To investigate this a joined programme is being elaborated with the University of Toulouse to study the gas gain characteristics of large size TEPCs at low gas densities.

TEPC measurements were performed at different institutes continuing the expertise work accumulated in the laboratory for a large range of reference and practical radiation fields. A survey of dose equivalent distributions was performed around a cyclotron for radiosotope production dedicated for clinical use using the ambient monitor HANDI. Using the laboratory system, TEPC measurements were performed at the research reactor Silène (CEA, Valduc) within the frame of an experiment aimed at spectrometry measurements around the shielded source for investigation of criticality accidents. Measurements were used to control the radiation quality and the dosimetry of radiobiological experiments at the research reactor TAPIRO (ENEA, Casaccia). The evaluation of these experiments is in progress.

Kerma measurements were performed using PCs made of carbon and A-150 plastic in neutrons fields with energies of 28 and 39 MeV at the Paul Scherrer Institute (CH). This experiment was achieved in collaboration with the PTB and with the Université Catholique de Louvain (project 2) to determine kerma factors for carbon and A-150. The kerma measurements with the PCs were evaluated with those obtained at the PTB in the range from 14 to 20 MeV by in improving calibration techniques and critically assessing the basic physical data required to reduce experimental uncertainties.

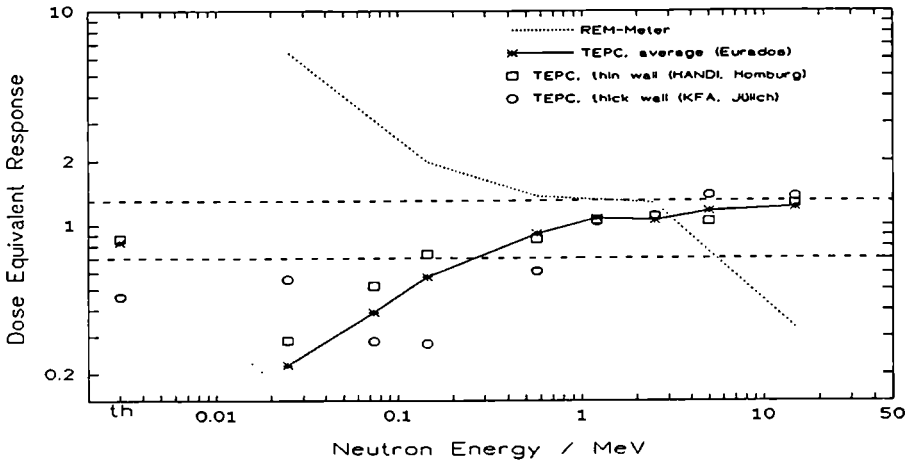

Figure 1. Response of different TEPCs compared with that of a rem-meter. The response interval expected after optimisation of the counter characteristics is indicated (horizontal lines).

Scientific staff :

S. Gerdung, A. Kunz, T. Lim, P. Pihet

Other research group(s) collaborating actively to this project :

Universität Basel (Dr. R. Henneck) and Paul Scherrer Institute, PSI Villigen (CH)

Univ. Catholique de Louvain-la-Neuve, Unit. Phys. Nucl. (Prof. J.P. Meulders) (B)

Centro Ricerche Energia Casaccia, ENEA, Lab. di Patologia (Prof. M. Coppola) (I)

Contract BI7*0051F "Dosimetry and spectroscopy measurements of the leakage radiation fields from the Silene reactor" (Dr. R. Medioni, Dr. H.J. Delafield)

Publications :

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2) Kunz, A., Pihet, P., Arend, E. and Menzel, H.G. An easy-to-operate portable pulse-height analysis system for area monitoring with TEPC in radiation protection. Nucl. Instr. and Meth. Phys. Res., A229, 696-701 (1990)

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4) Pihet, P., Guldbakke, S., Menzel, H.G. and Schuhmacher, H. Measurements of kerma factors for carbon and A-150 plastic for neutrons from 13.9 to 20.0 MeV. In preparation.

Head of Project 2: Dr. Brede

II Objectives for the reporting period

Investigation of reference neutron fields (E_n > 20 MeV) for detector calibration.

Determination of neutron kerma factors in A-150 plastic and carbon (E_n < 40 MeV) combining low-pressure proportional counters and neutron fluence measurement techniques.

Development of a transfer device for dose equivalent quantities.

III Objectives for next period

Expansion and improvement of the neutron fluence determination for neutron energies between 20 and 80 MeV with NE213 scintillation detectors.

Adaptation of a proton recoil telescope for neutron fluence determination above E_n= 50 MeV. Reduction of the uncertainties by comparing Monte Carlo simulations and measurements.

Determination of A-150 plastic and carbon fluence-to-kerma conversation factors for E_n > 40 MeV.

Mathematical optimisation of tissue equivalent proportional counters with regard to application as area monitor and dose equivalent transfer instrument in collaboration with University of Saarland.

IV Progress achieved including publications

PTB has been provided the neutron reference fields for neutron energies below 20 MeV, including the information on the spectral neutron and photon fluences and related instrumentation.

The suitability of the tissue-equivalent proportional counter (TEPC) as a tranfer device for dose-equivalent quantities, in particular as a reference instrument for the "field calibration" of individual dosimeters, has been investigated.

For neutron energies higher than 15 MeV, basic data for dosimetry such as W-values, kerma and fluence-to-kerma conversion factors have been investigated using the low-pressure proportional counter (PC) technique.

For neutron energies above 20 MeV, the necessary information on field characteristics (photon and neutron fractions, absolute and spectral neutron fluence) has been improved by using TOF techniques with PCs, a proton recoil telescope and spectrometry with an NE213 scintillation detector.

a) Investigation of reference neutron fields

The first step in obtaining and characterizing of reference neutron fields (40 MeV > E_n > 20 MeV) has been successfully taken at the Paul-Scherrer-Institute (PSI), Villigen, Switzerland. Using the pulsed proton beam of the cyclotron and a 2 mm thick Be target for the neutron production, time-of-flight (TOF) spectrometry has been applied with NE213 scintillation detectors and low-pressure proportional counters to separate neutron and photon dose fractions (Schrewe et al.).

A written proposal has been made to create the experimental prerequisites for setting up reference neutron fields in the European Community at neutron energies between 20 and 90 MeV. An external pulsing system for the cyclotron beam at the

Université Catholique de Louvain (UCL), Louvain-la-Neuve, Belgium, has been proposed (Brede et al.) which allows TOF spectrometry with low-pressure proportional counters and scintillation detectors to be applied. Realization of this project will considerably improve the experimental conditions.

Spectrometry with NE213 scintillation detectors :

The spectral fluence of the neutron field has been obtained by converting the TOF spectra of the scintillation detectors into energy spectra. For energies between 2 and 20 MeV the use of scintillation detectors allowed the neutron fluence to be determined within an uncertainty of 3 %. For neutron energies above 25 MeV, however, the agreement between the measured response functions of the NE213 scintillation detector and the efficiency predicted by calculations (Monte Carlo codes SCINFUL and CECIL developed by Oak Ridge and Kent State University (USA) respectively) was not satisfactory. Discrepancies up to 30 % in the efficency as a function of the detector energy threshold have been found.

Fluence determination with a proton recoil telescope :

At PSI the absolute fluence of the neutron fields has been determined within an uncertainty of about 8 % using a proton recoil telescope (PRT) which consists of a hydrogen-containing radiator, two gas-filled proportional counters and two semiconductor silicon detectors operating in coincidence. Extensive Monte Carlo simulations of the experimental arrangement have been performed in order to avoid systematic uncertainties in the neutron fluence analysis.

b) Determination of kerma factors :

Kerma factors for carbon and A-150 tissue-equivalent plastic were determined in almost monoenergetic neutron beams with nominal energies between 14 and 20 MeV at PTB and neutron energies of 26 and 38 MeV at PSI (Ch) (Schuhmacher et al.). The kerma was measured with low-pressure proportional counters with walls made of graphite and A-150 plastic in collaboration with University of Saarlandes. The neutron fluence was measured with a proton recoil telescope and the spectral neutron fluence with an NE213 scintillation detector. Spectral fluence measurements were performed in collaboration with University of Louvain (B). The kerma factor ratio for ICRU muscle tissue to A-150 plastic calculated on the basis of the new kerma factors is about 0.93 for neutron energies above 14 MeV.

c) Development of a transfer device :

Neutron transport calculations and charged particle energy deposition spectra in small volumes have been performed in order to assess the dose-equivalent response of a small size TEPC in a phantom. The phantom that has been modelled by a sphere made of A-150 plastic will be used for the calibration of instruments in units of dose equivalent. Such approach will in particular enable the comparison of the TEPC as transfer instrument with other dose-equivalent meters developed by other groups within the framework of the present contract.

Scientific staff :

R. Nolte, U.J. Schrewe, H. Schuhmacher, B. Siebert

Other research group(s) collaborating actively to this project :

Universität Basel (Dr. R. Henneck) and Paul Scherrer Institute, PSI Villigen (CH)

Univ. Catholique de Louvain-la-Neuve, Unit. Phys. Nucl. (Prof. J.P. Meulders) (B)

University of Göttingen, Zweites Physikalisches Institut (Prof. F. Smend) (D)

Publications:

1) H.J. Brede, W. Beverung, and R. Böttger. An External Pulsing System for the Louvain-la-Neuve Cyclotron. Internal PTB-Report: PTB 7.31-90-1 (1990)

2) U.J. Schrewe, H.J. Brede, F. Langner and H. Schuhmacher. The Use of Microdosimetric Detectors Combined with Time-of-Flight Techniques. Nucl. Instrum. Meth. A299 (1990) 226 - 230

3) H. Schuhmacher, H.J. Brede, R. Henneck, A. Kunz, J.P. Meulders, P. Pihet and U.J. Schrewe. Measurement of Neutron Kerma Factors for Carbon and A-150 Plastic at Neutron Energies of 26 MeV and 38 MeV. Submitted for publication in: Physics in Medicine and Biology.

Head of Project 3: Dr. Zoetelief

II Objectives for the reporting period

To develop a field instrument for measuring dose equivalent in mixed n-γ fields based on high-pressure ionization chambers (HPIC). To measure the pressure dependence of the reading and the ion recombination for TE and Al HPIC's filled with methane, a hydrogen-rich gas, and argon, using gas pressures ranging from 0.1 to 8 MPa and variable collecting potentials ranging from 50 to 600 V.

To perform measurements at the TNO around a Van de Graff generator producing 15 MeV neutrons, for different positions relevant for radiation protection situations, in the generator hall and in the maze. To compare HPIC measurements with respect to radiation quality and other physical characteristics of the ambient field with those from a proportional counter, a Geiger-Müller counter, and a rem counter.

III Objectives for next period

In the second year of the contract the measurements will be extended to other neutron energies. The results will be analyzed in terms of cavity size effects and ion recombination theory. The Caswell and Coyne code will be used to perform calculations of energy deposition, ion yield and cavity size effects (insiders, stoppers, starters and crossers), and the results of the measurements will be compared with the calculations. In addition ion recombination theories will be studied. Analysis of the results have to be made in terms of e.g. columnar recombination (Jaffé theory) or cluster recombination (Kara-Michailova-Lea theory).

IV Progress achieved including publications

The use of high pressure ionisation chambers (HPIC) for determination of dose equivalent is based on increased sensitivity of the chambers with increased gas pressure whereas the pressure dependence as well as the ion recombination characteristics can be used to provide information on radiation quality.

To benefit from the increased sensitivity of HPICs of importance is that leakage currents are kept relatively small. Therefore, leakage currents have been measured in order to test their influence and reproducibility. It was found that there is little dependence of the leakage current on gas pressure or type of gas, but a strong dependence on the voltage applied. For a collecting potential of 600 volt the leakage current was approximately $(1.0\pm0.3).10^{-13}$ A. Its contribution to the total current measured at 600 volt at positions 2m 0° (i.e. 2 meter distance from the target and at an angle of 0°) and 2m 45° varies from about 3% to 50% for pressure applied of 8 and 0.1 MPa respectively. Measurements of leakage currents show hysteresis (fig.1), but this hysteresis is only partly responsible for the hysteresis in the charge measurements during irradiation, amounting up to 30%. Leakage current measurements performed before or after an irradiation session are different from another. After irradiation a radiation induced leakage current was observed, but for all HPIC's it is leaks away with a half time of few minutes. Furthermore charge measurements of the tissue-equivalent (TE) HPIC irradiated under vacuum conditions were not found significantly different from the normal leakage rate, confirming that there is little or no radiation induced leakage.

It was concluded that the leakage current of the present chambers is too large. Since variations in the leakage current determine the lower level of detection, additional work would be required to reduce it by a factor of 10 to 100 to reach a suitable value taking into account usual ionization currents for neutron irradiation. As shown the leakage current is dependent collecting potential and the minimum detectable absorbed dose rate will be optimised by testing the various gas/HPIC combinations.

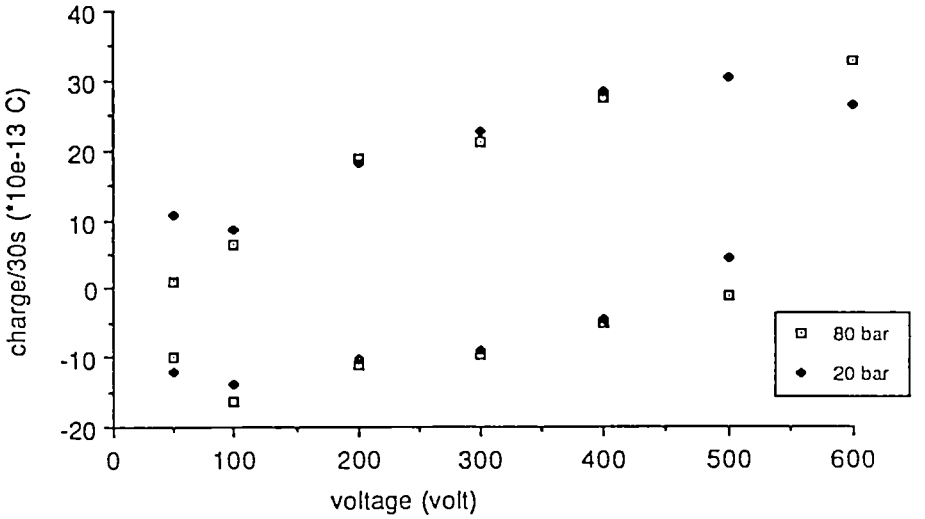


Figure 1 Typical pattern for leakage currents for the TE HPIC filled with methane for different gas pressures related to the collecting potential applied.

Until now, measurements were performed with the HIPCs at various neutron energies (ranging from 0.9 to 15 MeV) in the direct beam at relatively high dose rates (in the order of 10 $mGy.min^{-1}$). The present investigations are aimed at studying the response of the system under conditions more relevant for radiation protection situations. From the gases previously used, methane was selected to obtain a detector with high neutron sensitivity and argon to obtain a photon sensitive device. To study the influence of the chamber wall both TE and Al HPICs were used.

Measurements were planned using the TE and Al HPICs employing CH_4 and Ar as filling gases respectively at seven different positions in the generator hall and maze entrance at our institute. Gas pressures are ranging from 0.1 to 8 MPa and collecting potentials from 50 to 600 V. Until now the measurements at positions 2m 0° and 2m 45° have been systematically completed for the four combinations of HPIC and filling gas. The relative readings, defined as the quotient of the reading at 600 V for a given pressure to that at 600 V for 1 MPa, have been calculated as a function of the pressure for the TE and Al chambers at the two positions. As an example the results of the TE chamber filled with methane at position 2m 45° are shown in fig. 2. The relative readings for the same chamber and gas obtained at position 2m 0° were not found significantly different from the results at position 2m 45°. More precisely, the results indicated that for the TE HPIC, used as the total dose meter, no significant variation in radiation quality could be detected when radiation quality information is derived from pressure dependence. The same conclusion was drawn on the basis of ion recombination derived from the reading versus collecting potential curve. This observation is in agreement with our previous finding that the quality factors obtained from TEPC measurements are not significantly different at the two positions. The relative readings of the Al chamber filled with Ar are about 10% lower compared to that of the TE chamber filled with Ar.

In the future we intend to apply the same technique and analysis at the maze entrance of the neutron generator hall where larger variations of radiation quality are expected. These measurements will be used for demonstrating the suitability of the HPIC technique to provide reliable radiation quality information using differences in the pressure dependence curve as well as differences in ion recombination.

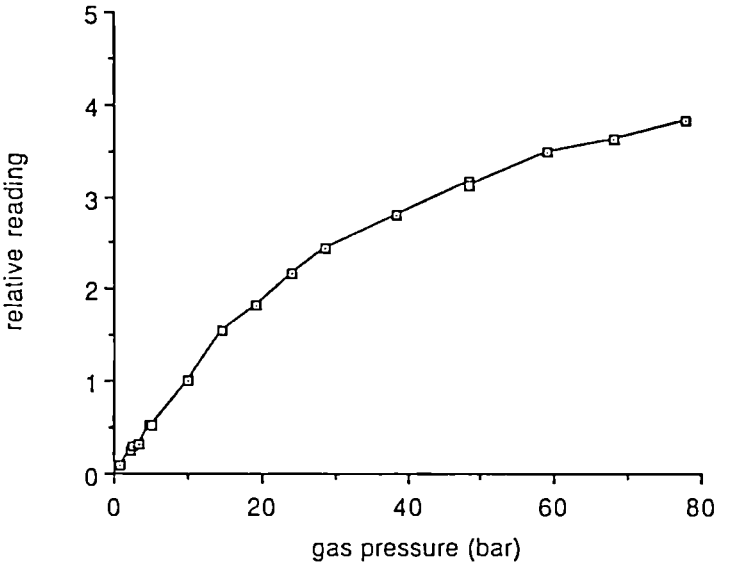


Figure 2 Relative reading at 600 volt versus gas pressure for a methane filled TE HPIC measured at position 2m 45°.

Scientific staff :

J.J. Broerse, F.S. Draaisma

Other research group(s) collaborating actively to this project :

Institute of Atomic Energy, Swierk (Dr. N. Golnik) (P)

National Institute of Standards and Technology, Gaithersburg (Dr. J.J. Coyne) (USA)

Publications :

1) H.M. Gerstenberg, J.W. Hansen, J.J. Coyne, J. Zoetelief. Calculations of the relative effectiveness of alanine detectors for neutrons with energies up to 17.1 MeV. Radiat.Prot.Dosim. **31**, 85-89, 1990

2) J.J. Coyne, R.S. Caswell, J. Zoetelief, B.R.L. Siebert. Calculations of microdosimetric spectra for low energy neutrons. Radiat.Prot.Dosim. **31**, 217-221,1990

3) D.R. Schlegel-Bickman, H.J. Brede, S. Guldbakke, V.E. Lewis, and J. Zoetelief. Measurements of k_u values of argon-filled magnesium ionization chambers. Phys.Med.Biol. **35**, 717-730, 1990

Head of Project 4: Dr. Schmitz

II Objectives for the reporting period

Investigation of the properties of semiconductor devices as microdosimetric detectors in mixed neutron gamma radiation fields;

Design of a prototyp semiconductor detector layout;

Adaption of the Monte Carlo Neutron/Photon transport Code (MCNP) for calculations in a humanoid phantom;

Preparation of a humanoid phantom for MCNP;

Preparation of a computer code for energy deposition calculations following the transport calculations.

III Objectives for next period

Experimental investigations of semiconductor detector properties in neutron fields using DRAM (dynamic random access memory) chips as microdosimetric detector;

Optimisation of the detector design with respect to the thickness of tissue equivalent layers for neutron radiations

Investigation on the relation of microdosimetric spectra measured with semiconductor detectors and tissue equivalent counters

Calculation of energy deposition spectra in organs for radiation fields of different composition and with different geometrical properties

Collection of relevant biological data to enable an investigation on the correlation between energy depostion spectra and biological effects.

IV Progress achieved including publications

The most innovative part of the coupled semiconductor ionisation chamber dosimeter is the semiconductor detector. Therefore, the developement of this part of the detector was started first. Since the semiconductor detector is supposed to give information on the radiation quality it will be operated as microdosimetric ionisation yield detector. The design of this detector part is based on a sandwich, where the semiconductor device is covered by a layer of tissue equivalent material (Fig. 1a). The minimum thickness of this layer will be such that secondary charged particle equilibrium would be established at the position of the semiconductor-tissue interface, if the semiconductor would be tissue equivalent. The effect of the interface on the performance of such detectors must be further studied and is partly subject of the next reporting period.

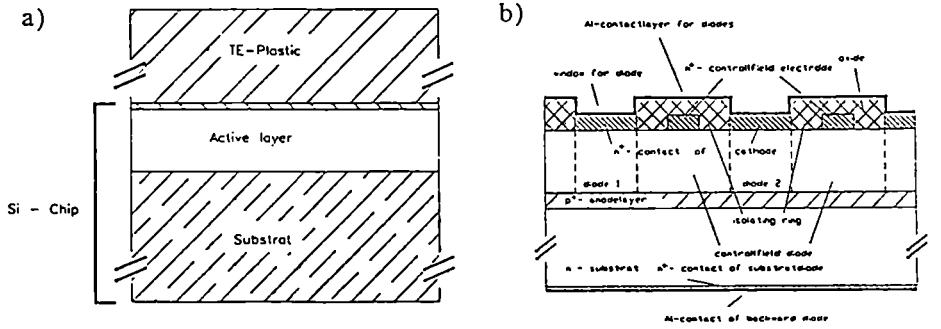


Figure 1 Silicon Detector design. a) Basic design showing the sandwich of a tissue equivalent plastic layer and a silicon detector chip; b) Scetch of the detector chip design.

Charged particles generated by the radiation in the tissue equivalent layer enter the semiconductor material - Silicon - and are slowed down. The sensitive elements (detector elements) are specially constructed diodes, whose depletion layer is almost cubical in shape with a length of side in the range of 2 μm to 4 μm (Fig. 1b). The size of the diode is one critical parameter and 2 μm is near to the technological border. Another critical parameter is the thickness of the so called dead layer above the diodes, which charged particles have to pass. This layer has to be as thin as possible. The layout shown shematically in Figure 1b is one proposed version of a microdosimetric semiconductor detector. Possibilities of producing such a detector chip are right now evaluated.

A further critical parameter is the signal to noise ratio of a detector like the one proposed. Due to the experience available with semiconductor detectors, it is expected that the minimum number of ionisations, which have to be produced by charged particles in a diode is about 1000 to 2000. The energy required to produce an ion pair is 3.6 eV in Silicon. Consequently, the minimum energy deposition which would be detectable is roughly 5 keV.

In the framework of applying the methodology of biological response functions to get information on radiation quality, the neutron photon transport code MCNP was adapted. Furtheron, a humanoid phantom was implemented. It is based on the male adult phantom (Adam) described by R.Kramer et al. (Kramer, R., Zankl, M., Williams, G., Drexler, G. (1982). The calculation of dose from external photon exposures using reference human phantoms and Monte-Carlo methods. GSF-Bericht S-885). Transport calculations were then performed for a variety of monoenergetic photon and neutron fields as well as a ^{252}Cf neutron source and for mixed neutron photon fields. Target organs included the brain, the kidneys and the thyroid.

Following the transport calculations microdosimetric calculations were performed. For this purpose, a program developed to simulate proportional counter measurements was modified. Fig. 2 shows microdosimetric spectra in three organs assuming isotropic irradiation by a ^{252}Cf source. The spectra are normalized to unit incident neutron fluence. The spectrum used as input for the microdosimetric calculations for a specific organ is averaged over this organ.

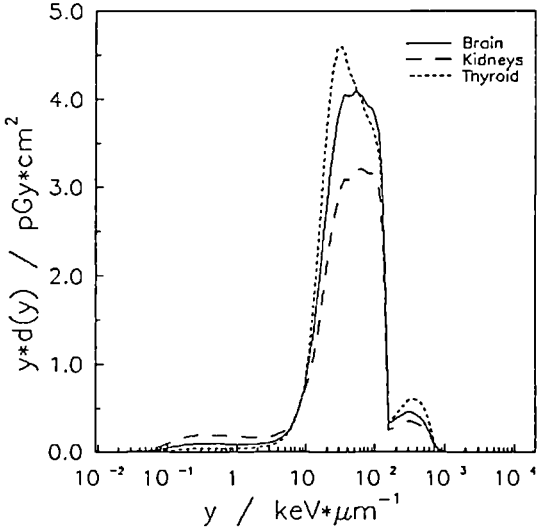


Figure 2 Dose distributions in different organs in a humanoid phantom. The diameter of the sensitive side is 1 μm. The irradiation was isotropic coming from a ^{252}Cf source.

Scientific staff :

L.E. Feinendegen, M. Kopec, O. Schröder

Other research group(s) collaborating actively to this project :

Institute of Nuclear Physics, Krakau (Dr. P. Olko) (P)

Institute of Physics and Nuclear Techniques of AGH, Krakau (Dr. K. Morstin) (P)

Publications:

1) Olko, P., Booz, J. Photon Induced Microdosimetric Distributions in Nanometer and Micrometer Regions. Radiat. Prot. Dosim 31, Nos. 1-4, pp. 205-209 1990.

2) Schmitz, Th., Morstin, K., Olko, P., Booz, J. The KFA Counter: A Dosimetry System for Use in Radiation Protection. Radiat. Prot. Dosim 31, Nos. 1-4, pp. 371-375 1990.

3) Schmitz, Th. Microdosimetry Health Physics Instrumentation. In: Advances in Radiation Protection, Oberhofer, M. (ed.), pp. 171-197 1991.

4) Schmitz, Th., Booz, J., Feinendegen, L.E. Das Problem der Risikoabschätzung im Bereich der Umweltbelastung mit ionisierenden Strahlen. AGF-Tagung 'Umwelt und Krebs', Gockel, E., Schulte-Hostede, S. (ed.), Arbeitsgemeinschaft der Großforschungseinrichtungen (AGF), Thenée Druck Bonn, pp. 52-55 1990.

Head of Project 5: Dr. Segur

II Objectives for the reporting period

Our main objective for the present period was the determination of electron-molecule collision cross-sections at low energy for propane and isobutane.

Furthermore preliminar calculations were made of the electric field and the corresponding gaseous gain characteristics in low pressure proportional counters with complex geometries.

III Objectives for next period

For the next period, we plan to extend our cross-sections determinations in propane and isobutane to higher energies (until relativistic energies), in order to be able to calculate swarm parameters in gas mixtures (TE propane and isobutane based mixtures) of relevance in microdosimetry. Calculations of electron W values for these gases will also be carried out.

We also plan to apply gas gain modelling studies in some practical situations (for proportional counters developed in Homburg, Jülich or Bromwich) in order to try to determine, from the calculations, the best working mode of these counters as a function of various parameters (geometry, gas, pressure, etc...) and their limit of operation.

IV Progress achieved including publications

The determination of electron-molecule collision cross-sections in organic vapours is of paramount importance not only for modelling the motion of electrons in proportional counters, but also for the determination of energy losses and consequently the W values for these various gases, in particular gases of relevance for dosimetry and microdosimetry techniques. Furthermore, the theoretical study of the slowing down of electrons is required from very high (a few MeV) to very low (a few eV) energies and cannot expected to be made without a good knowledge of the cross-sections for all interaction processes involved in electrical discharge mecanisms (ionisation, excitation, vibration, attachment), for the whole range of energies and for the gas of interest.

Unfortunately, the knowledge of the cross-sections for organic vapours is actually very poor. The only gas whose cross-sections are well known is methane (see our previous report, contract n° B16-A-292-F). But in the case of propane and isobutane, very few informations are available. Neither experimental nor theoretical determination of their cross-sections were made so far. Furthermore, with respect to theoretical determination, quantum mechanical approach would be yet unable to give valuable data since the interaction potential between electrons and these molecules is not well known due to the complex structures of propane or isobutane molecules.

The only approach to determine these cross-sections is to use informations coming from experimental determination of swarm parameters (i.e. drift velocities, diffusion coefficients, ionisation coefficients, etc..). All these coefficients depend on the ratio of the electric field divided by the pressure (E/P or E/N where N is the density of the background gas). The various swarm parameters are defined by the quadrature of the distribution function of electrons and the electron-molecule cross-sections. It follows that the knowledge of the variation versus E/N of these parameters allows, through an unfolding procedure, to determine the energy variation of the cross-sections.

The unfolding procedure usually does not provide an unique set of cross-sections,

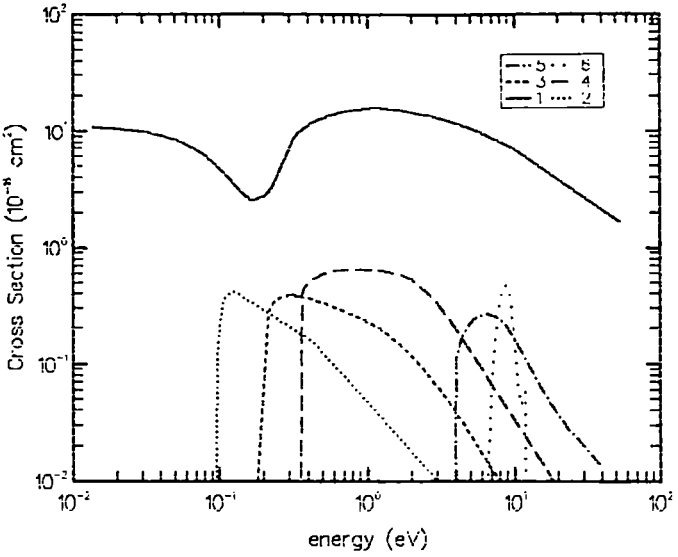


Figure 1 Ajusted cross section in propane : (1) momentum transfer; (2,3,4) vibrational excitation; (5) total ionisation; (6) (x1000) attachment.

but, if the number of data derived from experimental measurements of swarm parameters are large enough (coefficients determined in pure gas and in different mixtures for example), the results obtained are at least as acccurate as those from direct measurements of the cross-sections since the accuracy of measurements in swarm experiments is very high (less than 3% in most cases).

To illustrate the results obtained, Figure 1 shows the low energy set of cross-sections derived for pure propane. To our knowledge they are the first working set of cross-sections available in the literature. The 25 different vibrational processes occuring in propane were assembled in three different cross-sections. Furthermore superelastic collisions in vibration were taken into account and were seen to be important at thermal and subthermal electron energies. Figure 2 compares the experimental swarm parameters and those calculated using the cross sections obtained by deconvolution for pure propane and argon-propane mixtures. Values are shown for drift velocities and characteristic energies. Taking into account the scatter between experimental data, the results show the good agreement between experimental and calculated values proving the suitability of the unfolding procedure.

The progress achieved for the knowledge of electron collision cross sections in organic vapors open a large field of investigations for the application of low pressure chambers for radiation protection dosimetry, and for basic dosimetry and microdosimetry research. It is intended to apply the gas gain modelling calculations to the gas detectors developed in the frame of the present contract with the aim to improve the understanding of their basic properties and to improve their performance. As an example, experiments to measure gas gain in cylindrical proportional counters (Jülich) are being compared with absolute gas gain calculations as a function of different parameters. First calculations are in progress. Similar studies are initiated within the frame of different contracts in collaboration with several institutes including e.g. the modelling of TEPC with complex geometry for individual dosimetry (CEA, France) and the simulation of test experiments for investigation of the spatial variation of gas gain in cylindrical PCs as a function of gas pressure, i.e. under equilibrium and non-equilibrium situations (INFN, Italy).

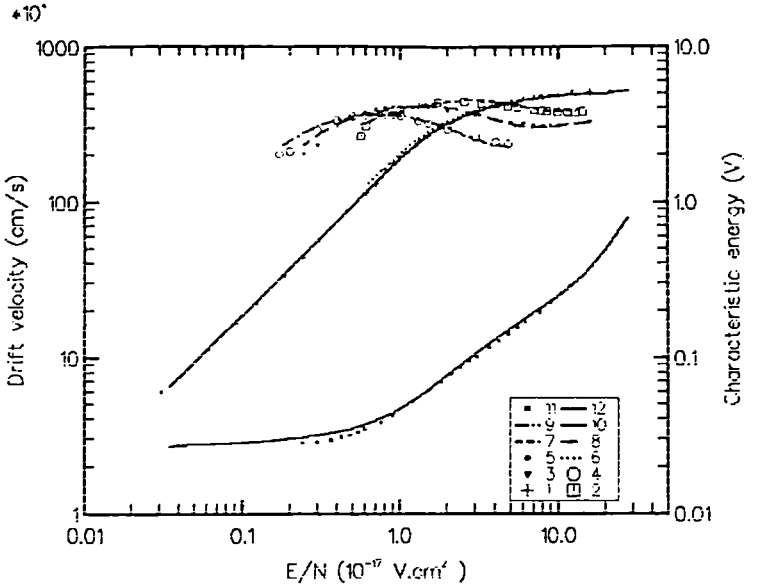


Figure 2 Experimental (symbols) and calculated (lines) values of the drift velocity and the characteristic energy in propane and argon-propane mixtures : (1,6) 90 % propane; (2,7) 20 % propane; (3,8) 10 % propane; (4,9) 5 % propane; (5,10) 100 % propane; (11,12) characteristic energy is only available in pure propane.

Scientific staff :

D. Blanc, M.C. Bordage, J.Y. Gosselin, T. Moutarde, I. Pérès, A. Schouki

Other research group(s) collaborating actively to this project :

Commissariat à l Energie Atomique, Fontenay-aux-Roses (Dr. J. Barthe) (F)

Istituto Nazionale di Fisica Nucleare, Laboratory di Legnaro (Dr. P. Colautti) (I)

Publications :

1) P. Ségur, I. Pérès, J. P. Boeuf and J. Barthe, Modelling of the electron and ion kinetics in cylindrical proportional counter. Proc 10th Symp. on Microdosimetry (Rome, 1989), J. Booz, J.A. Dennis and H.G. Menzel, eds., CEC, EUR 12864, Radiat. Prot. Dosim., 31, 107 (1990).

2) P. Ségur and M. C. Bordage, Recent Advances in the Solution of the Boltzmann Equation for the Motion of Electrons in a Weakly Ionized Gas. In Proc. XIX° Int. Conf. on Phenomena in Ionised Gases (Belgrade, 1989), V.J. Zigman, ed., 86-107 (1990)

3) M. C. Bordage, P. Ségur and I. Pérès, Calculation of Ionization coefficient at high E/N values in helium, argon and methane. In Proc. XIX° Int. Conf. on Phenomena in Ionised Gases (Belgrade, 1989), V.J. Zigman, ed., 566-567 (1990)

4) I. Pérès, M.C. Bordage, P. Ségur, Determination of methane cross-sections and calculation of hydrodynamic parameters in argon-methane mixtures. In Proc. ESCAMPIG 90 (Orléans, France), p 116 (1990).

5) I. Pérès, Modélisation da la cinétique électronique et ionique dans les compteurs proportionnels, thèse d'Université, Avril 1990, Toulouse.

Progress Report

Contract: Bi7-031 Sector: A12

Title: Determination and realisation of calibration fields for neutron protection dosimetry as derived from spectra encountered in routine surveillance.

1	Klein	PTB
2	Thomas	National Physical Laboratory
3	Chartier	CEA - FAR
4	Schraube	GSF Neuherberg

I. Summary of Project and Global Objectives

The objective of this collaborative project is to produce in the laboratory a few well characterised neutron fields that replicate typical spectral neutron fluence distributions encountered in radiation protection practice. These fields are needed for the calibration of neutron area and personal dosemeters which generally do not have the energy response required to determine dose-equivalent quantities. The project consists of four distinct parts, namely

- the measurement of the spectral neutron fluence typically encountered in practice,
- the preparation of a catalogue of all measured spectra in an agreed format including the calculation of relevant dose equivalent quantities and the response of commonly used neutron dosemeters
- the inspection of this catalogue in order to extract a few representative spectra and their expansion in terms of the calibration spectra already available or, if necessary, newly defined ones, and finally
- the computational prediction of configurations consisting of the usual neutron sources (accelerator, reactor or radionuclide based) and appropriate moderators in order to produce these reference fields in the laboratory.

Four European laboratories, well experienced in the field of neutron metrology and dosimetry, are cooperating in this project. Spectrometric measurements will be performed independently by each laboratory, chiefly in its own country. The setting up and analysis of the catalogue, however, require close cooperation.

Head of Project 1: Dr. Klein

II Objectives for the reporting period

i) Development of computer programs to make compendia of neutron spectra accessible and to evaluate them in terms of dosimetric quantities. Emphasis on program development
ii) Development and use of computer programs to improve the calculation of response functions for practical neutron spectrometers. Emphasis on proton recoil proportional counters and Bonner spheres.
iii) The main objective for the reporting period was to measure the spectral neutron fluence in work places, actually in the environment and at nuclear research laboratories, taking advantage of the spectrometers available at PTB. In addition, "candidates" for "realistic" calibration fields had to be investigated.

III Objectives for next period

i) Compiling and evaluating of newly measured neutron spectra. First results will be presented at the Symposium on Neutron Dosimetry in Berlin, October 1991.
ii) Application of recently developed methods and Monte Carlo programs with emphasis on ^{3}He proportional counters and Bonner spheres
iii) The measurement of spectral fluences in various neutron fields relevant to radiation dosimetry will be continued. The first intercomparison of different types of spectrometers and dosemeter systems will be performed at the new and unique Cadarache irradiation facility. Concerning the few-channel unfolding problem, the possibility of obtaining information on the uncertainties of the resulting spectral distribution will be considered and knowledge acquired on the problem will be applied in the routine unfolding codes in use.

IV Progress achieved including publications

i) A computer program for the handling of a data bank of neutron spectra encountered in radiation protection practice has been developed which allows spectra to be ordered according to various qualifiers such as author, location, experimental or calculational method, mean energy, mean dose equivalent. Some typical spectra have been introduced to this data bank and used to search for criteria in determining a small but sufficient and well accessible set of calibration spectra for neutron dosimetry.
ii) A new computer code has been written which allows an improved calculation of the neutron response function of spherical proton recoil proportional counters which are often used in radiation protection practice because of their isotropic response. The Monte Carlo code includes a treatment of the gas amplification based on the classic Townsend theory and a calculation of the electric field in the counter. Calculated and experimentally determined response functions agree quite well [1].
When this program is used to calculate the response matrix, a spherical proton recoil counter can be used to determine the spectral neutron fluence in absolute scale. Results will also be presented at the symposium in Berlin.
The neutron response functions of a large set of Bonner spheres have been calculated using MCNP and selfwritten transport codes. The comparison with an experimental calibration is in progress. These results will also be presented at the symposium in Berlin.

iii) Bonner spheres have the advantage of covering the whole neutron energy range of interest in radiation protection, from thermal to tens of MeV. Their disadvantage is that they give the spectrometric information in a few channels which are themselves correlated. Moreover, the data in the various channels (spheres) are not obtained simultaneously but consecutively. This shortcomming could cause serious problems when measuring in very low dose fields that require correspondingly long measuring times. In order to check such limitations two series of measurements were performed. In the first series, five Bonner spheres were used for measurements "free in air", at a place near the PTB research reactor (during a shut-down period). In the second series the measurements were performed in a work room at PTB, free of any radiation sources, using 11 Bonner spheres. In both cases the measuring times varied between 3 and 8 days for each sphere. The spectra obtained from these measurements are shown in Fig. 1. Even at dose-equivalent rates of only a few nSv/h, reasonable spectral distributions can be obtained.

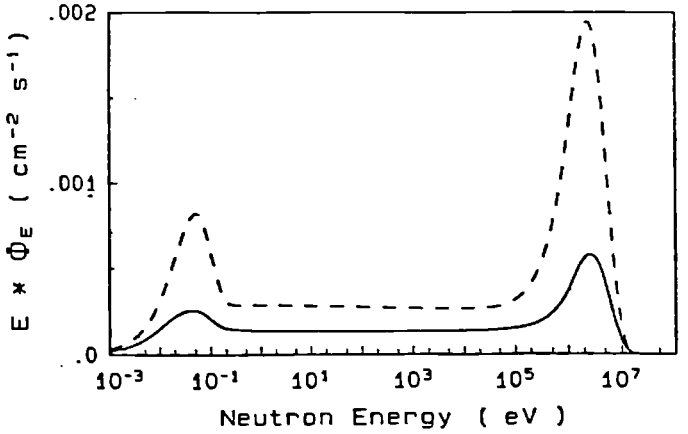


Fig. 1 Spectral fluences obtained from Bonner-sphere measurements "free in air" (dashed line) and in a work-room (full line) at PTB.

An interesting radiation protection situation arises in plasma experiments. The intense, but short "shots" make spectrometric measurements difficult. For this reason the neutron field of the TEXTOR plasma experiment in Juelich was partially simulated in the low-scatter area of the PTB accelerator facility, where 2.5 MeV monoenergetic neutrons were produced using the T(p,n) reaction. The geometry of this simulation is shown in Fig. 2, where the target (T) simulates the plasma, the iron plate the vessel and the piece of wood the roof of the TEXTOR experimental hall, which had to be installed to reduce the neutron skyshine during discharges. The fluence spectra obtained in three different geometries are given in Fig. 3. The target neutrons were measured in a free geometry, through 2.3 cm iron, and through 2.3 cm iron and 60 cm wood. The fluence of the 2.5 MeV neutrons as measured with a proton recoil telescope is confirmed by the free-geometry BS measurement with a difference of less than 4 %. A small background due to room- return neutrons (about 4.6 % in fluence) is identified. It is chiefly the inelastic scattering in iron that degrades the neutron energy, while the fluence is considerably reduced by the efficient moderation and absorption (about a factor of eight) of the wooden roof.

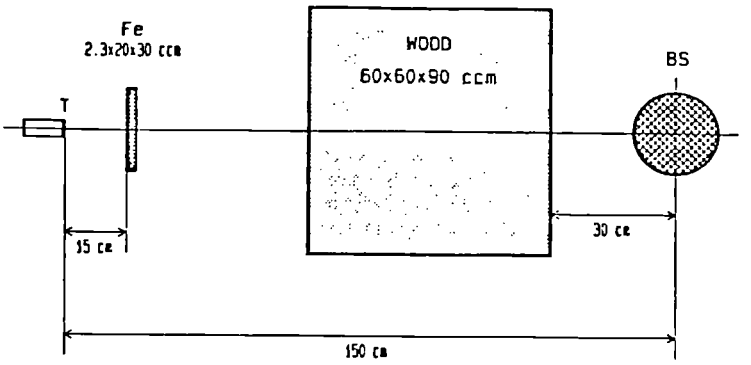


Fig. 2 The geometry of the TEXTOR experiment simulation.

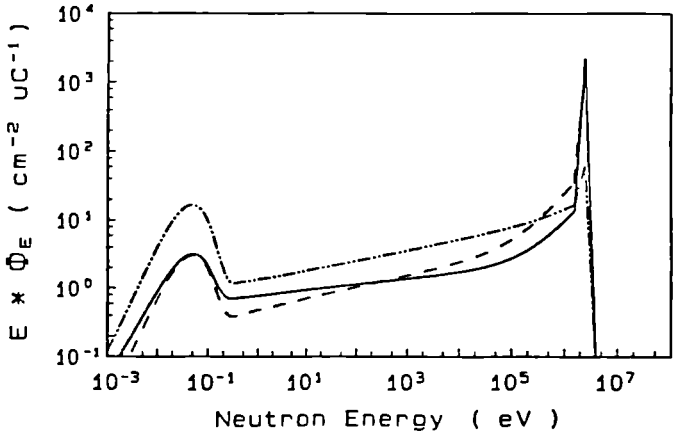


Fig. 3 The fluence spectra as derived from Bonner-sphere measurements in three geometries of the TEXTOR-experiment simulation: free geometry (full line), throgh iron (dashed line) and through both iron and wood (dot-dashed line).

The moderator technique is in principle inadequate for measuring neutron spectra containing strongly monoenergetic components. In order to treat such spectra and other "untypical" spectra properly, the unfolding code used was modified. Further improvements to the code are expected in the near future.
At PTB, bare or D_2O-moderated ^{252}Cf sources are employed for calibration purposes inside a "bunker" room (7 m x 7 m x 6.5 m). This room with its heavy concrete walls is much smaller than the low-scatter experimental facility used for the accelerator experiment, and therefore the room-return

neutrons make a considerable contribution to the total neutron fluence inside the "bunker". It is assumed that the spectral fluence of the room-return neutrons is well suited for use as a "realistic" calibration field for dosemeters. With Bonner spheres up to 30.48 cm (12") diameter, measurements were made in the "bunker" at a distance of 170 cm from the source centre. For each type of ^{252}Cf source (bare or moderated) and Bonner sphere the direct measurement was combined with one performed with a shadow shield placed between source and sphere. For both sources, the neutron fluence behind the shadow shield at a distance of 170 cm from the source was about half the total fluence. Again the Bonner spheres seemed to be successful in measuring integral fluences; the value obtained for the strength of the bare ^{252}Cf source differs by only 1.8 % from the value determined with the PTB water bath (< 1 % uncertainty). The final analysis of the measurement data sets and a comparison of the unfolded with the calculated spectral neutron fluences is in progress.

It is planned to make a brief presentation of all these results for the Seventh Symposium on Neutron Dosimetry, Berlin, 14 - 18 October 1991.

Publications

[1] K. Weise, M. Weyrauch, K. Knauf
Neutron Response of a Spherical Proton Recoil Proportional Counter
submitted for publication in NIM

Head of Project 2: Dr Thomas

II Objectives for the reporting period

The objective of the initial stage of this *realistic fields* project is to identify and characterise the types of neutron fields to which radiation workers are exposed. This involves establishing a data base of the fields encountered in nuclear sites, and this should be based on actual measurements of neutron spectra and dose equivalents. It is the intention to gather together existing information, from all sources worldwide, in a unified representation. NPL will concentrate mainly on compiling the data base, evaluating the spectra in this data base, and producing the catalogue, however, any opportunity to measure particularly relevant spectra will be taken.

III Objectives for next period

Completion of the analysis of data taken to date using the NPL Bonner sphere spectrometry system.
A questionaire has already been prepared and will be distributed, to groups who have been active in neutron spectrometry, asking for data on measured neutron spectra. This data, as it becomes available, will be consolidated into a catalogue where all the results are represented in a standard form allowing spectral shapes, dose equivalent quantities, etc., to be compared in a simple manner. The extent to which the total dose equivalent values for the various spectra are dependant on the detailed spectral shapes will be investigated.

IV Progress achieved including publications

NPL had intended to concentrate during the initial stages of the project on compiling the data base of measured neutron spectra. Some progress has been made in this direction. A literature survey of existing data has revealed a fair number of measurements, but there is little information about the quality of the resulting spectral distributions.

A questionaire, for circulation to all known groups involved in neutron spectrum measurements, has been prepared and distributed to other members of the collaboration for comments. A computer program written at PTB to simplify and standardise collection of spectral data from the measurers is being investigated.

It had not originally been our intention at NPL to pursue the measurement of new neutron spectra in working areas during this period, however, the opportunity arose of providing data for the neutron spectra in a particularly appropriate environment. Many of the people who are exposed to neutrons are in fact working with radionuclide neutron sources. These are manufactured in the UK and elsewhere, and it was thought to be consistent with the aims of this project to provide data on the neutron spectra encountered in radionuclide source production plants.

Primary neutron spectra were measured using a Bonner sphere (BS) spectrometry system. In addition, a microdosimetric system, developed by Leeds University, and based on the use of a tissue equivalent proportional counter (TEPC), was used to investigate the charged particle spectra. Finally measurements were also made with two commonly available area survey instruments.

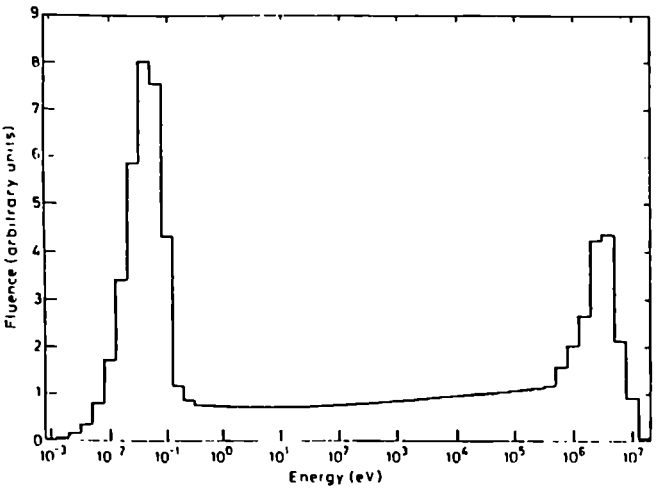
The BS system is a low resolution spectrometer, but has the advantage of providing information about the neutron spectrum over the full energy range from the thermal region to several MeV. Much work has been undertaken at NPL to validate this system, both in terms of deriving the response functions of the spheres[1] and in investigating the optimum

spectrum unfolding technique[2]. The sphere set used consisted of 9 polyethelene spheres ranging in size from 7.62 cm (3") to 28.1 cm (15"), and having as the central thermal neutron detector a spherical ^{3}He proportional counter. In addition to measurements with the spheres, the ^{3}He detector was used, both bare and under a 1 mm thick cadmium cover, to derive the thermal neutron intensity. The data was analysed using the spectrum unfolding code STAY'SL[3]

The microdosimetric system was based on a 12.7 cm (5") Far West Technology counter, and the two area survey instruments were: a Harwell model 0949 dose equivalent monitor, and the Nardeux device the DINEUTRON.

Measurements were performed at four sites within the plant. Two were simply measurements at a fixed distance from materials, americium oxide and a ceramic containing americium, which although not radionuclide neutron sources in the conventional sense, do emit significant numbers of neutrons when the americium content is high enough. The other two measurements were at the operator position for two glove boxes, one used for handling ^{244}Cm, and the other for preparing Am-Be radionuclide sources. This latter position was surrounded by other glove boxes where Am-Be sources are prepared and stored temporarily. One of the main interests of the exercise was to compare dose equivalent values measured by the spectrometry system with those derived from the TEPC, and those from the area survey instruments.

It is hoped to present all the data at the forthcoming Symposium on Neutron Dosimetry to be held in Berlin in October 91, however, the data for the Am-Be production area is presented here since it is probably the most directly relevant for the realistic field project. The neutron spectrum measured with the BS system is shown in the figure. From this it can be seen that there is a significant thermal neutron fluence in this field. This has consequences when comparing quantities derived from the BS system with those from the TEPC measurements.



The measured neutron spectrum of the operator position for one glove box in a line of similar glove boxes all used to prepare and store Am-Be radionuclide sources

Most of the dose equivalent is due to the neutrons in the high energy part of the spectrum (93% of the total is from neutrons with energies above 100 keV), and the result for the total dose equivalent derived from the BS system and the TEPC are in good agreement. However, the average quality factor for the spectrum is not dominated in the same way by the higher energy neutrons, and the value derived from the total neutron spectrum, as measured by the BS system, is significantly lower than that derived from the TEPC. This is because the response of the TEPC to lower energy neutrons is reduced compared to that for higher energies.

Like all presently available area survey instruments, the Harwell 0949 dose equivalent monitor over-reads in certain energy regions while under-reading in others. For the four sites measured, however, the readings of this instrument were high, but always within 10% of the value derived by spectrometry. The corresponding results for the DINEUTRON were varied, sometimes high and sometimes low, and the differences with the results from spectrometry were always greater.

References

1. CEC EURATOM Radiation Protection Programme Progress Report 1985-89, Vol. 1, p275.
2. A. Alevra et al. Unfolding Bonner-sphere Data: A European Intercomparison of Computer Codes, PTB Report PTB-7.22-90-1, Jan 1990.
3. F.G. Perey; Least-Squares Dosimetry Unfolding: The Program STAY'SL, Oak Ridge National Laboratory Report ORNL/TM-6062, ENDF-254.

Head of Project 3 : Dr. Chartier

II - Objectives for the reporting period

For the reporting period, the following objectives have been linked to the actions undertaken by the CEA-FAR:

a) the participation to measurement campaigns in french nuclear installations in order to obtain recent experimental data intended to be included in a up-to-date european neutron spectra catalog.

b) the improvement of neutron spectrometry techniques used for measurement in the field, and particularly concerning the unfolding codes.

c) the association of measurements, performed in the same radiation field, with Bonner sphere and proton, recoil techniques.

d) the computational prediction of neutron spectra, which could be produced by an experimental facility installed in the laboratory of SIDR/Cadarache.

e) the realization of the previously mentioned set-up and the organization of an inter-comparison exercice involving the different spectrometric equipments and instruments used by the contractors.

III - Objectives for the next period

The same actions as those described in paragraph II, being a part of a general program will be extended along the next period. According to the results and decisions taken by contractors at the next coordinating meeting, minor changes can be brought to a specific item. In particular, a second irradiation campaign has been suggested for testing other dosimetric instruments. The CEA program will be concentrated on the carrying out of spectrometric characterization at several workplaces, the investigation of accuracy in the simulation of realistic neutron spectra, and a closer association of multisphere and proton recoil techniques.

IV - Progress achieved including publications

IV.1 - New data for the neutron spectra catalog

Official contacts have been initiated with the radiation protection departments of several french installations involved in neutron hazards. Those laboratories belong to CEA-FAR, CEA-D.A.M. (in CEN Valduc). COGEMA (La Hague/Marcoule) and, in each center, a meeting has been held in order to obtain the necessary authorizations and the collaboration of staffs.

The Bonner sphere technique and the proton-recoil spectrometry have been used, in November 1990, to characterize the neutron spectrum at a workplace in CEA-Fontenay-aux-Roses. The provisional results (see Fig. 1) have been obtained in front of a glove box in which (Am-Be) neutron sources are manufactured. (For complementary information on the data processing, see paragraph IV.2.1.).

Furthermore, three other places of interest have been investigated to foresee and to organize the spectrometric measurements in the next 9 months. The following cases have been considered:

a) near a transport container at the reprocessing plant of La Hague (COGEMA),
b) in a laboratory in charge of Pu chemistry at Valduc (CEA-D.A.M.),
c) at Marcoule (COGEMA) where fuel elements (and especially MOX fuel) will be processed.

IV.2 - Improvements of the neutron spectrometry techniques

IV.2.1 - *Unfolding code for NE213 detector*

For the NE213 scintillator detector, the unfolding process (differentiation method) is based on simplified assumptions (step-response function) combined with analytical expressions, taking into account, with more or less accuracy, the parasitic effects in the detector and its surroudings. Based on mathematical elements formulations proposed in (1), (2), a new unfolding code has been written, in order to be used with the NE213 spectrometer. Several hypothesis have been combined :

a) the determination of the efficiency including the irradiation geometry (angular distribution), and a proton bias (detection threshold) (3),

b) the use of a trapezoid shape for the response function to correct the measured proton distribution,

c) the mathematical approach described in (4) to replace the differentiation technique by a convolution product, smoothing the result in terms of the energetic resolution of the detector.

The software has been checked with two "wide" neutron reference spectra : those of (Am-Be) and ^{252}Cf sources (5). From the measurements achieved with calibrated sources (in terms of emission rate), it has been possible:

a) to reproduce quite accurately the shape of the reference spectra for $E_n \geq 2$ MeV,

b) to determine the value of the proton bias (working as a "free" parameter), to fit the measured fluence to the reference fluence provided by the calibration certificate of the sources.

IV.2.2 - *Monoenergetic fluence references*

In neutron spectrometry, the availability of monoenergetic fluence references, for which the traceability has been proved, is essential. In France, the current state is not satisfactory and several consecutive campaigns of experiments may lead to inconsistent conclusions. Nevertheless, with those fluence references and after several checks (monitor, target preparation, target backing material, etc...), the main characteristics of the SP2 proportional counters (H_2 and CH_4 fillings): calibration curves, efficiency, energetic resolution..., have been determined again in February 1991.

IV.3 - Association of B.S. and P.R. techniques

Up to now, the complementarity of B.S. and P.R. techniques, has consisted in two independent evaluations of data, showing a systematic discrepancy between both results in the common energy range. The overevaluation by P.R. detectors has been assigned to the oversimple unfolding code of the NE213 detector. The satisfactory results, just obtained with the (Am-Be) and ^{252}Cf spectra (see IV.2.1.) give confidence in a better agreement between both techniques, and even in previous results which have to be reevaluated (see IV.1.).

IV.4 - Computational predictions of neutron spectra

The CEA Report 5398 (6) has released in the same document a series of realistic neutron spectra measured at several places of nuclear power plants and laboratories. Those spectra can be shortly described as follows:

a) a "High Energy" component: $E_n \geq 10$ keV
b) an "Intermediate" component: 0.1 eV $\leq E_n \leq$ 10 keV
c) a "Thermal" component: $E_n \leq 0.1$ eV

From the Report 5398, its appears that the relative contribution of a) and b) to the total dose equivalent may strongly vary. Preliminary calculations have shown that an assembly, composed of the following elements: [14.6 MeV neutrons] + [^{238}U converter] + [Fe shield] + [$(CH_2)_n$ channel] + [Scatterer D_2O (optional)] could replicate the most common type of neutron spectra.

The MCNP-3A code has been run for several configurations of the assembly, to calculate the neutron spectrum available in the "calibration zone": a spherical volume (diameter 30 cm) at a 30 cm distance from the $(CH_2)_n$ channel exit. The results have been confirmed by a similar calculation with the TRIPOLI code (CEA-Saclay-SERMA).

Several parameters interfere on the resulting spectrum: length of the channel, option: with or without scatterer, dimensions and material of the scatterer, position of the calibration zone, etc..., and those modifications have to be evaluated in connection with the requirements of the practical aspects of instruments calibration. A general presentation has been made at the coordinating meeting in PTB (Sept. 1990).

The computational evaluation is still in progress in order to evaluate as extensively as possible, the range of neutron spectra which could be available with such a set-up. In particular, the origin of the residual parasitic contribution of 14.6 MeV neutrons, and the means to reduce the corresponding fluence are being studied specifically.

As a conclusion, the flexibility of the set-up allows to replicate several realistic neutron spectra, the data of which having already been published. A synthesis of the subject has been proposed for presentation at the Berlin Symposium (Oct. 1991).

IV.5 - Preparation and realization of a spectrometry exercise

Another objective of the CEA-FAR program, was the preparation of the experimental set-up, used as a radiation source, for an intercomparison of the spectrometry techniques involved in the measurement of "wide" neutron spectra.

The arrangement, for which Monte-Carlo calculation have been performed, has been partly realized and installed in the experimental hall of the CEA/SIDR in Cadarache. Some modifications have been achieved on the 150 kV SAMES accelerator beam line in order to improve the monitoring system (associated-particle detectors), essential in the traceability of the multiple runs. It will be the basis for linking the partial results.

The detailed organization and the experimental conditions have been finalized at the PTB-CEA meeting in Cadarache (4-5 April 1991), on the basis of calculated data. The neutron spectrum involved in the intercomparison is given in Fig. 2, with the associated photon spectrum (Fig. 3). Preliminary measurements achieved in April 1991 on that configuration are presented in Fig. 4.

Publications

1 - J.L. Chartier, F. Posny, M. Buxerolle
Experimental assembly for the simulation of realistic neutron spectra
(Submitted for presentation at the 7th Neutron Symposium - Berlin Oct. 1991)

2 - F. Posny, J.L. Chartier, M. Buxerolle
Neutron spectrometry system for radiation protection measurements
(Submitted for presentation at the 7th Neutron Symposium - Berlin Oct. 1991)

IV - Bibliography

1 - W.H. Miller - Nucl. Instr. and Methods 153 (1978) 535-541

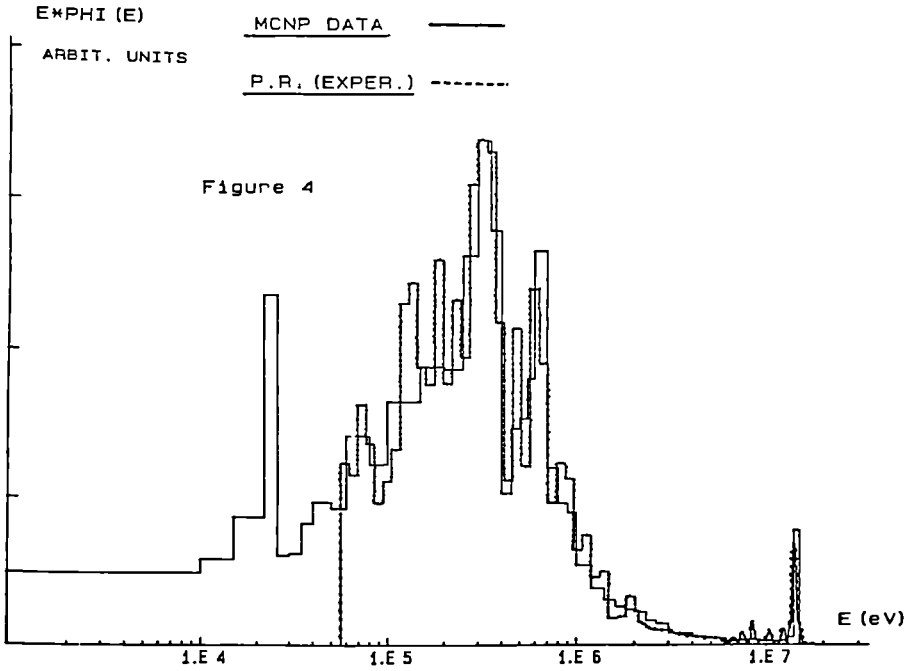
2 - W.H. Miller, K.W. Peterman - Nucl. Instr. and Methods 163 (1979) 253-255

3 - M.J. Coolbaugh - R.E. Faw - W. Meyer - USAEC - Document No COO-2049-7 (1971)

4 - R.H. Johnson - ORNL - RSIC - 40 (1976)

5 - International ISO Standard 8529

6 - M. Buxerolle et al. - CEA Report 5398 (1987)



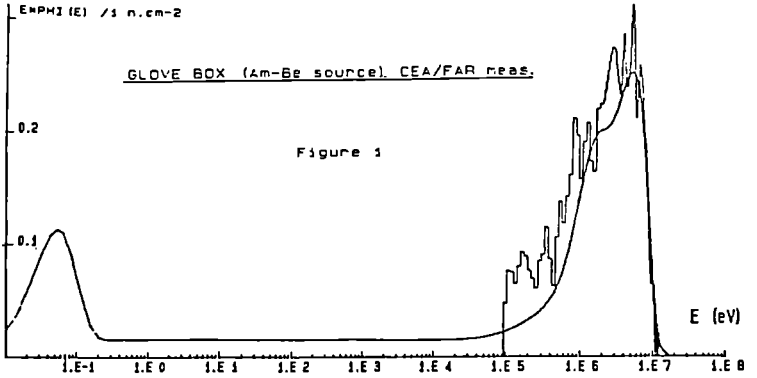


Figure 1

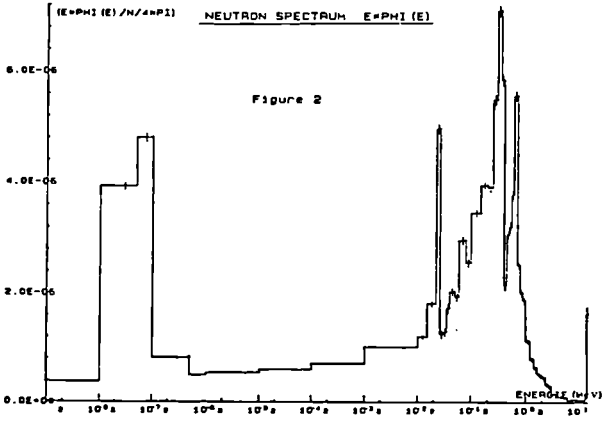


Figure 2

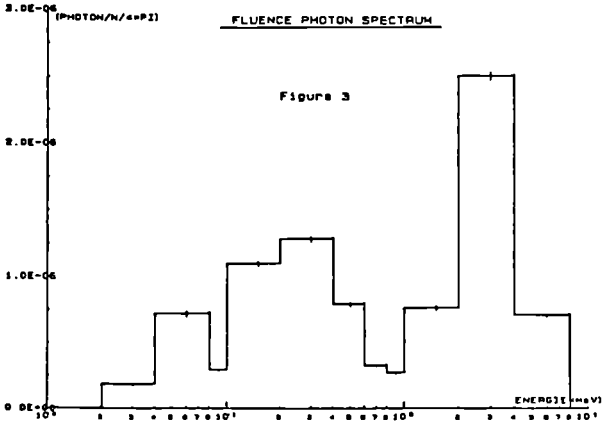


Figure 3

Head of Project 4: Dr. Schraube

II Objectives for the reporting period

1. Calculation of the response matrix of a Bonner sphere spectrometer set with spherical 3He-counter as the basis for unfolding of Bonner sphere data which had been experimentally determined at working places. This part will be described in some detail below. 2. Collating and sifting of published data for their aptitude of being included into the envisaged spectra catalogue. An extensive collection of nearly 90 neutron spectra in Russian language was translated and all Bonner sphere data extracted and transfered into the format of the new catalogue.

III Objectives for next period

1. Adaptation of the calculated response matrix to a Bonner sphere system with 6LiI detector.
2. Unfolding of own experimental data as measured at working places.
3. Completion of the spectra catalogue with these data and data from literature examinations.

IV Progress achieved including publications

During the calculation of the Bonner sphere matrix three items were studied: i) the pressure dependence of response, when the gas pressure of the 3He counter is varied, ii) the influence of the polyethylene density of the spheres to the response, and iii) the possibility of representing of the whole matrix in the sphere diameter domain by a log-normal distribution (Zaborowski-technique).

The Monte Carlo calculations of BSS response were performed for a commercially available 3He spherical proportional counter with a diameter of 3.2 cm placed in the center of the polyethylene moderating spheres with different diameters in the range of 2 inch to 15 inch.

The gas filling was assumed to be 172 kPa, the figure which has been used during a comparative benchmark study, initiated by the European Dosimetry group EURADOS-CENDOS, and which is close to experimentally determined values.

The density of the spherical polyethylene moderators was assumed to be=0.95 g.cm-3. The response of the BSS to neutrons was calculated for broad parallel beam geometry for 27 values of neutron energy in the range from 1.0.10-8 to 30 MeV.

The Monte Carlo code used in the present calculations was the MCNP code version 3B3 distributed through RSIC by the NEA Data Bank. It represents the most extensive Monte Carlo program that is available in the public

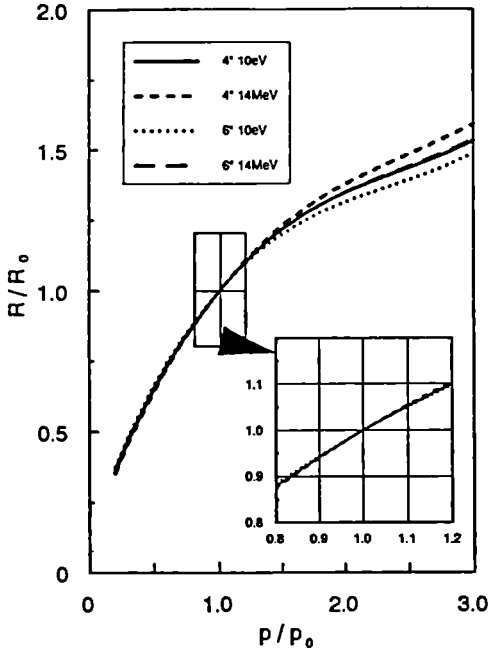


Fig.1 Dependence of response ratio R/Ro on the gas pressure p normalised to the standard pressure conditions (po=172 kPa). The data points, which fit the curves within +-2% are omitted for clearness.

domain and is continually updated to take advantage of advances in computer hardware and software.
The MCNP code which is written in Fortran 77 was implemented to run under UNIX operation system on the GSF CONVEX C220S computer, which is a very fast parallel super computer with vector architecture.

The modelling of the broad parallel beam geometry was performed in the following way: The positions of the starting particles were sampled uniformly on the surface of a disk source which is centered on and perpendicular to an axis of the sphere. All neutron tracks were parallel to the source-detector axis, and fully included the whole surface of the moderating sphere. The space between source and Bonner sphere was assumed to be vacuum.

To derive the pressure dependence of response, the calculations were performed for different counter gas pressures for several Bonner spheres and neutron energies. Figure 1 shows as an example the dependence of response on the pressure for the 4" and 6" spheres and for neutron energies 10 eV and 14.8 MeV, normalised to the standard pressure (po=172 kPa) condition. Cubic polynomials are fitted through the data points with a maximum uncertainty of +-2%. It can be seen that for instance an increase

of the pressure by 20 %, results in a 10 % increase compared with the response at the standard pressure. At higher pressures the increase of the response starts to depend somewhat on sphere diameter and neutron energy. A reduction of the pressure by the same amount, reduces the response by 12% for the four conditions under consideration.

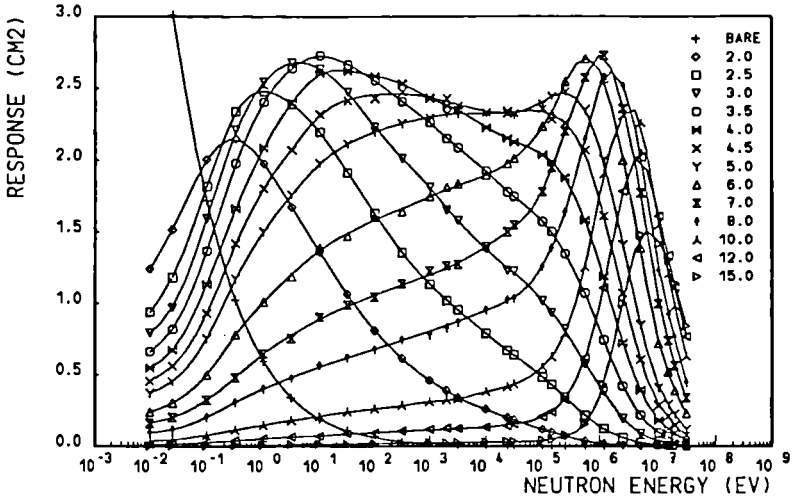


Fig.2 Energy response of BSS for MCNP calculated spheres (data points). The lines connect the responses at the 49 energies of the final matrix.

The response data obtained from the MCNP calculation were then interpolated to generate the response matrix with 49 energy points in log-equidistant intervals (i.e. 5 per decade) from 0.01 eV to 30 MeV for spheres from 2 to 15 inch in diameter steps of 0.5 inch. The responses for the additional Bonner spheres were interpolated in the sphere diameter domain for each respective neutron energy using the cubic spline interpolation method which smoothes out the estimated statistical uncertainties at the same time. The same method was then used for the interpolation in the (logarithmic) energy domain for all response data obtained both from MCNP calculation and from the sphere diameter interpolation.

A first comparison with recent experimental data which were determined during an EG sponsored experimental study, exhibits excellent agreement even in absolute scale.

Publication:
V.Mares, G.Schraube and H.Schraube: Calculated Response of a Bonner Sphere Spectrometer with 3He Counter. Accepted for publication by Nucl.Instr.Meth. (1991)

Progress Report

Contract: Bi7-021 Sector: A13
Title: Calculation and measurement of doses from particulate radioactive source.

1 Charles Nuclear Electric
2 Herbaut CEA - Grenoble
3 Patau Univ. Toulouse III

I. Summary of Project and Global Objectives

The evaluation of the hazard posed to the skin by very small radioactive sources (diameter < 1 mm) has become popularly known as the 'hot particle' problem. Hot particles appear to be a considerable practical problem on US PWR reactors though European experience to date is much less severe. Hot particle exposures continue to pose a radiological hazard in clean up operations at Chernobyl and the IAEA have recently set up a task group to consider the problem. Recently two ICRP and NCRP task groups have reviewed the basis of skin dose limitation and provided guide-lines relating to hot particle exposures. Both groups agree that the end point of practical concern following hot particle exposures is skin ulceration and that the risk of cancer mortality is negligible. The ICRP have used primarily UK pig skin data to suggest a threshold dose of 1 Sv over an area of 1 cm^2 at a depth of about 100-150 μm which would prevent the occurrence of even superficial transient ulceration. The NCRP have used primarily US pig skin data and have produced a criterion which is aimed to prevent the occurrence of a more severe response of acute deep ulceration. The NCRP suggested exposure limit, which is given in terms of a total beta particle exposure of 10^{10}, is less restrictive than that suggested by the ICRP task group by a factor of about 4. The disparity reflects the different levels of acceptable damage and possibly some differences in dose estimates used in the animal studies on which the threshold criteria are based. Both criteria are significantly less restrictive than the hot particle exposure limits previously employed. It is clear from a recent CEC/DOE/EURADOS skin dosimetry meeting (Dublin, May 1991) that there remains considerable uncertainty in the measurement and calculation of doses from radioactive particle sources. This effects both the interpretation of biological data on which dose limits are based and the subsequent extent to which the dose limit can be complied with by routine monitoring. There is therefore an urgent need to validate current methods of measuring and calculating doses from hot particles. The aim of this project is to produce standard, idealised hot particle (initially Co-60) sources for dose measurements by several techniques. These dose measurements will then be compared with Monte Carlo code and widely used semi-empirical tabular methods of dose evaluation. It should then be possible to recommend suitable methods for dose evaluation in order to interpret biological and epidemiological data, and to guide routine monitoring of hot particle exposures. This work is carried out under the auspices of EURADOS working group 2.

Head of Project 1: Dr. Charles

II Objectives for the reporting period

1990-91: Production of spherical low activity (1 MBq) Co-60 sources (100 μm diameter). Extrapolation chamber and radiochromoc dye film measurements of dose rate. Initial development of a Monte-Carlo code and comparison with initial dosimetry data. Since the contract commenced in late 1990 this reporting period covers only part of the first years objectives as stated above.

III Objectives for next period

1991-92: Production of larger and higher activity sources (to be used in pig-skin studies). Possible use of TLD and film in addition to extrapolation chamber and radiochromic film for dose evaluation. Refinement of Monte-Carlo code and comparison with semi-empirical methods of dose calculation such as VARSKIN MOD2. Extension of studies to include other beta emitters such as Tm-170 (E_{MAX} 1 MeV).

IV Progress achieved including publications

Due to the closure of the DIDO and PLUTO neutron irradiation facilities in the UK it has been necessary to review the production of neutron activated particles for this study. Following consideration of alternative facilities and after some initial delays it was eventually decided to use the Petten reactor facility and the first activated Co-60 samples were received in April 1991. These samples are currently being evaluated at Berkeley using extrapolation chamber and radiochromic dye (in collaboration with the National Institute of Standards and Technology, Washington DC). They will be passed to CEA Grenoble for extraplation chamber and TLD evaluation.

During the initial part of this contract we have made a critical evaluation of the use of the extrapolation chamber for the measurement of doses from point sources and made comparisons with radiochromic dye films. We have used a commercial 1 mm diameter Sr/Y-90 source which was utilised in the past for pig and mouse skin studies of hot particle effects. These evaluations have been carried out in collaboration with the Brookhaven National Laboratory and the National Institite of Standards and Technology in the USA. This work has provided a better understanding of the problems involved in hot particle dosimetry and enables us to proceed with an intercomparison study using a variety of different measurement techniques.

EXTRAPOLATION CHAMBER MEASUREMENTS

The tissue equivalent parallel plate extrapolation chamber potentially provides an absolute method for determining dose-rates from beta emitting sources. The extrapolation chamber was originally intended for application in situations where the chamber volume could, at least at the smallest

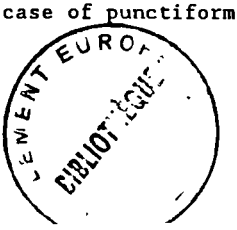
plate separtions, be reasonably assumed to be uniformly irradiated (eg at large distances from extended sources). Such instruments have more recently been adopted for measuring surface doses from planar sources of limited extent and for investigating dose distributions in the immediate vicinity of punctiform sources. Successful application in such circumstances has required an improved understanding of the effect of dose non-uniformity on the form of the relationship between chamber ionisation current and electrode separation. Extensive automation of chamber operation has also minimised operator doses and as a result has permitted the extensive programme of measurements required for the determination of spatial dose distributions. This interim contract report provides examples of the use of the automated extrapolation chamber for the determination of dose-rates from both planar and punctiform $^{90}Sr/^{90}Y$ sources. Examination of the shape of the observed ionisation current/electrode spacing characteristics, together with the predictions of a simple theoretical model for an idealised point source, indicate that for small source/small electrode combinations, significant curvature may still be present at the smallest practically attainable electrode separations. Dose estimates from both linear and non-linear extrapolation models have therefore been obtained and compared with those provided by radiochromic dye film dosemeters (GAFCHROMIC) calibrated using 400 kVp X-ray exposures and read out using manual and Scanning Laser Densitometric, Microphotometric and Automatic Image Analysis techniques. For the large area planar source the extrapolation chamber estimates obtained by conventional linear extrapolation are in reasonable agreement with the radiochromic film values. Non-linear extrapolation provides significantly improved agreement in the case of the punctiform source.

Dose Rate Estimation

Dose rates D/Gy s^{-1} are calculated using the conventional extrapolation chamber relationship

$$D = \frac{(W/e)\ (S_m/S_a)\ (dI/dx)_{I=0}}{\pi\ r^2\ P_a}$$

where; (W/e) is the mean energy per ion pair (J/C), (Sm/Sa) the ratio of stopping powers of the chamber mediun and air for the beta spectrum of the source being measured, $(dI/dx)_{I=0}$ is the terminal slope of the ionisation current (I) versus chamber electrode separation (x) curve, r the collecting electrode radius and P_a the density of air. In situations where the chamber is uniformly irradiated I will simply be a linear function of x and $(dI/dx)_{I=0}$ can be simply obtained from the slope of a linear regression line fitted to the observed data. Even in cases where the irradiation has been highly non-uniform eg at the surface of thin extended sources or near to small sources it has in the past been conventional to assume that at small enough electrode separations some linear region will exist which can be treated in a similar manner to obtain the required estimate of $(dI/dx)_{I=0}$. Discrepancies between the dose estimates obtained from extrapolation chamber measurements on this basis and those provided by alternative techniques have however more recently brought the procedure into question and have prompted a theoretical and practical reassessment of the method of estimating $(dI/dx)_{I=0}$ especially in the case of punctiform sources



HEORETICAL PREDICTION OF EXTRAPOLATION CHAMBER CHARACTERISTICS FOR UNCTIFORM SOURCES

del

imple model of the extrapolation chamber response has been developed by uming that the current is due to ionisation along the path of the beta icles traversing straight line tracks from the source and through the ber air volume between the chamber window and collecting electrode. Th ionisation current then depends on the number of beta particles and th path length through the detecting volume. This varies purely due to ch es in solid angle between the small source and the chamber. The ch es in geometry as the chamber spacing is varied depends on collector rad (r) source to window separation (a) and the chamber separation (x).

A si lified solution for the current I for small x (r/x>>1) can be derived which has the form:-

$$I \propto x - (a+x)*\mathrm{Log}_e((a+x)/r) - a*\mathrm{Log}_e(r/a)$$

and th gradient, dI/dx is given by:-

$$/dx = \mathrm{Log}_e(1 + (r^2/(a+x)^2)$$

The pre cted variation in chamber current (I) vs electrode spacing (x) is non line r, especially for small collecting electrode size (r). The gradient(d /dx) increases as the chamber spacing is reduced even below 0.1 mm, the effect being particularly noticeable for the smaller collector electrode sizes. Measurements of gradient made using a linear fit for spacings in he range of say 0.1-0.5 mm would appear to underestimate dose by a factor f up to 3 for small electrodes (r = 0.55 mm) depending on the source to window separation. For the larger electrodes (r = 5mm) the underestimate is smaller, of the order of a few tens of percent.
The use of a uadratic fit;

$$I = \alpha + \beta x + \delta x^2$$

which as x->0 ends to linearity will reduce these underestimates considerably bt a slightly better fit is given by a higher order polynomial or a function of the form

$$I = \alpha + \beta x + \delta/x$$

AUTOMATED CHAMBER MEASUREMENTS ON PLANE AND PUNCTIFORM SOURCES

Testing of the automated chambers scanning facilities and practical evaluation of the effect of the assumed fitting function has been undertaken in a series of measurements on plane and punctiform $^{90}Sr/^{90}Y$ sources.

Plane Source

Typical scans made at 1mm spacings with a 1mm diameter collecting electrode across a 2 cm x 4 cm plaque source are shown in Figure 1. Effects due to source non-uniformity are clearly discernable and the apparent hot spot has been confirmed autoradiographically. Measurements over the central region were also made using the 1 cm collecting electrode. Dose estimates obtained using the two electrode sizes (spatially averaged in the cases of the 1mm electrode) and the three extrapolation methods are collated in Table 1.

Punctiform Source

Measurements aimed at simulating the evaluation of the contact dose rate from a "hot particle" have been performed using all 4 electrodes on a 1 mm diameter 300 MBq bead source. For each electrode the source position was critically adjusted both laterally (Y,Z), to obtain a maximal value of the slope of the linear current versus electrode separation and vertically until change in the intercept on the separation axis (x) indicated that the source was contacting and distending the window. Once the optimum source position had been obtained repeat measurements were made and the averaged current versus separation curves were fitted to the three extrapolation models. Reproducibility between measurements was good (coefficients of variation on measured currents at a given separation were typically $< 0.5\%$) and the standard errors quoted for the dose rates (Table 2) derived using the linear and quadratic models are those due to curve fitting alone (Errors for the $\alpha + \beta x + \delta/x$ model can not be directly obtained but are estimated to be typically $< 3\%$).

MEASUREMENTS USING RADIOCHROMIC DYE FILM DOSEMETERS

Attempts to measure surface dose rates from the above test sources have also been made using radiochromic dye film dosemeters as part of a recently instituted collaborative project with the National Institute of Standards and Technology. Dose rates beneath a single layer of the polyamide used for the window of the extrapolation chamber obtained using radiochromic films calibrated and read-out using a variety of techniques (Table 3) are included in Tables 1 and 2

DISCUSSION

Variation between measurements with the 1 mm electrode for the large plane Sr/Y-90 source is dominated by the source uniformity. Estimates which were spatially averaged over the central region are however in good agreement with those obtained using the 1 cm diameter electrode. Although the extrapolation chamber model exhibits similar regresssion coefficients the predicted dose values for the 1 cm electrode are significantly different. Estimates derived from the linear quadratic extrapolation model are in better agreement with those obtained using radiochromic film. The differences between the extrapolation models for the point source reduce markedly as the electrode size is increased. Both non-linear models however exhibit better correlation coefficients than the linear model for the small electrodes and the predicted doses are again in better agreement with those obtained using radiochromic films.

The extrapolation chamber will be used in subsequent studies for the evaluation of average doses over various areas from small radioactive sources. Extrapolation will extend to the smallest possible chamber spacings and the I vs x characteristics will be fitted with second or third order polynonials as necessary. Radiochromic film and TLD techniques will also be used.

PUBLICATIONS:

Darley P J, Charles M W, Hart C D, Wells J and Coleby M S E. Dosimetry of planar and punctiform beta sources using an automated extrapolation chamber and radiochromic dye films. CEC Skin dosimetry workshop. Dublin May 1991.

Charles M W. The hot particle problem. CEC Skin dosimetry workshop. Dublin May 1991.

Soares, C.G., Darley, P.J., Charles, M.W. and Baum, J.W. Hot Particle Dosimetry using Extrapolation Chambers and Radiochromic Foils. CEC Skin dosimetry workshop. Dublin May 1991.

FIGURE 1.

A scan of the dose rate across a plane Sr/Y-90 source (4 x 2 cm^2) using the automated extrapolation chamber. The various scans are slightly offset on the y-axis.

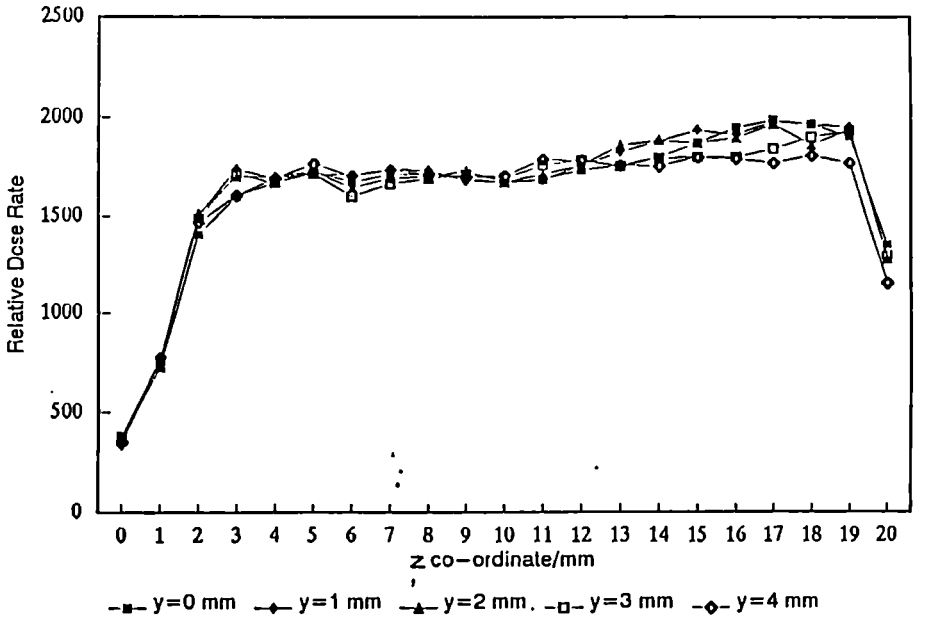


Table 1 Dose Estimates for 2 cm x 4 cm Sr/Y-90 Plane Source

Electrode Diameter		EXTRAPOLATION CHAMBER Extrapolation Model $\alpha+\beta x$	$\alpha+\beta x+\delta x^2$	$\alpha+\beta x+\delta/x$	RADIOCHROMIC Evaluation a
1mm	Correlation Coefficents	0.9995	0.9997	0.9997	n/a
	Mean Value/Gy min-1	0.91	0.96	1.00	0.94
	Std Reproducibility	0.03	0.06	0.11	0.03
	Errors Fitting	0.01	0.02	n/a	n/a
10mm	Correlation Coefficents	0.9996	0.9997	0.9998	n/a
	Mean Value/Gy min-1	0.90	0.96	1.01	0:94
	Std Reproducibility	0.01	0.03	0.04	n/a
	Errors Fitting	0.01	0.02	n/a	n/a

Table 2 Dose Estimates for 1 mm Punctiform Sr/Y-90 Source

Electrode Diameter		EXTRAPOLATION CHAMBER Extrapolation Model $\alpha+\beta x$	$\alpha+\beta x+\delta x^2$	$\alpha+\beta x+\delta/x$	RADIOCHROMIC Evaluation b	c	d
1mm	Correlation Coefficents	0.9718	0.9998	0.9997	n/a	n/a	n/a
	Mean Value/Gy s-1	0.87	1.5	1.9	2.6	2.4	1.9
	Coef variation/fitting(%)	7.6	1.3	n/a	n/a	n/a	n/a
3mm	Correlation Coefficents	0.9914	0.9999	0.9997	n/a	n/a	n/a
	Mean Value/Gy s-1	0.31	0.43	0.50	0.63	n/a	0.45
	Coef variation/fitting(%)	4.1	1.3	n/a	n/a	n/a	n/a
10mm	Correlation Coefficents	0.9978	1.0000	0.9999	n/a	n/a	n/a
	Mean Value/Gy s-1	0.049	0.059	0.067	0.080	n/a	0.055
	Coef variation/fitting(%)	2.1	0.8	n/a	n/a	n/a	n/a
30mm	Correlation Coefficents	0.9998	1.0000	1.0000	n/a	n/a	n/a
	Mean Value/Gy s-1	0.0071	0.0076	0.0083	0.009	n/a	n/a
	Coef variation/fitting(%)	0.7	1.1	n/a	n/a	n/a	n/a

Table 3 Radiochromic Evaluation Methods

Method	Batch	Calibration Source	Standarisation	Read-out Technique	Institute
a	II	400 kVp X-ray	Ion Chamber Fricke	Manual Densitometry	BNL
b	I	^{60}Co (gamma) $^{90}Sr + ^{90}Y$		Scanning Laser Densitometry	NIST
c	I	400 kVp X-ray	Ion Chamber	Manual Scan Microphotometry	BNL
d	II	400 kVp X-ray	Ion Chamber Fricke	Magiscan Image Analyser	BNL

Head of Project 2: Dr. Herbaut

II Objectives for the reporting period (1990)

Study of characteristics of a type PTW extrapolation chamber for different beta rays-sources (^{147}Pm - ^{204}Tl ^{90}Sr + ^{90}Y) with various sized collection electrodes.
Study of dosimetric characteristics of thermoluminescent dosimeters (LIF-PTFE discs - LIF - graphite loaded PTFE discs).

III Objectives for next period

Application of the above described dosimetric detectors to dose rate measurements from ^{60}Co particle sources produced by BNL.
Depth dose curves will be determined by polythene sheets before the window of the extrapolation chamber and by discs stack.
Our experimental measurements will be compared with calculated results obtained by Monte Carlo code (Toulouse).

IV Progress achieved including publications

1. Extrapolation chamber characteristics

1.1. General description

The PTW type 23391 extrapolation chamber consists of two parallel plate electrodes, one of them, the collecting electrode is movable with respect to the other (entrance window) in order that the thickness of the detector volume approaches zero. The thickness of the cavity can vary between the minimum thickness (0.6 mm) to 25 mm with a variable spacing of 0.001 mm increments. The collecting electrodes (10 mm, 15 mm, 20 mm, 30 mm, 40 mm in diameter), and the guard ring are made of A-150 tissue equivalent plastic material. The entrance window is made of polythene 7.05 $mg.cm^{-2}$ thick, corresponding to the thickness recommended for the determination of the dose of the skin. The cavity of the chamber is filled with air.
The electric field applied to PTW chamber is equal to 50 $V.mm^{-1}$.
The PTW chamber is connected to a Keithley electrometer (model 642 with a measuring limit of 10^{17} A).

An on-line HP VECTRA ES12 computer carries out the data acquisition (including ambient parameters), the data processing and the computation of the dose with its uncertainty.

1.2. Saturation effect

The ionization current varies with the applied high voltage.
Measurements has be done with different collecting potential values to determine the minimum polarity giving a collection efficiency fc equal to one. For a thickness chamber of 1 mm, we have applied a collecting potential U from ± 1 V (1 $V.mm^{-1}$) to ± 20 V (20 $V.mm^{-1}$), for two beta dose rates : 0.2 $Gy.h^{-1}$ and 10 $Gy.h^{-1}$ (table 1).
Table 1 - Collection efficiency

U (V)	± 1	± 5	± 15	± 17 U_{max}
fc	0.91	0.98	0.999	1.0

$$fc = \frac{I}{I_\infty}$$

I (I_∞) is the mean ionisation current obtained for positive and negative polarity of U (U_{max})

1.3. Leakage Current

For this chamber, it is of the order of 10^{-16} A, depending of the collecting electrode diameter and the depth of the chamber.

1.4. Collecting electrode areas

According to the Böhm's method /1/, we determine the area of the various collecting electrodes (Table 2).

Table 2 - Collecting electrodes areas

Nominal area cm^2 (diameter)	Experimental area cm^2	Standard deviation %
0.785 (10 mm)	0.9	0.8
1.77 (15 mm)	1.89	0.3
3.14 (20 mm)	3.31	0.3
7.07 (30 mm)	7.4	0.15
12.57 (40 mm)	13.01	0.2

1.5. Characterization of beta ray beams in term of absorbed dose rate in tissue.

The determinatiçon of the absorbed dose rate in tissue $\dot{D}_t$ at the specified depth of tissue is obtained from the dose rate measured in the gas of the cavity D_g at the same depth of tissue, by applying the relationships 1 and 2 and taking into account the necessary corrective terms /1/.

$$\dot{D}_t = \dot{D}_g \cdot S_g^t \qquad (1)$$

$$\dot{D}_g = \frac{\overline{W_g}}{e} \cdot \frac{1}{a\rho_g} \left[\frac{\Delta I}{\Delta y}\right]_{y \to o} \qquad (2)$$

where

S_g^t = ratio of average mass collision stopping powers for tissue and gas

$\overline{W_g}$ = average energy required to produce an ion pair for the beta rays in the gas

e = elementary charge

ρ_g = gas density (air)

a = effective collector area of the measuring volume

$\left[\frac{\Delta I}{\Delta y}\right]_{BG}$ = increment of the ionisation current ΔI per increment of the chamber depth Δy, obtained for Bragg-Gray conditions. $[\Delta I/\Delta y]_{BG}$ is calculated from the slope [dI/dy] of the function I(y).

Measurements have been dose in radiation fields, (^{204}Tl, $^{90}Sr + ^{90}Y$), delivered by beta irradiation facilities (Büchler or CEA). The beams are calibrated by PTB or LMRI, or by us, with an FWT-EIC 1 extrapolation chamber /2/.

The beam characteristics are given in table 3 and our experimental results compared with experience values are presented in table 4.

Table 3 - Beam characteristics

Radionucléide E_{max}	Source diameter (mm)	Filter yes or no	Irradiation Facility	Calibration distance (cm)	Beam Number
^{204}Tl E_{max} = 0.763 MeV	42	no	CEA	30	1
^{90}Sr + ^{90}Y E_{max} = 2.274 MeV	42	no	CEA	30	2
^{90}Sr + ^{90}Y E_{max} = 2.274 MeV	10	no	Büchler	50	3
^{90}Sr + ^{90}Y E_{max} = 2.274 MeV	10	no	Büchler	30	4

Table 4 - Experimental results - Reference date 01.01.1990

Beam Number	Absorbed dose rate ($mGy.h^{-1}$)		$\frac{(\dot{D}_t)_m}{(\dot{D}_t)_r}$
	measured value $(\dot{D}_t)_m$	Référence value $(\dot{D}_t)_r$	
1	46.9 ± 1.00	47.8 ± 0.8 (a)	0.981 ± 0.03
2	197 ± 1.2	196 ± 2.1 (b)	1.005 ± 0.02
3	79.02 ± 0.54	79.9 ± 0.6 (c)	0.989 ± 0.01
4	226 ± 1.3	228 ± 2 (c)	0.991 ± 0.01
(a) FWT.EIC 1 chamber - (b) LMRI value - (c) PTB value			

The uncertainties are given for one standard deviation ; our results are in good agreement with the reference values.
This extrapolation chamber will be used in the future for measuring absorbed dose rates of BNL ^{60}Co sources.

2. Thermoluminescent dosimeters

The study of dosimetric characteristics of LiF graphite loaded PTFE dics is in progress with a VINTEN-RIALTO reader, in photon beams and in beta rays beams described previously.

3. References

/1/ BOHM, J. PTB-DOS. 13 (1986.
/2/ HERBAUT, Y., HEEREN DE OLIVEIRA, A, VIVIA, R., LEROUX, J.B. DELAHAIE, M. Radiat. Prot. Dosim. 14(2) - 187-191 (1986).

Head of Project 3: Dr. Patau

II Objectives for the reporting period

The objectives for the period were to carry out and test a computer code able to calculate depth dose distributions in polystyrene around radioactive particles (hot particles) simulated by Co-60 sources. The results have to be compared with experimental measures made in the same conditions

III Objectives for next period

Adaptations of the present code to :
- dosimetric experiments with a polyethylene extrapolation chamber,
- TLD (LiF) dosimetry,
- Tl-204 disc sources.

Fitting with experimental results.

IV Progress achieved including publications

A computer program able to calculate energy spectra and local absorbed dose distributions, around high activity radioactive particulates ("hot particles") has been developed. For perfecting the project, idealised Co-60 beta spherical sources produced in Berkeley Nuclear Laboratories (U.K.) by neutron activation were considered.

For testing the reliability of the code the transport of beta particles was simulated in a complex geometrical configuration (figure 1) used for dosimetric measurements, both in B.N.L. and in the "Centre d'Etudes Nucléaires de Grenoble" (F.). The polystyrene frustrum of cylinder is representative of the electron moderator in which dosimetric measurements should be made with an extrapolation chamber. The main parts around the sensitive volume are made up with this material (1). It is sometimes used as a muscle substitute and its physical characteristics are fairly close to those of the skin (2). This medium is only separated from the source by a ten micrometers depth aluminium sheet. The space exterior to the sphere between support and sheet is filled with an adhesive which is temporarily assumed to have the composition of polystyrene.

PROCESSING OF THE PROBLEM

The energy spectral emission of electrons is sampled from a beta-ray spectrum of allowed transition (log ft = 7.5), whose maximal and mean energies are respectively 317.89 keV and 95.81 keV (3). The spectrum was calculated according to the

beta-decay FERMI's theory (4). Each nuclear disintegration is also followed by two gamma-ray emissions which are not considered, their mean free path in tissue equivalent materials exceeding 10 centimeters, while beta-particles release their energy within 0.07 centimeter.

The electron transport code takes into account the exact configuration shown on the figure 1. The greatest care was taken for precisely obtaining energy spectra of incoming and outgoing electrons and absorbed energy in the frustrum of cylinder. The latter may be sliced perpendicularly to its axis, so that depth distribution of absorbed energy inside itself may be calculated. The positions of surfaces S1, S2, and S3 are determined so that electrons reaching one of them have no chance to come or come back into the cylinder.

Monte Carlo methods were all along used for sampling each beta-ray emission position, direction and energy, and for simulating the slowing down up to 30 keV (5). According to the continuous slowing down approximation the residual pathlength for that energy in polystyrene is 16.8 µm (6), while the residual range is about 12 µm. Every history proceeds as a succession of steps delimited by preselected energies. In the course of each one, an electron undergoes many Coulomb interactions with atomic nuclei and bounded electrons loosing a small part of its kinetic energy and being deflected. Some interactions give rise to secondary electrons of energy greater than 30 keV; their histories are then simulated. For each step and for each material the pathlength and deflection distributions have to be calculated and sampled. The first are derived from LANDAU theory (7) and the latter from GOUDSMIT and SAUNDERSON theory (8). The probability of inelastic scattering with energy transfer greater than 30 keV, as well as the energy spectra of secondary electrons are derived from the MOLLER cross-section (9).

RESULTS

The computer code was executed for a set of source radii ranging from 0.001 cm to 0.1 cm. The truncated cylinder was 0.07 cm thick and had a radius of 1 cm.

The first investigation estimated the self-absorption in the source and the proportion of the emitted energy which is absorbed inside the cylinder (figure 2) versus the radius of the source. For a source sphere of radius 0.01 cm only 15 per cent of the beta initial energy is leaving the sphere and about 1.5 per cent of this same energy is absorbed inside the cylinder. This fact required to increase the sampling efficiency without altering the results in the cylinder by leaving electrons emitted at locations from where they couldn't reach the cylinder.

The figure 3 allows to compare energy spectra of beta-rays emitted, electrons leaving the sphere, and entering the cylinder for a source radius of 0.001 cm. The reduction of the number of electrons reaching the cylinder shows a significant energy absorption.

The depth distribution of energy deposition in the cylinder per beta-ray emitted in the sphere is represented with a resolution of 0.001 cm (figure 4) for five source radii. The attenuation is roughly exponential but becomes more pronounced as the source radius is increasing. Besides the more the radius is large, the less an electron emitted in the sphere has a chance to enter the cylinder; that explains the shifting between the five curves. Statistical fluctuations enlarge in end of range because free moving electrons become scarce.

CONCLUSION

The results shown here are only some exemples. Other practical cases will be provided in the very near future : replacement of polystyrene by polyethylene in the cylinder in order to match another model of extrapolation chamber, and calculations for (LiF) thermo- luminescent dosimetry. Other beta-emitter radionuclides should be considered.

To a certain extent some parameters and theories used may be fitted or improved. This will be carried out when experimental results obtained in the same conditions are available. The lowering of the 30 keV cut-off is possible; the directional distribution of electrons when their history is left is not so far isotropic.

On modern micro-computers required results can be obtained in less than one hour. It may be hoped that such codes could be of great use when experimentation is too expensive, difficult, or even impossible.

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(4) see R. D. EVANS : The Atomic Nucleus, Ed. Mc Graw Hill, New York, 1955

(5) J. P. PATAU : Thèse Dr. ès Sciences Physiques, TOULOUSE, N° 478, 1972
J. P. PATAU, M. TERRISSOL, M. MALBERT : J. Microsc. Spectr. Electr. 5, 393, 1980

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(8) S. GOUDSMIT, J. L. SAUNDERSON, Phys. Rev. 57, 24, 1940
S. GOUDSMIT, J. L. SAUNDERSON, Phys. Rev. 58, 36, 1940

(9) C. MOLLER, Ann. der PHYSik, 14, 531, 1932

FIGURE 1

GEOMETRICAL CONFIGURATION

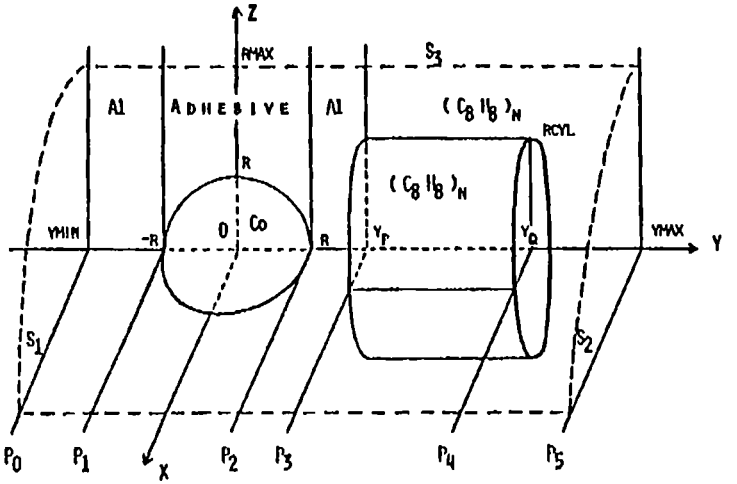


FIGURE 2

□ SELF-ABSORPTION : ENERGY LEAVING THE SOURCE

\+ ENERGY ABSORBED IN THE CYLINDER

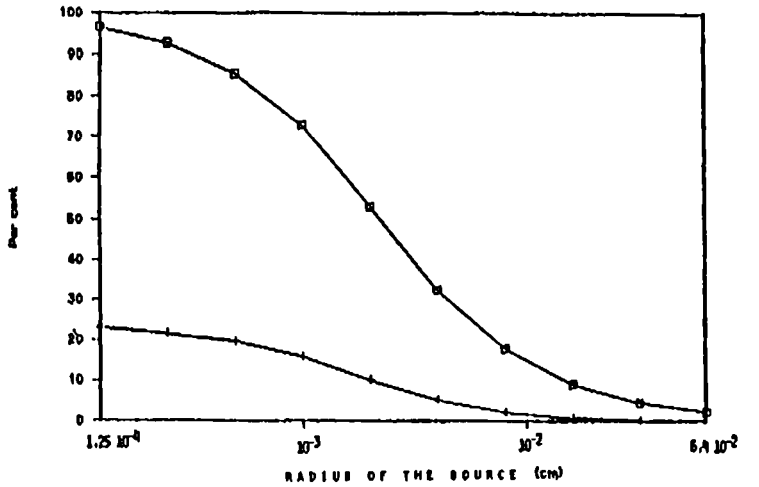


FIGURE 3 ENERGY SPECTRA OF ELECTRONS

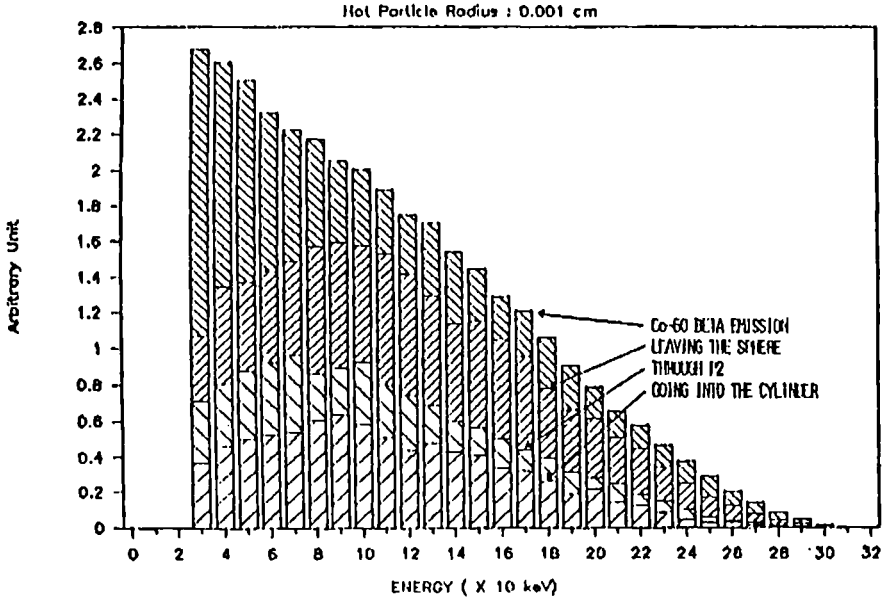
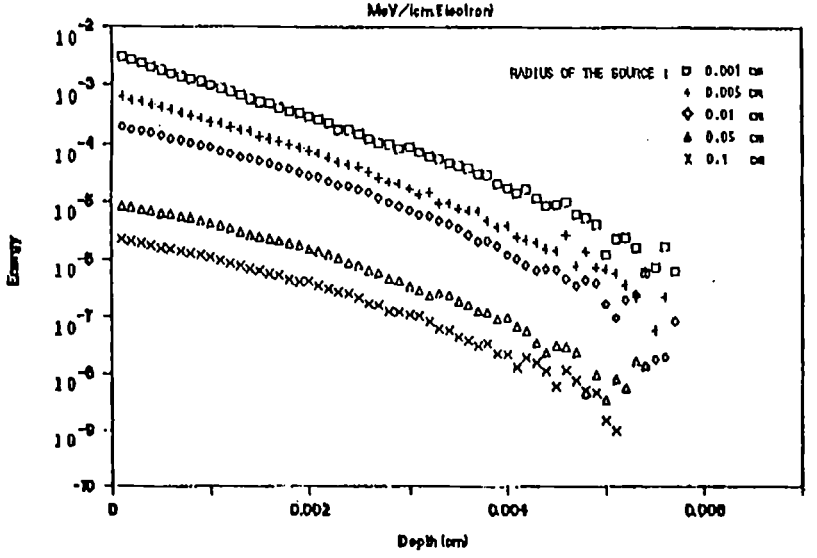


FIGURE 4 DEPTH ENERGY DEPOSITION



Progress Report

Contract: Bi6-347b Sector: A14

Title: The calculation of doses from intakes of radionuclides by inhalation or ingestion implementation of the operational dose quantities into radiation protection dosimetry (NRPB Association)

1	Bailey	NRPB
2	Kendall	NRPB
3	Stahlhofen	GSF Frankfurt
4	Roy	CEA - FAR
5	Patrick	MRC Radiobiological Unit
6	Kaul	Bundesamt für Strahlenschutz
7	Taylor	KfK Karlsruhe

I. Summary of Project and Global Objectives

This co-ordinated project was formed from two proposals submitted to the CEC Radiation Protection Programme: 890029 "Modelling and experimental studies of inhaled radionuclides in the human respiratory tract"; and 890082 "The calculation of doses from intakes of radionuclides by inhalation and ingestion". The participants originally consisted of four out of the seven organisations that had submitted Proposal 890029: NRPB (Bailey); GSF; CEA and MRC; and one of the two organisations that submitted Proposal 890082: NRPB (Kendall). The other participant in 890082 (BFS) joined towards the end of 1990. It has recently been agreed that proposal 0333 "Calculations on committed dose equivalents for internal exposures. Consequences of new ICRP recommendations" (Prof D M Taylor, KfK), should be incorporated into it.

The project is thus concerned with the development and implementation of models for evaluating doses to internal organs from intakes of radionuclides, by both inhalation and ingestion. However, for the inhalation route the project includes experimental studies designed to provide data required to improve models. Much of the work is closely linked to the development of ICRP models and recommendations relating to the dosimetry of internally deposited radionuclides. This is a particularly active area at present and will continue to be for the next several years, with the revision of the respiratory tract model, reference man, biokinetic models for individual elements (in conjunction with age-dependent models), and possible new models for bone and GI Tract. These are expected to lead to a full revision of ICRP Publication 30.

During this reporting period the implications were explored for doses per unit intake, and for ALIs, of proposed changes to ICRP recommendations relevant to internal dosimetry: ie, changes in tissue weighting factors, and in the limit on committed effective dose. Particular attention was paid to the question of whether there was a continuing requirement for additional limits on individual organ doses. Consideration has been given to developments in computer programs needed to take account of: the proposed new respiratory tract model; radioactive decay products with biokinetic behaviour different from the parent; and age-dependent parameters.

Considerable effort went towards the work of the ICRP Task Group on Human Respiratory Tract Models for Radiological Protection, which is due to complete its report in 1991, and of which three participants are members. In particular, extensive reviews were carried out of information relating to respiratory tract physiology, and to particle deposition and clearance. Models were developed to predict aerosol deposition in each respiratory tract region, and particle clearance kinetics. Reference values were recommended and consideration given to associated uncertainties and intersubject variations. A microcomputer program was developed to examine the practical application and radiological implications of the proposed model.

The ICRP Task Group has identified the bronchial epithelium as being of particularly high sensitivity to radiation among the respiratory tract tissues. It has traditionally been assumed that particles were cleared from this region within a few hours, but recent studies, notably at GSF and MRC, have indicated that a significant fraction (~ 20%) may be cleared much more slowly. This has been included in the proposed new ICRP model, and calculations have shown that it is the dominant factor in the contribution to effective dose from irradiation of the respiratory tract from long-lived α-emitters. However, it is acknowledged that there are major uncertainties associated with this phenomenon. Further human studies are therefore planned at GSF, and also at NRPB. New animal experiments were started at MRC to address the problem. The use of animals enables the amounts of activity associated with the trachea to be determined directly, and also permits investigation into the important question of an extent to which delayed clearance applies to particles cleared into the region from more distal airways.

Human experimental studies were also carried out to determine the effect of infection (rhinitis) on nasal deposition, and to investigate deposition mechanisms. Improved experimental techniques are being developed at CEA, GSF and NRPB, to extend the range (eg, in particle size) of controlled exposures to test aerosols that can be performed on humans, in preparation for studies planned for next year.

Direct collaboration between participants is an important feature of this project. A number of links existed before it was set up. Noteworthy examples during this reporting period include collaboration on the work of the ICRP Task Group on Human Respiratory Tract Models for Radiological Protection, and an intercomparison between the NRPB and BFS compilations of dose per unit intake values.

Task Group

Human volunteer studies of the effect of particle size and breathing pattern on nasal

deposition and clearance will commence. The first stage will be to carry out pilot studies using insoluble ^{99m}Tc-labelled aerosols. These will be generated using a spinning top aerosol generator for particles with diameters less than 7 μm, and with a vibrating orifice aerosol generator for larger particles. Work will continue on the development of a method for labelling polystyrene aerosols with ^{97}Ru, following which full scale human volunteer studies will commence. The use of arrays of small sodium iodide scintillation detectors to measure regional deposition in the nasal airways will be investigated.

Studies by Stahlhofen et al at GSF using boluses injected at different points in the breathing cycle have produced evidence for a slowly- cleared component of material deposited in the tracheo-bronchial airways. Interpretation of these results depends on the assumption that a bolus injected over a small part of the breathing cycle deposits at a well-defined lung depth. To investigate this, it is proposed, in collaboration with the Royal Free Hospital, London, to use the laser aerosol photometer/spirometer to enable boluses of sub-micron ^{99m}Tc- labelled aerosols to be administered to human volunteers. Images of regional deposition will then be measured using a gamma camera.

Finally, it is planned to submit proposals to carry out investigations of human bronchial clearance which will be complementary to those carried out with radiolabelled boluses at GSF. However, it is expected that these studies will not commence until 1992.

Head of Project 1: Dr Bailey

II. Objectives for the reporting period

Develop models for respiratory tract clearance and dosimetry of inhaled radionuclides, focusing on development and implementation of the proposed new ICRP lung model.

III. Objectives for next period

The review of particle clearance from the respiratory tract and the selection of reference values for the proposed ICRP compartment model for mechanical transport will be completed. The main outstanding issues are particle clearance and retention in the bronchial and bronchiolar regions, and estimation of the uncertainties and inter-subject variations associated with the reference values.

The rate of translocation of material to blood from particles deposited in the respiratory tract is in general time-dependent. To simplify calculations, the ICRP Task Group proposes to represent this behaviour with a combination of compartments that clear at constant rates. Work will continue on the determination of parameters for use with this model. Data will be derived from the published results of animal and human inhalation studies for a range of compounds of radiologically important radionuclides. A subsidiary source of information will be the time-dependent translocation rates derived by an NCRP.

IV. Progress achieved including publications

As a contribution on the work of the ICRP Task Group on Human Respiratory Tract Models for Radiological Protection, an extensive review of the literature relating to particle clearance from the human respiratory tract was carried out. Draft documents were completed on: justification of the approach and assumptions in the proposed clearance model; particle clearance from the extrathoracic airways; particle clearance from the alveolar-interstial region; and particle retention in the thoracic lymph nodes. While there have been numerous measurements of muco-ciliary clearance in the posterior nasal passage, from which intersubject variation and the effect of various factors can be quantified, data on clearance from the anterior nasal passage are sparse. Alveolar clearance has been quantified in experimental studies up to a year after inhalation. Measurements following accidental intakes are difficult to interpret, but suggest a component of much longer retention in the lung than assumed in the ICRP Publication 30 model. There is particular uncertainty about the extent of delayed clearance from the bronchi and whether it applies to material in transit from more distal airways. The results were used to select reference values for the proposed compartment model to describe particle clearance from the human respiratory tract.

The model proposed by the ICRP Task Group is more comprehensive than that used in ICRP Publication 30, and is intended for both dosimetry and bioassay. The most fundamental difference is that target cells have been identified in each respiratory tract region, and the doses to these have to be evaluated, instead of simply the mean lung dose. These factors in turn require deposition, clearance and dosimetry to be treated in more detail than in the ICRP Publication 30

model. In order to examine the practical application and radiological implications of the proposed model, a microcomputer program has been developed in a modular form so that changes can be made as the model develops. LUDEP (Lung Dose Evaluation Program) is a user-friendly menu-driven program which runs on IBM- compatible personal computers. It calculates: (a) doses to each region of the respiratory tract (b) doses to all other specified body organs (c) excretion rates and retention curves for bioassay purposes. Major changes were made during this reporting period as a result of the Task Group's decision to divide the tracheo-bronchial region into two: bronchial and bronchiolar.

Use of the program to investigate the implications of the proposed new ICRP model has begun. For example, for a 1 μm AMAD aerosol of a long-lived α-emitter such as ^{239}Pu in a relatively insoluble form (eg, oxide) or in a moderately soluble form (eg, nitrate) it was calculated that the main contribution to the effective dose from irradiation of the respiratory tract, arises from the dose to the bronchial epithelium. This results from the assumption that a significant fraction (20%) of the material deposited in the bronchial region, or cleared to it from the alveolar and bronchiolar regions, is cleared slowly ($t_{½}$ ~ 20 d). Doses to other organs are similar to those calculated with the ICRP Publication 30 model for ^{239}Pu-nitrate, because a similar fraction of the inhaled activity eventually reaches the blood, but are lower for ^{239}Pu-oxide, because of lower deposition in the alveolar region.

Publications covering work of reporting period

Bailey, M R, Birchall, A, Cuddihy, R G, James, A C and Roy, M. Compartment models for the mechanical clearance of particles from the respiratory tract of humans and laboratory animals (abstract). Proceedings of the Symposium on Particle-Lung Interactions: "Overload" Related Phenomena, Rochester, New York, May 17-18, 1990. J. Aerosol Medicine. 3, 68 (1990).

Bailey, M R, Birchall, A, Cuddihy, R G, James, A C and Roy, M. Respiratory tract clearance model for dosimetry and bioassay of inhaled radionuclides. Proceedings of the Third International Workshop on Respiratory Tract Dosimetry, Albuquerque NM, July 1-3, 1990. Radiol. Prot. Dosim. (in press).

Birchall, A, Bailey, M R and James, A C. LUDEP: A lung dose evaluation program. Proceedings of the Third International Workshop on Respiratory Tract Dosimetry, Albuquerque NM, July 1-3, 1990. Radiol. Prot. Dosim. (in press).

James, A C and Birchall, A. Implications of the ICRP Task Group's proposed lung model for internal dose assessments in the mineral sands industry. Proceedings of Minesafe International, Perth, Western Australia, September 10-14, 1990 (in press).

James, A C, Gehr, P, Masse, R, Cuddihy, R G, Cross, F T, Birchall, A, Durham, J S and Briant, J K. Dosimetry model for bronchial and extrathoracic tissues of the respiratory tract. Proceedings of the Third International Workshop on Respiratory Tract Dosimetry, Albuquerque NM, July 1-3, 1990. Radiol. Prot. Dosim. (in press).

Head of Project 2: Dr Kendall

II. Objectives for the reporting period

Validate the RAPID database of dose per unit intake factors. Conduct an intercomparison of dose per unit intake factors derived at NRPB with those derived at BGA (now Bundesamt für Strahlenschutz). Explore the implications of the latest ICRP proposed weighting factors.

III. Objectives for next period

(i) Complete and write up the work started in 1990.

(ii) Continue to support the work of the Task Group on Age-dependent dosimetry.

(iii) Further develop the models used for calculating dose per unit intake factors. Particular attention is likely to be given to:

(a) fetal dosimetry;

(b) the new ICRP Lung Model.

This work will be carried out in conjunction with other participants in the research contract.

(iv) Quality control on the dose calculations. This will involve intercomparisons with other research participants. It is unlikely that anything on the scale of the recent BGA intercomparison will be undertaken. Nevertheless, cross checks on specific nuclides will prove useful to all concerned.

IV. Progress achieved including publications

Work has concentrated in two main areas, comparing the NRPB compendium of dose per unit intake values with data from the Bundesamt für Strahlenschutz and on exploring the implications of the most recent draft recommendations of the ICRP.

Agreement between NRPB and BFS dose per unit intake values for adults was generally very good (97% of nuclides agreeing to within 10%). No serious disagreements were found for important nuclides and differences could usually be traced to specific features of the modelling. For children, agreement was still good, though the differences were larger than for adults. This is, in part, due to the use of different growth functions and different ways of dealing with the age dependence of Specific Effective Energies. A number of discrepancies were found, many of which could be traced to different assumptions in the modelling.

A good deal of work has been carried out to explore the implications of the draft recommendations of the ICRP so far as internal dosimetry is concerned. The proposals of February 1990 led to a number of difficulties, most of which have been overcome in the June 1990 suggestions. However, the low weighting factor now suggested for bone surfaces still allows a dose of 1 Sv to this tissue from one year's intake of certain nuclides. This could be reduced by introducing a specific organ dose limit or by increasing the bone surface weighting factor. However, the problem may not be a real one in practice:

a) The old organ dose limit was designed to protect against non-stochastic (deterministic) effects. Many of the nuclides involved are α emitters for which a quality factor of twenty has been used. This is the value recommended for stochastic effects; for deterministic effects a lower value may be more appropriate.

b) Many of the nuclides are fairly uncommon (eg, ^{93}Zr) and, in practical situations they are likely to be encountered in mixtures with other materials which do not preferentially irradiate bone surfaces.

c) We have considered committed doses, ie, doses received in the fifty years after intake. The ICRP Draft Recommendations (paras 171 and 174) refer to the limitation of organ dose rather than committed dose. If a material has a long physical and biological half life then a substantial fraction of the dose may be incurred decades after exposure and, perhaps, not within the lifespan of the individual.

Publications covering work of the reporting period

J W Stather, G M Kendall and A W Phipps. "Implications of the ICRP draft recommendations for ALIs" Radiol. Prot. Bull. 113, 9-14 (1990).

B W Kennedy, A W Phipps, T P Fell and G M Kendall. "The calculation of doses from internal emitters using a new computer program: Quality Control on the RAPID Database". NRPB-M215 Chilton (1990).

A W Phipps, G M Kendall and J W Stather. "Effectance, Committed Effective Dose Equivalent and Annual Limits on Intakes: Comments on the Implementation of the ICRP Proposals. NRPB-M242 Chilton (1990).

Head of Project 3: Dr Stahlhofen

II. Objectives for the reporting period

(i) Development of an improved statistical and algebraic model of regional deposition in the human respiratory tract.

(ii) Investigation of human tracheobronchial clearance by measuring lung retention of radio-labelled particles inhaled as a bolus.

III. Objectives for next period

(i) Bolus dispersion will be studied in models approximating various airway geometries (cylindrical and tapered tubes, and systems of tubes of different diameters; models of the larynx, of bifurcations, and of the tracheobronchial tree). The results will be compared with model calculations.

(ii) The radioactive Indium-111 oxide aerosol will be used for inhalation experiments to study the total deposition of submicron particles in the human respiratory tract. By scintillation counting the retention of these particles in the lungs will be measured to derive regional deposition. Also, with these particles, the retention of aerosol boluses delivered to various lung depths will be studied.

(iii) The algebraic regional deposition model will be extended to describe the mean as well as the median of regional deposition (ie, regional deposition at optimum absolute and relative accuracy). The dependence of regional deposition on age will be expressed explicitly (without the need for theoretical scaling factors). Furthermore, the algebraic model will be extended to express mass deposition of polydisperse aerosols explicitly as a function of the geometric standard deviation of the particle size distribution (ie, no integration routine is needed to obtain mass deposition). The influence of the morphologic and physiologic parameters of different ethnic groups will be studied.

(iv) ^{99m}Tc-labelled Fe_2O_3 particles will be produced with various particle sizes and with sufficient concentration to obtain γ-camera images of the distribution of the deposited particles. Mucociliary clearance will be studied with boluses injected to various volumetric regions of the TB tract, by scintillation counting and by γ-camera imaging.

(v) Magnetometric measurements of inhaled Fe_3O_4 particles will be conducted on healthy subjects to obtain standard values for long-term clearance, magnetic relaxation, intracellular viscosity, and macrophage activity. Smokers and nonsmokers will be studied separately (both clearance and relaxation have been shown to depend on smoking habit). Standard values being available, investigations on subjects with defined lung diseases will be studied. Furthermore, the studies will be extended to cultured macrophages, which will allow different agents to be added to influence certain properties of the cells.

IV. Progress achieved including publications

(i) An electronically controlled servo pump for dispersion experiments in models of airways has been constructed. First experiments with geometrical airway models have been conducted. In humans, the effects of breath-hold periods up to 1 minute on the dispersion of very small

($V = 20\ cm^3$) inhaled aerosol boluses have been studied. If the bolus is applied to volumetric lung depths > 50 cm^3, a marked increase, followed by a subsequent decrease in the standard deviation of the exhaled bolus with breath-hold time is observed. This is interpreted as an effect of cardiogenic mixing, followed by an increasing loss of particles due to gravitational settling.

(ii) A method has been developed to produce submicron monodisperse radioactive particles at a sufficiently high concentration for measurements of clearance and regional deposition in humans. Particles of indium chelate obtained from controlled condensation of APDC vapour are degraded to stable indium oxide particles in a high temperature furnace.

(iii) The algebraic regional deposition model was based on a statistical analysis of the available data. For extrathoracic and nasal aerodynamic deposition the model is based completely on experimental data. For tracheobronchial and alveolar deposition, where insufficient experimental data are available, theoretical results have also been used. The model has been extended to include dependence on age and sex, data for diseased subjects, and the natural biological variability of regional deposition. Deposition is expressed as a function of semi-empirical parameters which reflect particle transport and the respiratory data of the subject group considered. Additional scaling factors account for the dependence on sex, age and disease, and for natural biological variability. Depending on the factor used, the mean, and the upper and lower 95% confidence limits, both of the data and of the mean, for the whole group and for sub-groups, are obtained by the same function.

(iv) The retention of radioactive particles deposited from small aerosol boluses in the conducting airways has been measured for different sized particles. The slowly-cleared portion has been found to decrease from 60% for 1.6 μm particles to about 25% for 6 μm particles. Assuming these proportions to be representative of tracheobronchial clearance, the fast-cleared and slow-cleared fractions of thoracic deposition at steady-state breathing have been corrected to obtain estimates of tracheobronchial and alveolar deposition as functions of particle size. It is shown that the possible error introduced by assuming the whole tracheobronchial tree to be cleared rapidly is significant; it is however within the natural biological variability of the data.

(v) An improved spinning top aerosol generator has been constructed to produce spherical Fe_3O_4 particles with a high degree of monodispersity and a high particle number concentration. Using these particles the intracellular viscosity and the cell activity of lung macrophages have been studied in vivo. The intracellular viscosity has been found to be 65.3 Pa s. The macrophage activity has been characterized as a randomization process with a randomization energy of 6.7×10^{18} J.

Publications covering work of reporting period

James, A C Stahlhofen, W, Rudolf, G, Egan, M J, Nixon, W, Gehr, P, and Briant, J K (1990). The respiratory tract deposition model proposed by the ICRP Task Group. Proceedings of the Third International Workshop on Respiratory Tract Dosimetry, Albuquerque, July 1-3 1990. Radiat. Prot. Dosim. (in press).

Möller, W, Roth, C, and Stahlhofen, W (1990). Improved spinning-top aerosol generator for the production of highly concentrated ferromagnetic aerosols. J. Aerosol Sci. 21(8).

Roth, C, and Stahlhofen, W (1990). Radioactively labelled ultrafine particles for clearance measurements. J. Aerosol Sci. 21(8).

Rudolf, G, Köbrich, R, and Stahlhofen, W (1990). Modelling and algebraic formulation of regional aerosol deposition in man. J. Aerosol Sci. 21(8).

Scheuch, G, and Stahlhofen, W (1990). Dispersion of aerosol boluses in the human tracheobronchial tract. J. Aerosol Sci. 21(8).

Stahlhofen, W and Möller, W (1990). Magnetic measurement of macrophage activity in human lungs. In: Environmental Hygiene II, Eds. N H Seemayer and W Hadnagy, Springer, Berlin, FRG, 213-216.

Stahlhofen, W and Scheuch, G (1990). Messung der menschlichen Mukoziliarclearance. Pneumologie 44, 422-432.

Stahlhofen, W, Möller, W, and Godleski, J (1990). Relaxation measurements with spherical magnetic particles in the human lungs. J. Aerosol Sci. 21(3), 355-362.

Stahlhofen, W, Rudolf, G, and Scheuch, G (1990). Short-term and long-term clearance of particles in the human respiratory tract as function of particle size. J. Aerosol Sci. 21(8).

Stahlhofen, W and Möller, W (1990). Using spherical magnetic particles for testing the intracellular viscosity. J. Aerosol Sci. 21(8).

Stahlhofen, W (1990). Über die Ablagerungswahrscheinlichkeit von Aerosolteilchen im menschlichen Atemtrakt. In: Lokaltherapie von Luftwegsinfektionen, Eds. H Ganz and E Grill, Georg Thieme, Stuttgart, FRG, 40-44.

Head of Project 4: Dr Roy

II. Objectives for the reporting period

Measurement of nasal deposition of inhaled particles in the size range 0.5-6 μm in healthy adults and in adult patients suffering from rhinitis.

Modification of the methodology for aerosol exposure to apply to particles larger than 3 μm aerodynamic diameter, and to the normalization of breathing patterns.

Contribution to the work of the ICRP Task Group on Human Respiratory Tract Models for Radiological Protection.

III. Objectives for next period

(i) Measurement of total respiratory tract deposition of particles in the size range 0.5-6 μm inhaled by healthy adults and children and by patients with restrictive lung function.

In order to avoid sedimentation and impaction during controlled breathing, the aerosol generation and exposure system is in the process of being adapted to these particle sizes. Storage of inhaled and expired aerosols has been replaced by a constant aerosol flowrate system in which the subject breathes, inhaling and expiring without any separating valve. The inspired volumes are controlled by a single Fleisch tube. Particle deposition is calculated from the variations of concentrations in the aerosol flow, downstream of the subject.

Standardization breathing parameters, at rest and light exercise, will be chosen suitable both for adults and children, with the aim of keeping the breathing pattern constant for all ages, and making clearer a possible relationship between aerosol deposition and lung dimensions, especially for age effects.

(ii) Measurement of nasal deposition in adults and children of various ethnic groups.

The study will be made with the same inspiratory flow rate in order to allow comparison of nasal filtering efficiency, related only to nasal features and particle sizes;

- between adults and children,
- between adults of different ethnic groups.

IV. Progress achieved including publications

(i) Breathing parameters and lung volumes for use in respiratory tract dosimetric models.

As a contribution to the work of the ICRP Task Group on Human Respiratory Tract Models for Radiological Protection, information relating to parameters that determine the amount of

gases and particles inhaled and deposited in the respiratory tract was reviewed. Choices were made for adults and children (new-born, 3 months, 1, 5, 10, 15 years) concerning:

- lung volumes: total lung capacity (TLC), vital capacity (VC), functional residual capacity (FRC) and dead space (V_D);
- ventilation rates: tidal volume (TV) and respiratory frequency (f_R) at rest, at light and at heavy exercise;
- time-budgets and residences of population categories, according to their age, gender and occupation.

Reference values were thus recommended for lung modelling in several ethnic groups for whom there was sufficient information: Caucasians, Chinese, Japanese, Africans, Afro-Americans, Native Americans, etc.

(ii) Experimental study of deposition of inhaled particles in the nose of adults suffering from rhinitis.

Nasal deposition of a polydisperse aerosol of isopropyl myristate (aerodynamic diameter, d_{ae}, between 0.5 and 10 μm) was measured in a group of 25 healthy subjects and 24 suffering from infectious rhinitis.

Eight subjects were studied twice: while healthy and while suffering from the disease. Anterior rhinomanometry was measured in each subject. Mass deposition was measured by comparing inhaled and expired aerosol concentrations, once by nose, once by mouth controlled-breathing. Nasal depositions of masses of particles with $0.5 < d_{ae} < 3$ μm (60% of the aerosol) and $3 < d_{ae} < 6$ μm (37% of the aerosol) were found to be significantly higher in subjects with rhinitis than in the healthy ones, by the nonparametric MANN and WITHNEY test. The eight subjects studied twice whose data were not included in the statistical treatment, showed clear differences in nasal resistances, pressure drop and aerosol deposition, before and during rhinitis.

Publications covering work of reporting period

M ROY, M H BECQUEMIN and A BOUCHIKHI - Ventilation rates and lung volumes for lung modelling purposes in ethnic groups. Radiat. Prot. Dosim. (in press).

Monique ROY and Catherine COURTAY - Daily activities and breathing parameters for use in respiratory tract dosimetry. Radiat. Prot. Dosim. (in press).

M H BECQUEMIN, D L SWIFT, A BOUCHIKHI, M ROY and A TEILLAC - Particle deposition and resistance in the noses of adults and children. Eur. Respir. J. (in press).

M H BECQUEMIN, C P YU and M ROY - Total deposition of inhaled particles related to age: comparison with age-dependent model calculations. Radiat. Prot. Dosim. (in press).

Head of Project 5: Dr Patrick

II. Objectives for the reporting period

Studies in the F-344 rat of the slow clearance from large airways of particles administered by inhalation.

III. Objectives for next period

Further studies will be made of the long-term retention of particles on the airway surface in the rat and also, it is hoped, in another species.

The study of the functional morphology of the large airways will be commenced, by establishing the necessary histological and image analysis procedures for a quantitative inter-species comparison.

IV. Progress achieved including publications

An investigation has been made under the first of the main objectives, to explore the slow clearance from the large airways of particles administered to rats by inhalation.

Fischer-344 rats have been exposed to an aerosol of fused aluminosilicate particles labelled with ^{57}Co. At 1, 7, 28 and 112 days after inhalation animals were sacrificed and the tracheas examined for ^{57}Co. A measured length of trachea was defined in situ, then removed from the carcass and washed repeatedly with saline. The region of the first bifurcation was similarly removed and washed. The ^{57}Co content in the washings and the airway wall was determined by scintillation counting.

From 3 days after inhalation the animals were regularly monitored in a small animal whole body counter. Excretion measurements of ^{57}Co were made over a period of 3-7 days before sacrifice.

From this data the retention of particles in the trachea has been analysed into three functional compartments: Tf = material being rapidly cleared, as by the muco-ciliary mechanism; Ts = slow or stationary material remaining on the surface of the epithelium for extended period; and Tw = particles sequestered in the airway wall.

Expressed as a fraction of the whole-body retention at 1 day after inhalation, multiplied by 10^5 and normalised to a measured tracheal length of 20 mm, Tf decreased from 22 at 7 days to 0.6 after 112 days. Such a large decrease was to be expected for rapid muco-ciliary clearance. In contrast, Ts was 39 at 7 days but only fell to 37 by 28 days and to 7.0 by 112 days. Tw remained at between 10 to 15 throughout the experiment.

Thus the fraction of the inhaled particles estimated to remain stationary or slow-moving on the epithelial surface was the largest of the three compartments at 7 and 28 days after inhalation,

and was approximately an order of magnitude greater than the rapidly cleared component at 28 and 112 days. The fraction of particles sequestered in the tracheal wall was similar to earlier findings in this laboratory.

The tracheal washings were examined as autoradiographs of cytospin preparations. At the later times it was found that nearly all the ^{57}Co was within macrophages.

Taken together, the study has demonstrated in the rat that long-term retention of particles on the surface of large airways is possible, and suggests that this is brought about by "airway macrophages" which reside there and can keep particles there for some months.

Head of Project 6: Prof Kaul

II. Objectives for the reporting period

Preparatory studies for the research project:

(i) to continue to develop a new computer code for calculating age-dependent dose coefficients
(ii) to start to search and evaluate literature.

III. Objectives for next period

The development of the new computer code for the calculation of age-dependent dose coefficients will be continued. The new ICRP biokinetic and dosimetric respiratory tract model will be incorporated. Implementation of age-dependence into the code will be started.

Work will start to assess uniformly the biokinetic data of thorium, to perform a sensitivity analysis of biokinetic parameters and to identify those parameters which are of primary importance for the dose of ALI result. The same work will be performed with further selected elements.

IV. Progress achieved including publications

Preparatory studies for the research project are in progress:

The development of a new computer code for the calculation of age-dependent dose coefficients using general biokinetic models is continuing. First tests of calculating nuclear transformations according to the standard model of ICRP Publication 30 for the ingestion of radionuclides by adults were successful. Conceptually, the inhalation calculations and the consideration of daughter nuclides are clear, even with biokinetic data that are different from those of the parent nuclide. The study of the draft of the new ICRP respiratory tract model showed that it can easily be incorporated into the computer code.

The first item to be examined within the scope of the research project is the biokinetic behaviour of thorium. For this purpose, the reference data bank developed at our Institute for thorium/thorotrast was enlarged by key words from the source material on biokinetics. The literature was searched by DIMDI and INKA/INIS. First evaluations of these data have been started.

Progress Report

Contract: Bi-024 **Sector: A14**

Title: The assessment of internal Dose: The establishment of registries of dose assessment, autopsy data and models

1. Gibson, Radiation Dosimetry Dept., AEA Environment & Energy, Harwell Laboratory, UK.

I. Summary of Project and Global Objectives

This proposal is designed to establish the basis for setting up three registries:

(i) a registry of internal dose assessments using information supplied by European laboratories and obtained from the literature;

(ii) a registry of autopsy data using information obtained by individual laboratories in each European country;

(iii) a registry of mathematical models used for internal dose assessments in European laboratories and in the literature.

The study contract will be used to establish protocols, in consultation with European laboratories through Eurados-Cendos Working Group 6 (E-C6) and with the US Uranium and Transuranium Registries, in order to produce compatible databases for ultimate exchange of information. An intercomparison of dose assessment methods, organised by E-C6, is already underway and will form the basis of the protocols. The ultimate objective is to ensure compatibility of internal dose records within Europe in the context of 1992.

It is intended that detailed protocols will be prepared for the 3 European Registries on: Internal Dose Assessments (ERIDA); Autopsy Date (ERAD); and Models for Internal Dosimetry (ERMID) for discussion by E-C6. These protocols will be used to provide the basis for a database for use under a new contract.

Head of Project 1: Mr Gibson, AEA Environment & Energy, Harwell Laboratory, UK

II. Objectives for the reporting period

In the first period, it is intended that a detailed protocol will be devised and discussed at Eurados-Cendos Working Group 6 and be circulated to corresponding members. The basis of the protocol for ERIDA will include:

(i) details of the initiating event;
(ii) information on PAS, chemical form, particle size, etc;
(iii) body monitoring data;
(iv) bioassay data;
(v) methodology used in calculations;
(vi) results of the assessment.

III. Objectives for next period

Completion of protocol description, development of the specification of a computer database and proposals to the CEC for a project to set up this European Database in 1992-1993.

IV. Progress achieved including publications

We are devising the specification for a database of internal dosimetry assessments in order to assist dosimetry services in tackling new or unfamiliar cases. The idea is not to compile an exhaustive list of assessments, but rather to file a set of generic assessments which will provide a useful source of reference for Internal Dosimetry Services (IDSs) in Europe.

For example, suppose that an IDS has to deal with an intake of ^{252}Cf. ICRP30 recommends the use of the standard plutonium biokinetic model for californium. However, UK experience shows that californium dissolves more readily in the lung and transfers to body fluids. This is of vital importance in assessing excretion data from a ^{252}Cf intake. The database will contain details of the californium assessment including modifications to the plutonium model. This information would then be readily available to other workers.

The structure of the database will be stored using Superbase 4, run via Microsoft Windows on an IBM-compatible PC. The essential information about each case will be entered in a 'box' format. On entry into the database, the user will be able to select the attributes of the assessments and the database will locate any available assessments which satisfy the requirements. For example, by scanning the list, of attributes and selecting, 'nuclide', the user could then enter 'Cf 252'. The database would then be searched for any assessments referring to this nuclide. The user can scan the selected database files to see if there is a Cf 252 assessment which is suitable for the case in hand. Upon selecting an assessment the user can 'click on' a particular function to obtain further information. Boxes will then be revealed which give textual descriptions of, for example, the model used in the calculations. Alternatively, files containing excretion data or in-vivo monitoring results, can be called. In cases where more detailed information is available in the open literature, an abstract of this material can be viewed by 'clicking' on the 'abstract' box. A full reference to the published work will be given.

We intend to include, not only our own assessments, but cases from Europe and beyond. All personal details and identification of the originating laboratory will be removed from material stored in the database.

Head of Project 2: Miss F A Fry, NRPB, Chilton, UK

II. Objectives for the reporting period

The objectives are:

(i) to establish a protocol for a European registry of radionuclide contents of human tissues at autopsy;

(ii) to determine the likely availability of autopsy data within the member states, bearing in mind potential difficulties arising from legal and confidential considerations;

(iii) to investigate the possibility of extending the registry to include other European countries that are not presently members of the European Community;

(iv) to establish a basis for exchange of information with the US registries;

(v) to make proposals to the CEC for the setting-up and management of a European registry.

III. Objectives for next period

Description of the UK protocol and management structure as a prelude to inviting cooperation from other European countries to provide data and to become part of the European Registry of Autopsy Data (ERAD).

IV. Progress achieved including publications

At the instigation of British Nuclear Fuels plc, the UK has established a management group of a UNIted Kingdom Occupational RadioNuclide Exposure Study (UNIKORNES) to devise the protocol for the UK Registry of autopsy data from former workers. An independent Advisory Group of eminent scientists and medical practitioners will be used to peer review the protocols and procedures. The Management Group intend to establish an organisational framework, and ensure dissemination of the information and knowledge gained from the studies is reported in the open literature. NRPB and the UKAEA are represented on the management committee and will provide a link to a European Registry of Autopsy Data (ERAD).

UNIKORNES has been set up independently of this Study Contract but is considered as a useful first step towards ERAD and other European countries will be kept informed of proposed protocols so that a wider-based registry can be established in the future. The UK Registry will have a close collaboration with USUR and USTR at Hanford USA.

Head of Project 3: Dr J Piechowski, CEA, Fontenay-aux-Roses, France

II. Objectives for the reporting period

It is vital, in accurate dose assessments, to provide appropriate mathematical models based upon actual measurements on humans through in-vivo monitoring; bioassay measurements of urine, faeces, blood, etc; comparison with personal air samples for lung intake or with measurements at wound sites; and ultimately comparison with autopsy data. It is important to consider the latest information on human anatomy and physiology and the routes of entry of radioactivity into the body.

III. Objectives for next period

To investigate the capabilities of 4th generation programming languages and expert systems in the context of the requirements of the present study.

To agree the basic specification of a computer-based model using an expert system with a mechanistic model based upon the latest models as for instance the one proposed by NRPB for internal dose assessment and make proposals to the CEC for a project to design and operate the computer model in 1991-1992.

IV. Progress achieved including publications

Internal Dose Assessments are instigated in the following cases:

as part of routine monitoring;
an incident occurs which requires an assessment of intake;
a Personal Air Sampler (PAS) measurement gives a high reading;
a routine bioassay sample gives a measurement above the threshold limit;
as part of an epidemiological study;
as a result of a claim for compensation;
as part of a research project.

In all cases bioassay data plays an important part but in certain instances data from other sources can also be relevant including:

PAS data including particle size analysis;
Identification of radionuclide composition by radiochemistry spectrometry;
In-vivo monitoring;
Nose blow data;
Wound monitoring.

The relative importance of each type of data depends on the circumstances of the intake. Bioassay data is very important in the case of actinides whereas in the case of Uranium and Thorium, with their low specific activities, the PAS measurements can be particularly useful. The radiochemistry and spectrometry can give some help for those nuclides which are not routinely bioassayed. Whole body measurement is useful in cases involving gamma-emitting nuclides and lung monitoring is useful following intakes of ^{241}Am.

Once it has been determined which are the important nuclides, the assessor tries to fit the experimental data to a standard model and so arrive at an estimate of the original intake and thence to the internal dose. At Harwell, the standard models are written in FACSIMILE and are currently run on the IBM Mainframe. A library of models for different nuclides and various particle sizes has been built up but if a relevant model has not been used before it can easily be produced. The CEA and NRPB have several programs available and under development, incorporating ICRP models, to help in assessments; as for instance BAP and LUDEP produced by NRPB, UK. These programs run on standard IBM PC compatibles. The matching of model to experiment is currently done manually.

The standard models are based on the ICRP models and are used in the case of 'sparse' data. If there are large amounts of data or it is particularly difficult to fit the data to the standard models, the assessor may decide to run non-standard instances of the model. Various parameters of the model are altered to arrive at a best fit; it is here that most judgement is required since it is not intuitively obvious to a non-specialist which parameters need the most tuning. If the model is changed, the ALI (or more fundamentally the dose/intake) has to be recalculated, whereas in cases using the standard model, once the intake has been estimated, the CDE can be obtained directly from ICRP 30 tables.

Scope for Automation

The majority of assessment are relatively straightforward but a small fraction, of the order of 10%, can be classed as difficult in that substantial judgement and experience have to be brought to bear on the problem. A critical factor in determining the difficulty of a particular case is whether the time of intake is known; if it is, the case can often reduce to number crunching; if it is not known, some expert judgement may be required but there appear to be some well founded rules for using related data to arrive at a good estimate for it. The difficult cases are generally those for which the intake date is completely unknown.

There are two areas of the problem in which expert system techniques could be applied:

a) preprocessing parameters to pass to programs or modules based on ICRP models;
b) judgement of the correctness of the results.

Most bioassay and internal dose assessment programs have default parameters which can be altered by the user to suit particular cases. Deciding how to alter these defaults requires a good understanding of the model and the specific needs of the assessment in question. It should be possible to determine rules which could be used to provide an expert assistant front end to modules taken from proven programs currently in use.

Running the models with chosen parameters is only the first step. An expert judgement must be made as to how correct the results are, especially when sparse data produces conflicting dose assessments. Several iterations through the cycle of choosing input parameters - running the model - analysing the results - modifying input parameters may be needed. Again there are likely to be rules that an expert follows to resolve conflicts and which could be incorporated into an expert assistant.

The cases which are straight-forward could be automated to the extent where little or no assessor intervention would be required. The final report could be produced automatically, ready for

checking and signing. Relatively straightforward cases could also be automated. Some of the inbuilt rules would be used but prompting the assessor for guidance could largely be avoided. The difficult cases enter the provenance of a true expert system; although some quantitative processing could be done, many qualitative decisions would need to be made based on an expert assessor's knowledge and experience. It is unlikely that these cases could be assessed without some assessor intervention. Also the expert system would be useful to organisations who carry out dose assessments only occasionally or to dosimetry services who usually carry out one type of assessment but may occasionally have to deal with others.

In writing the expert system, knowledge input would be required from an expert in internal dose assessments and also an expert in the modelling of the biological processes involved.

There are very many expert system shells and knowledge-based languages available, each with their own strengths and weaknesses. Harwell has had experience in using two products in the field of Criticality Dose Assessment. Such approach may be used for internal dose assessment with a reasonable confidence that this proposal is feasible. However, a careful examination of currently available tools is required. There are some requirements that can be immediately stated that will narrow the field e.g. the final product must run on a moderately powerful IBM PC compatible, possibly in the Windows-3 environment.

Previous experience at Harwell indicates that the area of internal dose assessments is a good candidate for an expert system. The combination of expert assistant front and rear ends to existing model codes should provide a useful system. We conclude that the use of knowledge-based techniques in dose assessments could provide substantial benefits; at the very least it would free the assessor from most of the routine work and allow him to concentrate on the more intractable cases.

Contract: Bi7-029 Sector: A14

Title: Assessment of internal dose from radionuclides using stable isotope tracer techniques in man.

1	Roth	GSF Frankfurt
2	Molho	Università degli Studi di Milano
3	Hislop	AEA Technology Harwell Laboratory
4	Taylor	KfK Karlsruhe
5	Henrichs	GSF Neuherberg

I. Summary of Project and Global Objectives

The principal objective of the programme is to investigate the use of stable isotope tracers to improve source terms for models of internal dosimetry from environmental exposure to radioactive materials. There is a continuing and accepted need for more realistic human metabolic data, especially with respect to the variability of dose values for non-standard, non-occupational conditions. Much of the information on which the current radiation protection recommendations are based is derived from animal studies and broad assumptions are made where information is lacking. The common way by which metabolic data in humans are obtained is either from measurements after accidental exposure or from experimental studies where radioactive nuclides are used as tracers. Human studies with radioactive tracers, however, whilst very valuable, have become extremely restricted to perform, especially when healthy persons or critical groups like children are involved. A promising possibility of undertaking metabolic studies in man seems to be the use of isotopically enriched stable elements as tracers.

This project is aimed to obtain biokinetic data in humans by a novel stable isotope approach and to assess radiation doses for several radionuclides for which the current state of knowledge is poor. The elements identified are Sr, Ce, Ba, Ru, Te, Zr, Pu and higher actinides. Whereas Sr, Ce, Ba, Ru, Te and Zr can be studied by substituting the radioactive nuclides by stable isotopes of the same element as tracers, for Pu and the higher actinides stable analogues will be used as surrogates. Promising candidates are Eu and Gd as analogues for Am and Cm respectively, whereas Hf and Sm may be considered as Pu analogues.

The goals of this project require the collaboration of several laboratories to contribute with their special expertise and experience in the different aspects involved.

Head of Project 1: Dr. Roth

II Objectives for the reporting period

1. To develop analytical methods for measurements of tellurium in biologic materials;
2. To investigate tellurium biokinetics in animals as a prerequisite for human studies;
3. To evaluate the variability in the intestinal uptake and excretion of tellurium in man.

III Objectives for next period

1. Measurement of strontium uptake following intake of contaminated biological material;
2. Variation of intestinal strontium uptake with strontium doses.
3. Compilation of the experimental results obtained at GSF Frankfurt and University of Milan on animals and humans to develop a new metabolic model for Te metabolism in man.

IV Progress achieved including publications

Analytical methods for the measurement of Te concentrations in biological materials have been developed successfully. By the use of graphite furnace atomic absorption spectrometry (GFAAS), detection limits of 10 μg Te / kg of wet weight was achieved for plant material (cress), and 0.2 μg Te / l was achieved for body fluids (blood plasma, urine). Te measurements in foodstuffs and tissue samples revealed Te concentrations that are substantially lower (up to a factor of 10^3) as compared to the currently recognized values.

As a test for the biokinetics of Te in mammals and to evaluate the organ distribution after Te application, Te was administered perorally and intravenously in three swine. These investigations, which were performed in collaboration with GSF Neuherberg, served also as a preliminary step before the human studies.

A series of studies have been performed to evaluate intestinal absorption and excretion of Te in man. A total of 12 investigations were performed in 5 healthy male volunteers. Since it is known that the chemical form can affect the fractional gut uptake, Te was administered as tellurate, tellurite and metallic tellurium (powdered, particle diameter about 1 μm). In order to provide a form of ingestion of Te which is similar to Te in foodstuffs, cress was cultivated on a Te containing medium and the edible parts of the plant were administered orally, either as raw cress or together with a dressing. No toxic symptoms could be observed in any of the experiments with the amounts of Te administered orally (15 - 55 μg). Fractional intestinal uptake (f_1-values) were derived from the cumulated excretion curves. For tellurate the mean fractional

absorption was 28% with a standard deviation of 11%. For metallic tellurium the absorbed fraction was 16% ± 3%. The excretion of Te after ingestion of intrisically labelled cress is delayed, indicating a delayed absorption of Te out of organic matter as compared to a Te salt. Fractional intestinal absorption, however, was not significantly different (26% ± 10%). However, administration of the cress together with a dressing decreases the f_1-values to about 10%. Excretion of tellurite is slower than that of tellurate, which might be an explanation for the higher toxicity of the tetravalent tellurium compounds.

For isotopic tracer studies with Te, the analytical techniques are a more difficult problem. Preliminary investigations with mass spectrometric methods were not very encouraging. With thermal ionization (TI) and a sector field mass spectrometer (MAT 212), no satisfying detection limits could be achieved, and the application of a secondary ion mass spectrometer (SIMS) at GSF Neuherberg provided no reproducible results. A more promising way seems to be the method of proton nuclear activation analysis (PNA), which is currently optimized at the University of Milan as part of this joint contract (see project No. 2).

For the measurement of intestinal strontium absorption from food, the methods to determine Sr concentrations in biological samples by GFAAS were successfully established. First experiments were carried out to measure the abundances of strontium isotopes in blood and urine by means of TI-MS. The accuracy and reproducibility achieved as well as the sensitivity of the method are such that metabolic studies in humans are possible by this approach.

Publications

1. Kron, T., Hansen, Ch., Werner, E.: Bestimmung von Tellur im Blutplasma von Kaninchen, *In*: 5. Colloquium atomspektrometrische Spurenanalytik (Ed.: B. Welz). Überlingen: Perkin-Elmer GmbH, 809-814 (1990).

2. Kron, T., Voigt, G., Hansen, Ch., Werner, E.: Tellurium distribution in the tissue od swine after peroral and intravenous administration of potassium tellurite. *In*: 6th International Trace Element Symposium. As, B, Br, Co, Cr, F, Fe, Mn, Ni, Sb, Sc, Si, Sn and other Ultra Trace Elements (Eds.: M. Anke et al.). Leipzig: Eigenverlag Karl-Marx-Universität, 1342-1347 (1990).

3. Kron, T., Hansen, Ch., Werner, E.: Tellurium ingestion with foodstuffs. *J Food Comp Anal* (in press 1991).

Head of Project 2: Prof.Dr. Molho

II Objectives for the reporting period

Two main objectives were related to the first year of the programme:

1. The projection and construction of a new facility for the activation of isotopes producing short living radionuclei;

2. The optimization of irradiation parameters for some of the stable isotopes of interest.

III Objectives for next period

1. Completion of the irradiation facility and test on beam;

2. Optimization of irradiation parameters for ruthenium and cerium.

3. Assessment of biokinetic data for ruthenium in animal experiments as a prerequisite fur human investigations; in cooperation with GSF Frankfurt (Project 1 of this programme).

IV Progress achieved including publications

The mechanical construction of the irradiation chamber has been completed together with the computer controlled driving system. A specific program has been written for the driving system in order to allow a very flexible use of the irradiation setup. Up to 40 samples can be mounted in the irradiation chamber and each sample can have different and specifically programmed irradiation times. Moreover, each sample can be dismounted by means of a mechanism activated from the control room; in this way it is possible to take out the samples from the irradiation room without any direct intervention.

With the old facility the optimization of the proton activation methodology for the specific determination of stable tellurium isotopes in plasma samples was performed. From a preliminary study of the possible nuclear reactions induced by protons on the stable tellurium isotopes included in the natural isotope mixture it results that ^{124}Te and ^{126}Te are the most suitable isotopes to be used as tracers. In fact, via (p,n)-reactions they produce radioactive nuclei with sufficiently long half lifes (4.18 days and 13.02 days) to allow an off-line detection and a significant reduction of the background due to the matrix. Moreover, the decay characteristics of these radionuclei are suitable for our detecting systems.

In order to optimize the conditions of measurement, the yields of the induced reactions were measured. The data obtained allow us to choose the best incident proton energy of 11.2 MeV. Moreover, the same data show that at this energy there is no cross interference in the production of the two radionuclides. For the chosen incident energy interfering nuclear reactions on nuclei of the plasma matrix which can produce the same radioisotopes as those of interest were investigated.

Another important test was performed to check the linear relationship between the intensities of the specific gamma rays and the amounts of ^{124}Te and ^{126}Te present in the samples. The measured ^{124}Te and ^{126}Te content values in samples doped with different but known amounts of the isotopes show a very good correspondence with the added values. This fact implies that all the procedure is not affected by hidden systematic errors and in particular that there are no problems of volatilization of radioactive products from the sample. To check the presence of interferences with the gamma transitions of interest, the half lifes were measured by means of the same lines. The values so determined (4.35 ± 0.3 days and 13.04 ± 0.2 days for ^{124}I and ^{126}I respectively) are in very good agreement with the values reported in literature (4.18 days and 13.20 days).

To determine the detection limits of proton nuclear activation analysis (PNA) for ^{124}Te and ^{126}Te, plasma samples enriched with known amounts of these isotopes were analyzed. Assuming a minimum detectable quantity as the amount corresponding to a peak area equal to three times the subtended background statistical fluctuation, we obtained as minimum measurable concentration in plasma the values of 4 ng/ml for ^{124}Te and of 8 ng/ml for ^{126}Te.

Subsequently, a test for intestinal tellurium absoprtion has been performed on a rabbit by means of the double tracer technique, in cooperation with GSF Frankfurt. For intravenous injection 261 μg of ^{126}Te from an enriched solution were used and 1.39 mg of ^{124}Te from an enriched solution were administered orally. The data aquired from the PNA analysis of the plasma samples withdrawn at different times allowed to evaluate a percentage absorption of 5.0 ± 0.8.

The whole of these data allows us to state that isotopic tracer studies on Te metabolism in humans are well feasible.

Some very preliminary analysis on ruthenium and cerium isotopes seem to indicate that also for these elements the possibility is open for metabolic measurements with stable isotopes.

Publications

M.C. Cantone, D. de Bartolo, G. Gambarini, N. Molho, L. Pirola, Ch. Hansen, P. Roth, E. Werner.
Te metabolism study by means of stable tracers: investigation in animals.
World Congress on Medical Physics and Biomedical Engineering (1991).

M.C. Cantone, D. de Bartolo, G. Gambarini, N. Molho, L. Pirola, Ch. Hansen, P. Roth, E. Werner.
L'attivazione nucleare con protoni per studi metabolici di Te.
LXXVI Congresso Nazionaler Societa Italiana di Fisica (1990).

Head of Project 3: Dr. Hislop

II Objectives for the reporting period

1. Development for measurement procedures for barium and lanthanide series elements in human excretion samples.

2. Measurement of the uptake and excretion of lanthanide series elements and barium following oral and intravenous administration to humans.

3. Measurement of the kinetics of excretion of strontium by renal and faecal routes following oral and intravenous administration.

III Objectives for next period

1. Measurement of strontium uptake and excretion in infants following oral administration.

2. Extension of barium and lanthanide gut uptake studies to larger population groups including women.

3. Extension of gut uptake studies of lanthanides and barium to consider the effect of dietary status.

IV Progress achieved including publications

A series of studies have been carried out to assess uptake and excretion of a number of stable isotopes, as analogous of radionuclides. As part of this programme, the human intake (diet), uptake and excretion of the rare earth elements have been measured using inductively-coupled plasma mass spectrometry (ICP-MS). Procedures have been developed to separate and concentrate the rare earth elements from matrices such as faecal ash and urine, using selected radiotracers to estimate yield. These methods involve ashing of the samples to remove organic matter, followed by dissolution in a mixture of hydrochloric, nitric and hydrofluoric acid, with silica removed as H_2SiF_6. The rare earths are then separated on an ion exchange column and determined by ICP-MS.

The data obtained in this study are summarised in Fig. 1, which shows the rare earth abundance levels (g/tonne crust), intake (mean of 6 whole diet samples in μg), faecal excretion (mean of 6 individuals in μg), and urinary excretion (mean of 6 individuals in μg).

Three conclusions are apparent from Fig. 1: the close similarity in the form of the abundance curve and the intake and excretion curves shows that little or no differentiation is seen between each of the elements across the rare earth group. This confirms that the human uptake and excretion mechanisms are insensitive to the slight chemical differences across the rare earth group of elements, and therefore possibly, to the small differences between the rare earth and the actinides, their intended analogues. Secondly, absolute faecal excretion levels are very similar to the levels found in the standard diet samples. The final point which emerges is that the urinary excretion is about 10% of the intake and faecal excretion levels. This effect, if confirmed in the

volunteer studies described below, may show that the uptake of actinides/rare earths is greater than hitherto supposed.

The absolute data in Fig. 1 are similar to data in a recent publication (i), which lends support to the validity of the analytical approach adopted. Data from the current study by ICP-MS for urine are shown compared in Table 1 with data from Ref (i), by neutron activation or atomic absorption analysis.

A volunteer study has been carried out with two volunteers simultaneously ingesting and being injected with different enriched stable isotopes of strontium, barium and a number of lanthanide series elements. The quantities of each isotope used were similar to those occuring naturally in the diet and in the blood and are listed in Table 2. Barium is of interest as an analogue of radium, and the lantanides as analogues of the actinides, as stated earlier. Blood samples were collected at 24 hours post-intake, and then at increasing intervals up to four weeks. A total excretion collection was carried out over the first seven days for balance purposes. 24 hour urine and faecal collections were then carried out on one day of each week for the following three weeks. The samples have been processed under the regime outlined above and measurements of isotope concentration and ratio carried out by ICP-MS. The data are currently in the process of being evaluated, and will be used for experimental design purposes for subsequent studies.

References

(i) Minoia, C., et al: Trace element reference values in tissues from inhabitants of the European Community. I. A study of 46 elements in urine, blood and serum of Italian subjects. *Sci. Total Environ.* 95 (1990) 89-105.

Publications

(1) Dalgarno, B.G., Brown, R.M., and Pickford, C.J.:
Strontium metabolism - A study of uptake using the stable isotope ^{86}Sr as a tracer.
Journal of Trace Elements in Experimental Medicine (in press).

Table 1: Lanthanides - urine excretion

Element	Urine (ng/24h) Harwell [1] ICP-MS	Urine(ng/24h) Minoia et al. [2] NAA/ICP-AES/GFAAS
Eu	126 ± 128	110 ± 80
Gd	75 ± 23	< 1000
La	400 ± 149	730 ± 55
Lu	< 9	50 ± 40
Nd	540 ± 87	3840 ± 190
Sm	30 ± 13	55 ± 38
Yb	37 ± 11	28 ± 20

1. Mean of six replicates (four subjects), plus or minus one standard deviation.
2. Mean of six to twentyeight subjects, plus or minus one standard deviation.

Table 2: Intakes of strontium, barium and lanthanide elements

Element	Intravenous Intake	Oral intake
Strontium	100 µg ^{84}Sr	2 mg ^{86}Sr
Barium	20 µg ^{135}Ba	100 µg ^{134}Ba
Neodynium	1 µg ^{145}Nd	20 µg ^{143}Nd
Samarium		10 µg ^{147}Sm
Europium		10 µg ^{151}Eu
Gadolinium		10 µg ^{157}Gd
Dysprosium	1 µg ^{160}Dy	10 µg ^{163}Dy

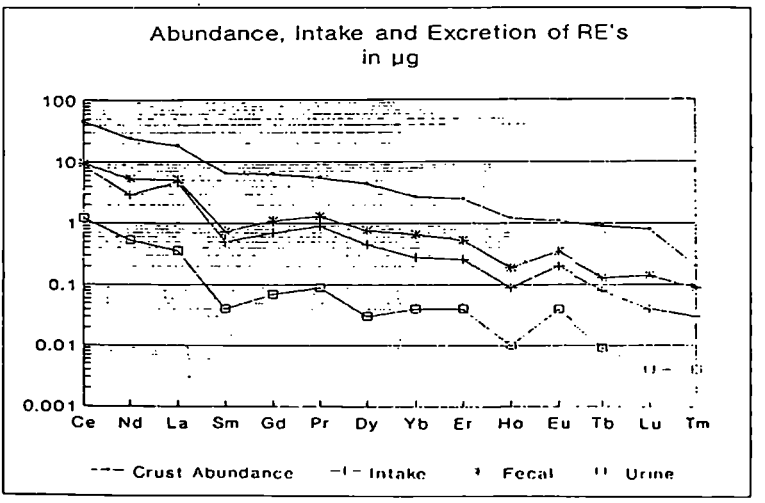


Fig 1: Abundance in g/tonne, and human intake and excretion levels in mg/day absolute, measured by inductively coupled plasma mass spectrometry, following chemical separation and preconcentration

Head of Project 4: Prof. Taylor

II Objectives for the reporting period

To assess the validity of the use of europium and gadolinium as surrogates for americium and curium by comparison of their binding to plasma proteins *in* vivo and in vitro.

III Objectives for next period

To complete the studies of the binding to the iron-transport protein transferrin and to measure the tissue distribution of europium and gadolinium in rats following the injection into rats and to compare this with previously published data for americium and curium.

IV Progress achieved including publications

Chromatographic studies using gel chromatography on Sephacryl, DEAE-Sepharose and Blue Sepharose established that both europium-152 and gadolinium-153 were bound to serum transferrin following labelling of serum either *in vivo*, by intravenous injection of the radionuclides as citrate complexes into rats, or *in vitro*, by the addition of the radionuclides, as the nitrilotriactetic acid complexes, to rat serum in vitro. Separation of the protein on the ion-exchange medium DEAE-Sepharose suggested that the Eu- and Gd-transferrin complexes tended to dissociate on the gel, however affinity chromatography on Blue Sepharose CL-6B established that about 20% of the total serum radioactivity was associated with transferrin.

Spectroscopic studies using UV-difference spectroscopy indicated that for both metals a maximum of two atoms of metal could be bound to each transferrin molecule. In addition saturation of Eu-152 and Gd-153 labelled transferrin with inactive iron resulted in the release of the bound lanthanide. Both these results suggest that the iron binding sites on the transferrin molecule are probably involved in the binding of these lanthanide metals. Preliminary studies using spectroscopy combined with computer speciation modelling suggest that the conditional stability constants for the formation of the europium and gadolinium complexes are low, probably ten or more orders of magnitude less than those for the Fe- or Pu-transferrin complexes.

Publications

Taylor, D.M., Duffield, J.R., Williams, D.R., Yule, L., Gaskin, P.W. and Unalkat, P.: Binding of f-elements to the iron-transport protein transferrin.
European Journal of Solid State and Inorganic Chemistry. 28 (Suppl.) 271-274, 1991.

Head of Project 5: Dr. Henrichs

II Objectives for the reporting period

- Review of the available data base for the elements strontium, cerium, barium, ruthenium, tellurium, zirconium, plutonium and higher actinides, relevant for the project.

- Preparatory investigations for the calculation of age dependent dose coefficients on the basis of the newly measured data.

III Objectives for next period

- Evaluation of the measured data aiming to improve the biokinetic data base (models) suitable for dosimetric purposes.

- Calculate dose conversion factors for the radionuclides of interest, taking into account dependencies on age and on chemical characteristics as well as influences of the nutritional composition as far as investigated experimentally.

IV Progress achieved including publications

By means of a literature review the scientific basis for the biokinetic models and data of all radionuclides tabled in ICRP Publication 30 was checked. Based on the results of this review and on the results of the German Reactor Safety Study (Phase B), those elements were identified which are of importance for the protection of the general public (in case of an accident in nuclear facilities) but for which relevant dosimetric data and their variabilities are not sufficiently well known. These elements were strontium, cerium, barium, ruthenium, tellurium, zirconium, plutonium and higher actinides:

- Strontium: Although many data have been published on the biokinetics of Sr in man, the importance of Sr isotopes in the context of possible reactor accidents makes it necessary to quantify the variability of biokinetic parameters with regard to the dosimetry with special emphasis on children;

- Zirconium and cerium: One of the most important parameters, the fractional uptake (f_1) of ingested activity from the gastro-intestinal tract to the blood, is very uncertain for those elements;

- Ruthenium and tellurium: The biokinetic data available are only taken from a few animal experiments, and excretion patterns in humans are completely unknown. For the interpretation of monitoring data reliable biokinetic information is urgently needed;

- Transuranium elements: Their high dose factors, their importance in nuclear industry, and the public concern of these elements urge the need to improve the

comparatively poor data base for the assessment of monitoring data as well as for the calculation of dose coefficients for various ages. Especially, the influence of uptakes with food on the fractional absorption into blood are nearly unknown.

Based on these results, the most important parameters to be investigated within this project were identified as the f_1-factors for the nuclides mentioned above as functions of age taking into account the influences of food constituents.

In cooperation with another CEC funded project (coordinated by the University of Lund) and as a preparation for the calculation of dose coefficients for children, first calculations of specific effective energies for photons (from 0.02 to 3 MeV) were performed for the phantom of a 7 week old baby. This phantom was produced as a voxel phantom based on numerous CT scans of a real baby. The first results are presently analyzed. If there are significant differences in comparison to the less sophisticated phantoms currently in use, it is planned to perform the same calculations for a child of 7 years and for an adult.

Progress Report

Contract: B16-0052-B Sector: A21
Title: Intercomparison and harmonization of methodologies, identification of future objectives in Radioecology, training and exchange of scientists

Head of Project: Dr. A. Aarkrog
President of IUR

Scientific Staff:
Wourking Group Leaders: H. Dahlgaard, L. Foulquier,
M. Frissel, G. Linsley,
C. Myttenaere, J. Van den Hoek.

I. Summary of Project and Global Objectives

1) Cooperation and the exchange of information between radioecologists in particular Soviet Scientists and those from countries outside EC and from countries which are not associated with the Radiation Protection Research Programme, in order to stimulate interactions that would increase our understanding of Radioecology problems;

2) Training of young scientists will be emphasized in the IUR new programme, in particular a summer school will be organized in 1990 (Mol);

3) IUR will develop a curriculum for a basic course in radioecology;

4) Furthermore IUR will play various roles in the field of informing the Public, i.e., "Information packages" and individual experience of IUR members previously involved in this difficult area.

Head of Project 1: Dr. A. Aarkrog

II. Objectives of the reporting period:

The main goals for the IUR Working Groups in the framework of the present proposal are:

1) to identify and review problems in the field of radioecology;

2) to provide input to training of young scientists;

3) to assist in the development of a curriculum for a basic course in radioecology.

III. Progress achieved (Summary):

1) Promotion of the formation of young scientists

The first Summer School on Radioecology was organized in Mol from the 8th to the 20th of July 1990 by the International Union of Radioecologists.

The realisation of such a project was made possible thanks to the financial support of the European Communities (DG XI and DG XII) and of the Belgian Nuclear Research Centre Mol.

The course was designed to fulfil the need of qualified scientists in the environmental radioactive protection areas of research centers, universities and industry.

Twenty nine people having a different classes of age have participated assiduously to the lecture given by 23 different eminent lecturers.

2) Development of Cooperation with the Soviet Scientists

The creation of a Soviet Branch of IUR increased tremendoesly the contacts and was one of the main reasons of the success of the Seminar on "Comparative Assessment of a Environmental Impact of Radionuclides released during the major accidents: Kyshtym, Windscale and Chernobyl" held in Luxembourg 1-5 October 1990 and organized by CCE (DG XI, DG XII) and IUR with the cooperation of SCOPE-RADPATH.

The Seminar was attented by about 200 participants of whom 46 were Soviet scientists.

At the Soviet invitation, 5 IUR representatives (A. Aarkrog, C. Myttenaere, M. Frissel, L. Foulquier and H. Dahlgaard) spent 10 days (7-17May 1990) in USSR.

In the framework of the "Mutual Agreement" 9 Soviet Scientists visited various radioecological laboratories (Belgium, France, United Kingdom, Denmark) in October 1990.

3) Contribution of IUR to International Programmes

-SCOPE-RADPATH:

the first case study Meeting, which comprised a technical workshop (focussing particularly on Sellafield discharges and Soviet radionuclide releases) was held from 26-30 March 1990 at University of Lancaster, U.K. In total 43 participants from some twelve nations, were present at the meeting. IUR supported the participation of 10 IUR members as IUR officially cooperates to the SCOPE-RADPATH programme.

- BIOMOVS:

The IUR "Environmental Assessment Modelling Working Group" has provided partial support for the attendance of several IUR Members to the BIOMOVS final meeting in Stockholm (8-12 October 1990).

- VAMP (IAEA):

The IUR supported the attendance of members to the VAMP meeting in Vienna as IUR is formally involved in the IAEA/CEC Environmental Model Validation Study.

- In April 1990, the WG Leader of the Environmental Assessment Modelling attended an International Seminar on the new recommendations of the International Commission on Radioecolocal Protection (ICRP) organized by CEC in Luxembourg and offered comments on the recommendations from the standpoint of environmental protection (see Information Bulletin N°12).

- IAEA - IUR: CRP set up in order to produce a Handbook of Transfer Parameter Values to Tropical and Sub-Tropical Environments. M. Frissel, IUR Representative, attended a two days meeting organized in Vienna, May 1990.

4) Working Groups Activities (in brief).

In the new CEC Radiation Protection programme, the cooperation between the European Countries is very important. But it is also important to assure contacts and exchange of information between those groups of European Laboratories and other European and especially non-European Scientists in the same fields.
The WG under IUR can act as an international forum for such contacts.

a) The WG "Radioecology of Continental Waters" (Leader: L.Foulquier) has continued the inventary and the updating of information in this field. A Report "Synthèse des travaux relatifs à l'impact de Tchernobyl sur écosystèmes aquatiques" has been issued and distributed during the CEC-IUR Luxembourg Seminar (Contract with CEC/DGXI).

b) WG "Marine Radioecology" (MARECO) Leader H.Dahlgaard, has represented MARECO at the second research coordination meeting of the CRP on "Sources of Radioactivity in the Marine Environment and this relative Contributions to Overall Dose Assessment from Marine Radioactivity" organized under IAEA in Riso 28 May-1 June 1990.

c) WG "Soil-to-Plant Transfer" (Leader : M.Frissel)
The WG organized a Workshop in Uppsala (27-29 September 1990) on "The contamination of crops because of Soil Adhesion". About 20 contribution were presented.
The WG's Data Bank received somewhat less data than last year, a relatively large fraction of them contained in so-called "Post-Chernobyl data on the uptake of Cs and Sr". Recently also TF values from the Chernobyl area were received from IUR Soviet members.

d) WG "Plant-to-Animal Transfer" (Leader: J.Van den Hoek)
The 3rd meeting of the WG took place at the Institut für Strahlenhygiene, Bundesamt für Strahlenschutz (Neuherberg, FRG) from 24-26 April 1990.
Twenty-two scientists from 15 Research Institutes, a representative of CEC (DGXII) and from IAEA, participated to this meeting. Furthermore 6 young scientists from four countries were invited to participate and to make a contribution.
The summaries of the contribution as well as the discussions and the areas of future research will appear in the Report of this WG meeting.
It must be also mentioned that there WG members have contributed to the Summer School on Radioecology (Mol, July 1990).

e) WG "IUR Environmental Modelling" (Leader: G.Linsley)
Activities 1989-90.
One of the main objectives of this Working Group has been to contribute to the validation of environmental assessment models. To this end it was proposed that IUR be one of the co-sponsoring organizations for the international BIOMOVS study. This was accepted and IUR has had this role since 1986. Similarly, the IUR is formally involved in the IAEA/CEC Environmental Model Validation Study (VAMP) and sends delegates to the Research Coordination Meetings of that study. The IUR has been represented at all meetings of the two international model validation studies.

The Environmental Assessment Modelling Working Group has provided partial support for the attendance of several IUR members to meetings of both BIOMOVS and VAMP. In the period 1989-90 the IUR supported the attendance of members to the VAMP meeting in Vienna (December 1989) and to the BIOMOVS final meeting in Stockholm (1990).

In 1989/90 there were no formal meetings of the Working Group, but many of the members are in regular contact through their involvement in the BIOMOVS and VAMP studies.

In April 1990, the leader of the Environmental Assessment Modelling attended an international seminar of the new recommendations of the International Commission on Radiological Protection (ICRP) organized by CEC in Luxembourg and offered comments on the recommendations from the standpoint of environmental protection.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Delta Institute for
Hydrobiological Research
Vierstraat 28
NL-4401 EA Yerseke

Contract no.: BI6-B-191-NL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. C. Heip
Delta Institute
Vierstraat 28
NL-4401 EA Yerseke

Telephone number: 1131/1920

Title of the research contract:

Transfer processes and modelling of plutonium species and gamma emitters in the Scheldt estuary ; redox and organic speciation in relation to aqueous and particulate fractionation.

List of projects:

1. Transfer processes and modelling of plutonium species and gamma emitters in the Scheldt estuary ; redox and organic speciation in relation to aqueous and particulate fractionation.

Title of the project no.: B16-B-191 NL

Transfer processes and modelling of plutonium species and gamma emitters in the Scheldt estuary; redox and organic speciation in relation to aqueous and particulate fractionation

Head(s) of project:
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I. Objectives of the project:

II. Objectives for the reporting period:

III. Progress achieved:

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

V. Publications:

Transfer Processes and Modelling of Plutonium Species and Gamma Emmitters in the Scheldt Estuary.

Participants in the programme:

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I. INTRODUCTION

In a previous study (Duursma et al., 1985), it has been shown that radioactive contamination at low levels, in the Dutch Delta of the main European rivers Rhine, Meuse and Scheldt, may be due to two or three specific sources additional to fallout of radionuclides. The case of the Scheldt is particularly interesting: besides the systematic seaward increase of plutonium concentrations in the sediments indicating a marine source of this element. ^{238}Pu distribution in the Western Scheldt estuary is altered by additional inputs in the estuary itself. On the other hand, the study of the distribution of Co-60 allowed the identification of the area where the highest contamination by nuclear power plants effluents occurs. This area does not correspond with the peak of excess Pu-238 indicating that this Pu isotope originates partly or completely from another source located in the watershed of the Scheldt.

A complementary study of the radionuclides in the Scheldt was undertaken in order to have a better understanding of the sources and the behaviour of the radioactive material in this estuary. The Western Scheldt is a rather ideal system which has already been intensively studied for stable elements. It is characterized by a long residence time of water and particles in the brackish zone where almost permanent anoxic conditions prevail. It is thus possible to investigate in this system the influence of the redox conditions on the behaviour of the radionuclides and especially on Pu. Furthermore, the region of Antwerp receives an important load of organic matter which allows an in-situ study of complexation of radionuclides in water and on particulate matter. Finally, it appeared that the artificial radionuclides are valuable tools for studying estuarine hydrodynamic processes.

II. SAMPLING

Deposited sediments, water and suspended matter (SM) were collected on board R/V LUCTOR from D.I.H.O. in the estuary and its major tributaries from 1979-1984 (before the Chernobyl accident) and from 1986-1988 (Sampling began about 2 months after the Chernobyl accident.). On the whole, about 75 samples were collected. Sampling locations are identified by the distance (km) from the mouth of the estuary.

Particle size of suspended matter was determined with pipet analysis and Coulter Counter and related to the total concentration, salinity and biological activity as determined by ETS-measurements. The organic content of the suspended matter was determined by ashing at 500 °C for eight hours. The base exchange capacity in bottom sediments was determined for comparison by exchanging Ca-acetate and NaCl and determining the Ca in the percolate. A series of measurements were made of in-situ particle size with in in-situ suspension camera, which was used in April 1989 at a series of stations from Temse to Vlissingen in a salinity range from fresh water (0.08-0.19 o/oo S) to almost seawater (27 o/oo S). The photonegatives were analysed with a recently completed automated image analysis system.

Large volumes of surface waters (200 l to 500 l) were sampled by pumping and particulate matters were recovered by continuous centrifugation.
Water samples were then filtered by pressure filtration on membrane filters of

0.45 um and immediately spiked with Pu-242, after acidification to pH 1.5 with HCl.

Plutonium isotopes were coprecipitated with $Fe(OH)_3$ using NH_4OH at pH 9. Hydroxides were recovered by filtration and oven-dried at 105°C. Suspended matter and hydroxides were later dissolved with HCl and with a mixture of HF + HCl + $HClO_4$ respectively, for plutonium analysis according to classical methods (Talvitie 1971, 1972; Wong, 1971; Ballestra et al., 1979). Briefly, the solution was radiochemically purified using the anionic resin Bio-Rad AG1X8 of 50 - 100 mesh and the plutonium was finally electroplated and counted by alpha-spectrometry with 300 mm^2 Si-Au surface barrier. Gamma emitters were measured using a high purity Ge Gamma-ray detector (32% relative efficiency and 1.80 KeV resolution at 1.33 MeV) placed into a 1 m^3 low-level lead shield of 15 cm thickness. Counting times ranged from 1 to 7 days per sample. Measured emitters include Cs-137, Ru-106, Sb-125, Co-60, small amounts of Cs-134 and traces of Mn-54, Co-58, Zr-95, Nb-95 and Ce-144. Only the Cs isotopes, Ru-106 and Sb-125 will be discussed in this report.

Aluminum content (2-6%) was analyzed in all S.M. and sediment samples and was used as an index of the abundance of clay minerals to normalize the concentrations (expressed in pCi/g Al) in order to distinguish activity variations controlled by grain size fluctuations from those due to other processes.

The major physicochemical parameters have been measured in each water sampling station (salinity, T, O_2, pH, DOC, POC, alkanility, Eh, dissolved iron and manganese, and suspended matter. Dissolved Co was analyzed by differential pulse cathodic stripping voltametry of its dimethyl glyoxime complex (Zhang et al., 1988).

III. SUSPENDED MATERIAL IN THE SCHELDT ESTUARY

Considerable variation in size of the suspended particles was found but no difference between freshwater and saline-water suspensions could be demonstrated. Maximum in-situ size of particles varied between 700 and 900 µm, median values were between 100 and 300 µm. Near the bottom particles tend to be somewhat larger. Particle size does not increase at the contact between fresh and saline water, salt flocculation does not appear to be an important process.

Coulter counter size measurements at stations with salinities higher than 4 o/oo S indicated large peaks of particles around 2 µm and around 10 µm. This clearly demostrates the destruction of the in-situ flocs by sampling and analysis; the in situ camera gives a more reliable estimate of particle size and surface area of the particulate matter in nature. Particle shape can also be analysed so that deviations from a spherical shape can be estimated, allowing a more precise estimate of surface area.

Variability in particle size seems to be regulated by local conditions of turbulence and particle settling. There is no relation with the organic matter content or the bulk composition. Changes in organic matter of the suspended material during transport through the estuary probably are due to a number of processes that may change the surface characteristics of the particles and thus alter their geochemical behaviour.

Mobilization of carbohydrates at low salinity, compositional changes from compounds of terrestrial origin to compounds of marine origin and changes in the isotopic composition may result in more porous, more surface active particles in the estuary than in both the freshwater or the sea.

At low salinities the particle size as measured with the pipet and Coulter Counter is smaller than in freshwater. The smaller size coincides with the area where carbohydrates are mobilized form the suspended material. They are probably present as polysaccharides or fulvic acids. Both types of organic compounds are very common in estuaries and are able to glue suspended particles together.

A turbidity maximum was present between Bath and Dendermonde, i.e. at low salinities and partly in the fresh water. Concentrations in the turbidity maximum reached 800 $mg.l^{-1}$ near the bottom and 200 $mg.l^{-1}$ in the surface waters. In the freshwater part up to Gent concentrations were between 40 and 60 $mg.l^{-1}$ and in the estuary downstream Bath between 25 and 60 $mg.l^{-1}$.

The organic matter content varied between 7.9 to 52.2 %. There is a general tendency for the organic matter content to be higher in the freshwater suspensions, but exceptions are frequent particularly because of primary production in the surface water. Because the automation of the scanning microprobe is not yet finished, the SEM analyses could not yet be carried out. From these data the origin of the suspended matter can be estimated.

IV. ORIGIN AND LONGITUDINAL DISTRIBUTION OF ARTIFICIAL RADIONUCLIDES

The distribution of artificial radionuclides will be discussed as a function of their major mode of introduction in the Scheldt estuary.

IV.1. The "marine" source : Sb-125, Ru-106, Cs-137 and Pu-239+240

The longitudinal distributions of Sb-125, Ru-106 and Pu-234-240 (fig.1) show a systematic seaward increase from about km 60, reaching a factor of 3 - 10 on a normalized basis. Cs-137 also increases seaward and Cs-134 was detected in the lower estuary with a constant but low Cs-134/Cs-137 activity ratio (about 0.05). This element will be discussed in more detail under section III.3 devoted to the Chernobyl accident.
These distributions suggest that the major source of Ru-106 and Sb-125, Pu 239+240 and to some extend Cs-137 is to be found in the contaminated coastal waters of the North Sea. Fixation of dissolved radionuclides in the saline waters by terrigenous Scheldt particles, or introduction of contaminated "marine" particles into the estuarine system may be considered.

The enrichment of artifical radionuclides in the North Sea has been directly related to the effluents released by the reprocessing plants of la Hague and Sellafield as previously discussed by DUURSMA et al., 1985. It must be added that near the mouth, dissolved Pu-239+240 and particulate Pu-239+240/Al ratio decreased by

30-50% from 1986 to 1988. These variations may correspond to a recent evolution of the contamination of the southern North Sea. Indeed Sellafield discharges have strongly decreased since a few years, but waters off the Scheldt mouth are essentially under the influence of Channel waters inputs and no recent data on plutonium releases at La Hague are available since 1985.

These results show that marine trace elements originating from the North Sea are introduced into the estuarine system and transported landwards up to km 60-90.

IV.2. Internal Sources : Co-60 and Pu-238

The longitudinal distribution of Pu 238/Pu 239+240 activity ratio and of Co-60 are given in figure 2. Both clearly indicates a maximum near km 60 for Co-60 and near km 100 for Pu-238+239 corresponding to the Doel power plant and the Rupel confluence respectively. These two sources would release a few mCi/y of Pu-238 and several hundred mCi of Co-60.

A more detailed study of the distribution and behaviour of cobalt was performed during this study. Six longitudinal profiles of dissolved cobalt obtained under various flow conditions indicate that the concentration of the stable element in the fresh water part is strongly affected by the river discharge suggesting that there is a continuous source of this element more or less diluted by the river flow. At mean values of the river discharge, the longitudinal profile enhibits a maximum near km 60 (fig. 3) which may be due to a local input. Measurements of total Co in the suspended matter show that the distribution coefficient of this element between the dissolved and the particulate phase is remarkably constant over all the salinity range and is equal to 3×10^5. Only a small fraction of the dissolved Co was found to be complexed by organic matter. However, in most cases the relation between the concentration of dissolved Co and salinity exhibits a curvature suggesting that Co is partly scavenged in the area close to the input of this element (km 60). Besides the nuclear power plant, there are many industrial waste water discharges in this area but it is nevertheless striking to see that the maximum of stable Co in the water column corresponds also to the maximum of Co-60 in the suspended matter and sediments.

IV.3. The Chernobyl accident : Cs-134/Cs-137 activity ratios

A short time after the accident (June 1986), significant enrichments in Cs-134, Cs-137, Ru-106 and Sb-125 were found in fresh water particles collected in various Scheldt tributaries, as well as in the estuarine sediment traps. No obvious plutonium enrichment was found. A few months later, owing to mixing with the various sources mentioned above, Chernobyl impact was hardly detectable near the mouth, and became weak close to the terrigenous sources

We will discuss here only the distribution of Cs-134, Cs-137 and of the ratio of these two isotopes before and after Chernobyl. Figure 4 shows the longitudinal distribution of Cs in the suspended matter and in the sediments before Chernobyl and in the suspended matter only after Chernobyl. As expected, the signal is especially important for Cs-134. Our measurements in aerosols collected in Brussels during the maximum of Chernobyl deposition give a Cs-134/Cs-137 ratio close to 0.51, a value

identical to the ratio found in Paris (Thomas and Martin, 1986). This value will be used as a reference of the Chernobyl signature. Figure 5 shows the evolution with time of the Cs-134/Cs-137 ratio in the Scheldt river and estuary.
Scheldt river ratios at Gent exponentially decrease with time but with a shorter apparent half-life. In the S.M. collected near the mouth at Schaar, the ratios are always lower than in the river and decrease irregularly with time. Measuremement by other institutions (Guegueniat et al., 1988, Anonymous, 1987; Nies and Wedekind, 1987; Nies, 1990) in coastal and non-coastal unfiltrated waters of the southern North Sea (51-53°N) show that ratios are systematically higher near the coast than at a certain distance. This distribution suggests that the higher coastal ratios are certainly due to a higher content of terrigenous particles in the more turbid waters.

These results clearly indicate the predominant continental origin of Cs-134, and its mixture with a North Sea stock poorly affected by Chernobyl fallout (by the end of June 1986, and later). Moreover, the ratio decay, which differs from theoretical decay of Chernobyl deposition, may be interpreted in term of a progressive mixing of Chernobyl-contaminated soil particulates with an increasing proportion of the Cs-137 global fallout inventory.
It is possible to calculate with these data the fraction (F) of Chernobyl-derived Cs in a given S.M. sample. This fraction was 90% in June 1986 and was still 60% after 2.5 years.
Therefore, although an important proportion of Chernobyl deposition may have been early eroded before the beginning of our sampling in June 1986, total elimination of Chernobyl Cs isotopes from the Scheldt watershed will still take many years.

The results mentioned above allow to envisage the use of Chernobylderived Cs as a terrigenous tracer in the estuarine system. Relative variations (i.e. normalized with respect to the corresponding river value) of the fraction (F) of Chernobyl-derived Cs-137, calculated as described above, are shown in figure 6 for various sampling cruises.

This figure shows that penetration of the terrigenous Cs in the lower estuary (downstream km 60 approximately) is very limited during periods of low and intermediate river discharge (75-125 m^3/s), whereas the S.M. stock upstream km 80 seems rather homogeneous and essentially land-derived. This conclusion is consistent with the results obtained above using the penetraµtion of the "marine" radioactive tracers. It agrees also with the well known fact that most of the continental S.M. transported by the river is trapped in the area of Antwerp and does not reach the sea except during exceptional high floods.

During the March 1988 flood (average river flow - 339 m^3/s at Schelle), the contrast between the upper and lower estuary has disappeared and a more linear distribution is found (fig. 6). The low value at km 90 (Rupel confluence) is probably due to resuspension of deposited sediment less contaminated due to the high velocity of water during the flood event. Also, the high value near the mouth (F = 53%) may be interpreted as the result of a seaward flushing of the estuarine S.M. stock during the flood, showing that most of the estuarine stock of S.M. is land-derived under such hydrological conditions.

V. ESTUARINE BEHAVIOUR OF ARIFICIAL RADIONUCLIDES

V.1. Pu isotopes

Except during the December 1986 cruise, dissolved $^{239,240}Pu$ activities in samples collected in the Scheldt estuary (fig. 7) were very low (< 0.003 to 0.008 fCi/l), while the activities of most marine samples (collected near the mouth of the estuary) are at least 5 to 10 times greater and relatively constant (0.05 to 0.08 fCi/l).

The dissolved $^{239,240}Pu$ activities found in the Scheldt estuary are very similar to those found by Sholkovitz et al. (1987) in four northeastern United States estuaries. Pu activities in waters collected in the Scheldt without tidal influence (Gent) and in some of its tributaries (Rupel, Albert Kanaal) are also very low and similar to estuarine stations. Although the data collected during this study are scarce. It seems nevertheless that dissolved Pu might have been removed in the Scheldt estuary. The decrease could be attributed to enhanced scavenging due to the presence of high suspended particulate load in the estuary and to long flushing time (about 2 to 3 months) as suggested by Scholkovitz et al. (1987) for American estuaries.

Owing to the very low dissolved plutonium (0.001 - 0.0550 fCi/l) activities, the study of redox partitioning between Pu (III + IV) and Pu (V + VI) initially planned was not possible. It has been decided instead to investigate the variation of the distribution coefficient (K_d) in relation with dissolved oxygen and to carry out Pu-237 adsorption experiments. Results are discussed below.

Relation with dissolved oxygen

Whereas Pu-239+240 Kd (fig. 8) is rather constant ($6 \pm 2 \times 10^5$) in oxygenated waters (10 mg/l dissolved oxygen) collected near the mouth, it increases up to 5×10^6 in the estuarine oxygen-depleted waters (0.3 mg/l), with a few exceptions. These variations are due to a strong removal of dissolved Pu, attributed to a probable redox control. Pu coupling with Fe-Mn redox cycles, or redox transformation of Pu itself, has been found in reducing fjords where Pu depletion coincides with the O_2-H_2S boundary (Sanchez et al., 1986) and in Pu-enriched deep anoxic estuarine waters (Sholkovitz et al., 1987). These authors have also shown that such depletion processes may be counter-balanced by later Fe-Mn oxide dissolution, and sorption inhibition by complexation with carbonate ions and dissolved organic matter. It seems that in the Scheldt Pu insolubilization in anoxic waters is the dominant process.

V.2. Sorption of radionuclides on suspended matter on short time scales

To investigate the sorption of the two main species of plutonium (Pu (III-IV) and Pu (V-VI)) as well as other important trace metals in oxic and anoxic conditions in the Scheldt estuary, a water sample of low salinity collected at Hemiksem in May 1988, was separated into four subsamples maintained under constant air or nitrogen bubbling during one week. Gas bubbling induced an increase of pH due to the desgassing of carbon dioxide. In each sample, a mixture of five trace metal isotopes (^{60}Co, ^{54}Mn, ^{65}Zn, ^{109}Cd and ^{134}Cs) was added in chloride form as well as Pu(IV) or Pu(VI) in nitric form, freshly prepared according to Lovett and Nelson (1981).

Aliquots were filtered at selected time intervals. Filters and filtrates were gamma counted.

For cobalt and cesium, a pseudo-equilibrium was rapidly attained, within less than four hours under oxic conditions. The distribution coefficient of cesium then increased slowly but significantly until the end of the experiment. Anoxic distribution coefficients were higher for cesium (compared to oxic distribution coefficients) and lower for cobalt.

In the case of manganese, as expected, the distribution coefficients were much higher and continuously increasing during the first hours in the oxic subsamples, while they were lower and reaching a pseudo-equilibrium in the anoxic subsamples. This evolution is likely due to the catalytic oxidation of Mn(II) into MnO_2 under oxic conditions or to the sorption of Mn(II) ions, under anoxic conditions.

Cadmium, zinc and surprisingly plutonium showed parallel evolutions. They reach an ill-defined pseudo-equilibrium after a few hours no systematic difference could be found between oxic and anoxic subsamples. We failed to notice any difference between the sorption behaviour of Pu (IV) and Pu(VI) in either oxic or anoxic conditions.

Data concerning the distribution coefficients K_d are summarized in fig. 9, together with former data obtained in December 1986 in the Scheldt estuary. Only the fresh water end member is considered here and only the K_d's after 3-4 hours incubation time have been plotted, i.e. after the establishment of the pseudo-equilibrium. Bars give the ranges of values for each series of samples. In December 1986, no bubbling was set, and the experimental medium initially anoxic remained anoxic.

The main significant difference between both series of data concerns manganese which was much more sorbed in May 1988, in oxic or anoxic conditions. Indeed, the December 1986 survey was carried out during strongly anoxic conditions, with high concentrations of dissolved iron and manganese. We argue that such important amounts of dissolved metals may have prevented manganese sorption during incubation by some kind of saturation effect. It is also possible that a strong anoxia might have dissolved iron and manganese oxides which are known to strongly bind Mn(II) ions and catalyze their oxidation.

The same trend is observed for cobalt. It is possibly due to the lack of manganese oxides in December 1986. These oxides are known to have a very high affinity for cobalt ions (Murray, 1975). A more detailed experiment of transfer of Co-60 along the Scheldt estuary has been performed in August 1988. The rate of transfer of this radionuclide from the dissolved to the particulate phase has been investigated over the whole salinity range. Figure 10 shows the distribution coefficient K_d obtained after an incubation of 15 hours. There is a pronounced maximum of K_d within the salinity range between 15‰ and 25‰. This salinity range corresponds also to the restoring of dissolved oxygen and the subsequent precipitation of Mn. This experiment confirms the very strong influences of redox conditions on the behaviour of trace elements and radionuclides in natural environments and the scavenging role of manganese for other trace elements.

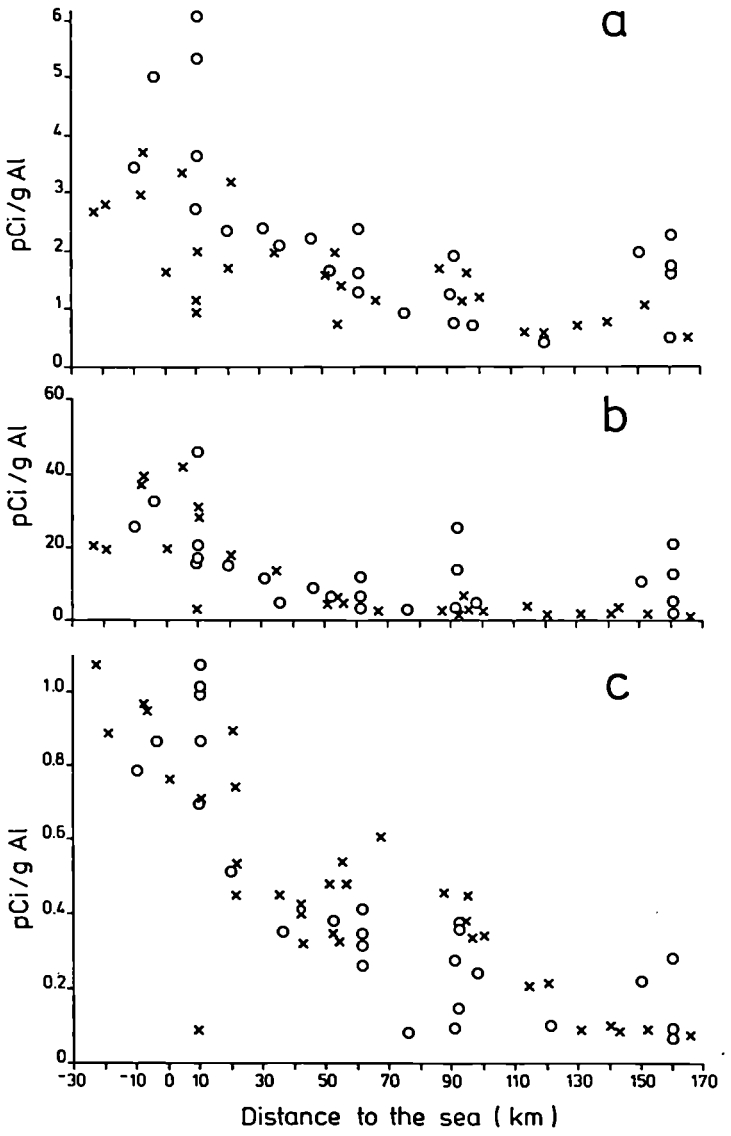

Fig.1 Longitudinal distribution of a) Sb-125, b) Ru-106, c) Pu 239+240 normalized with respect to aluminum in sediments and suspended matter of the Scheldt. X represents sediments and suspended matter collected before Chernobyl, and O denotes suspended matter collected after Chernobyl.

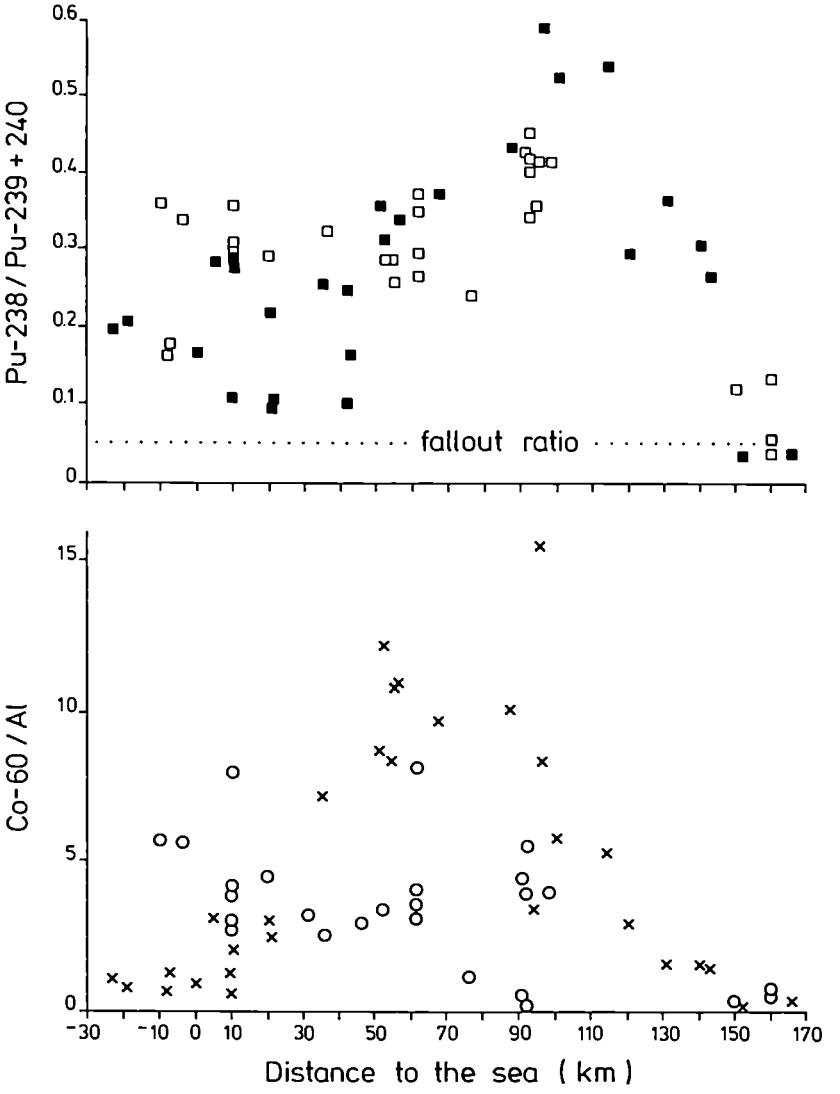

Fig.2 Longitudinal distribution of the Pu 238/Pu 239+240 ratio and of Co-60 normalized with respect to Al in the particulate matter and sediments of the Scheldt. ■ and □ for Pu refers to sediments and suspended matter respectively. Same symbols for Co as in figure 1.

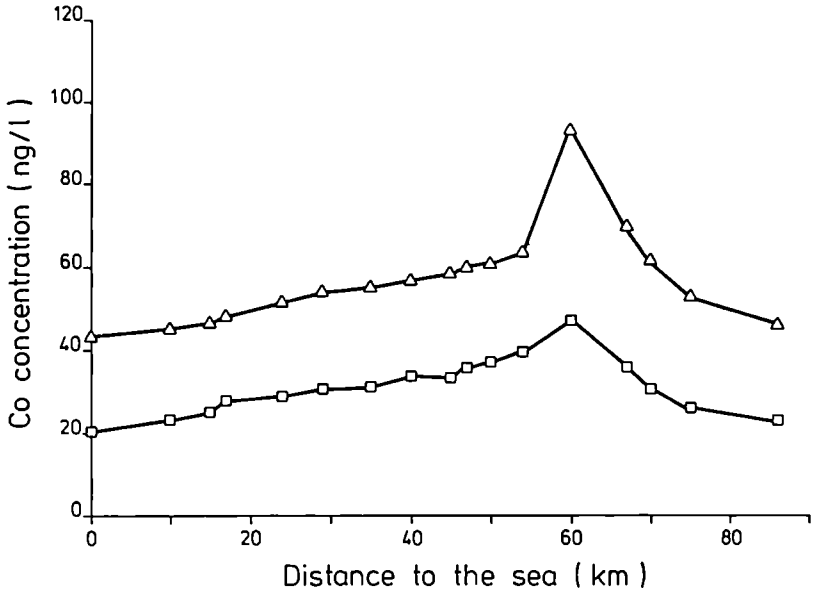

Fig.3 Longitudinal distribution of dissolved (□) and total (△) cobalt in the Scheldt for mean fresh water discharge (October 1988).

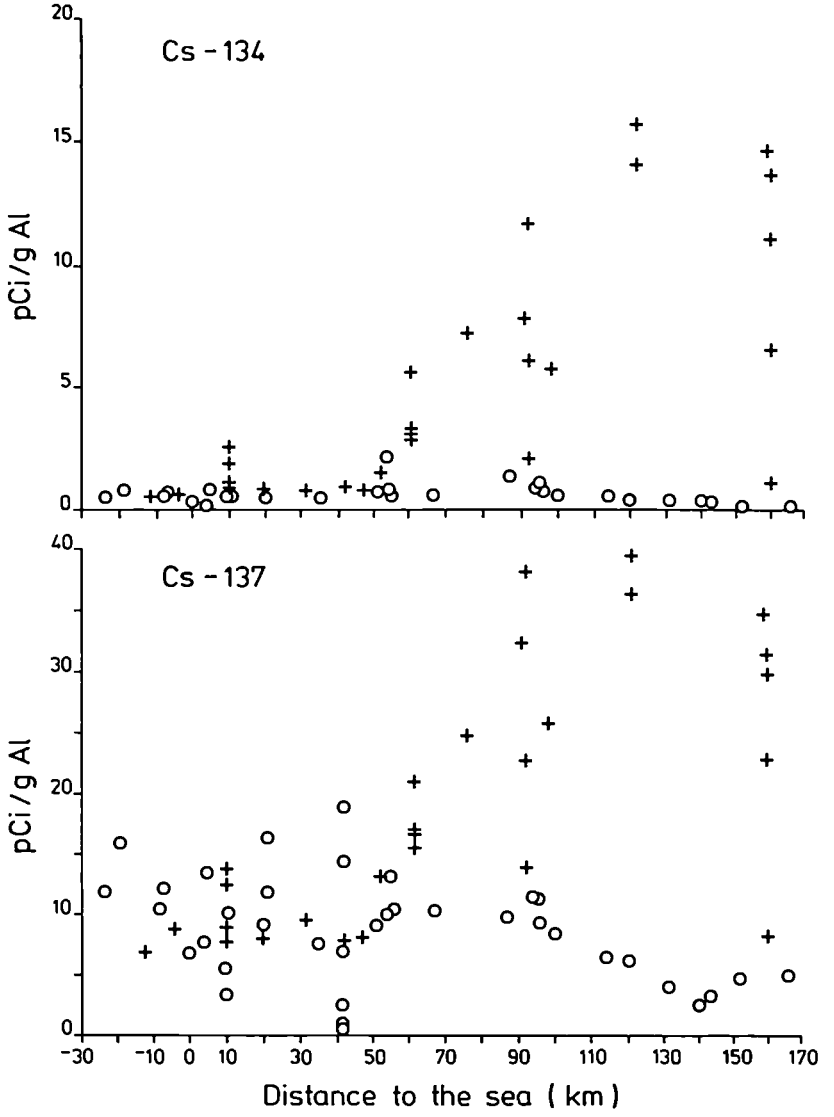

Fig.4 Longitudinal distribution of Cs-134 and Cs-137 normalized with respect to aluminum in the sediment and suspended matter of the Scheldt.

O represents sediments and suspended matter before Chernobyl and + denotes suspended matter after Chernobyl.

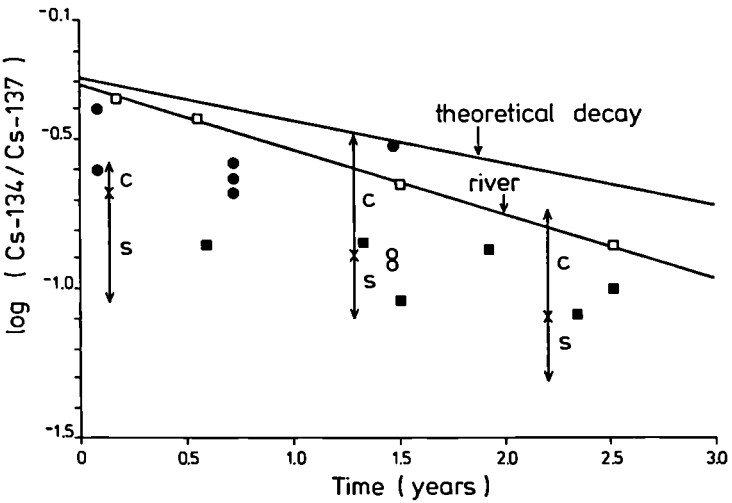

Fig.5 Cs-134/Cs-137 activity ratios versus time in the Scheldt river and estuary after the Chernobyl accident. Other measurements in unfiltered coastal and non-coastal Southern North Sea waters (51-53°N) are given for comparison

□ Scheldt river at Gent

■ Scheldt estuary near mouth

●,O Coastal and non-coastal sea water (Deutches Hydrographischer Institut, Hamburg).

C and S coastal and non-coastal sea-water (Laboratoire de Radio-ecologie Marine, La Hague)

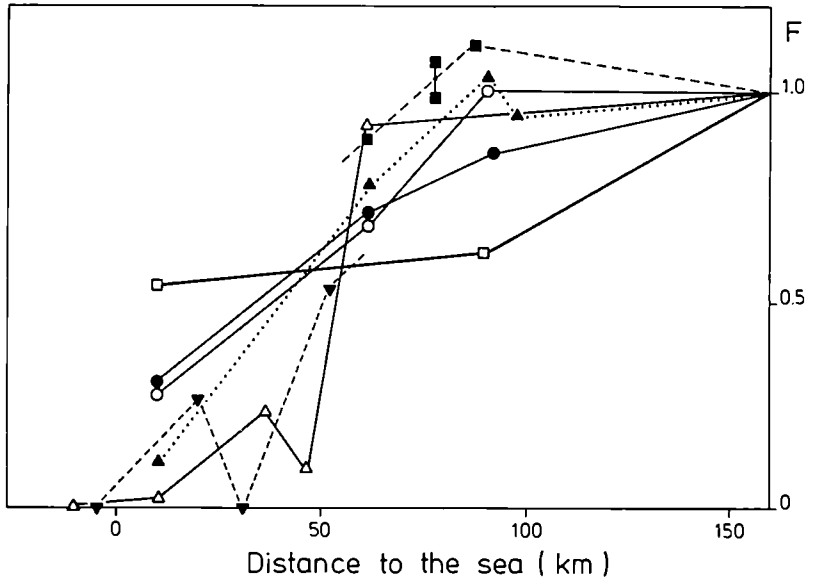

Fig.6 Fraction of the Chernobyl-derived Cs in the Western Scheldt suspended matter normalized with respect to the value observed at Gent.

■ July 1986, ● December 1986, ○ August 1987, ▲ October 1987, □ March 1988, △ August 1988, ▼ November 1988

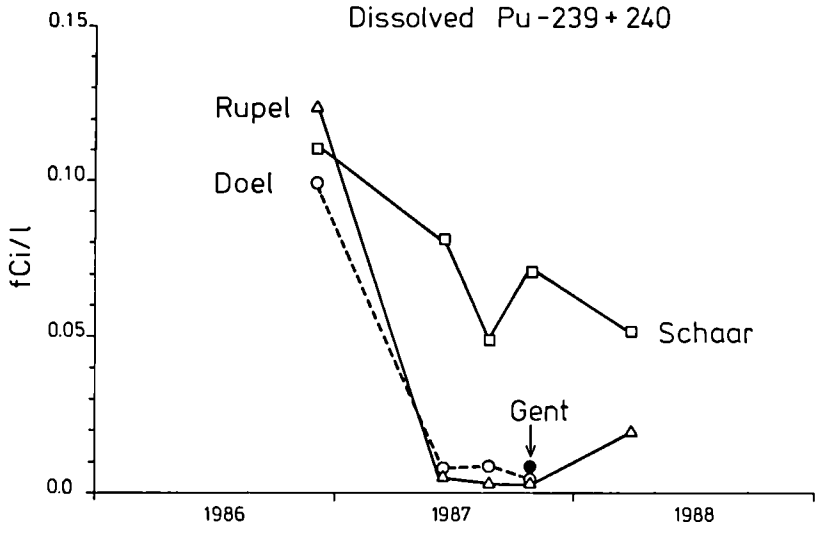

Fig.7 Activity of dissolved Pu-239+240 at Gent (km 160), Rupel (km 98), Doel (km 62) and Schaar (km 10) during the present study.

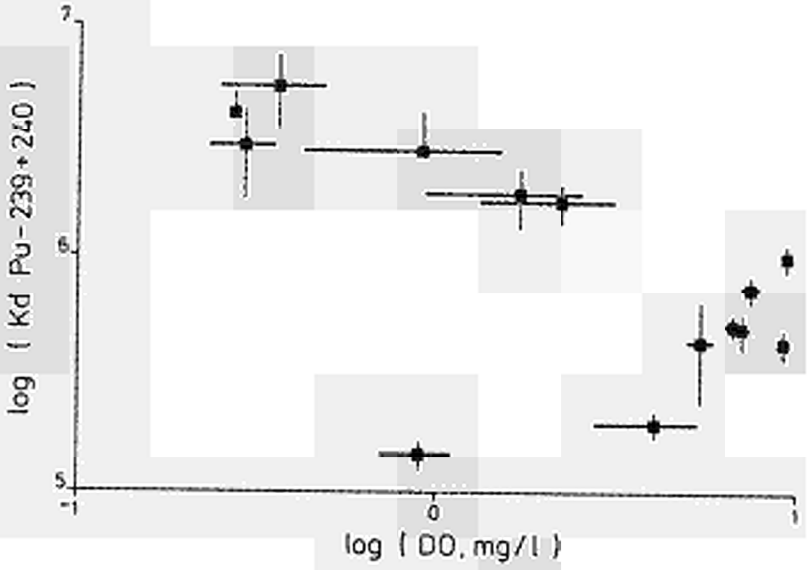

Fig.8 Distribution coefficient Kd of Pu-239+240 between the dissolved and particulate phases as a function of dissolved oxygen in the Scheldt estuary.

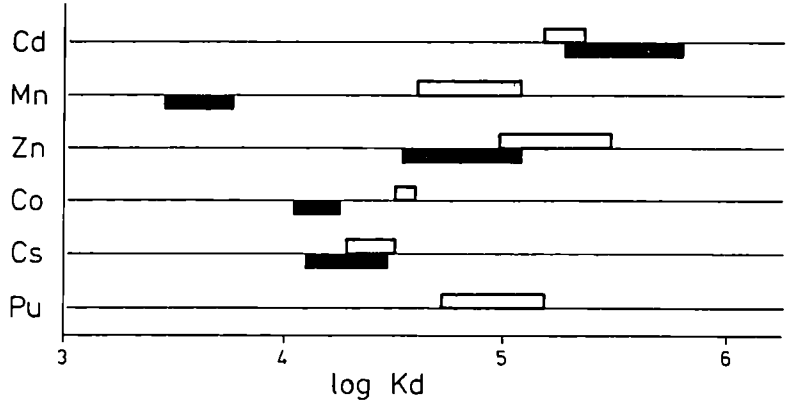

Fig.9 Fast distribution coefficients obtained during incubation of radionuclides after 4 hours with water samples of the Scheldt estuary in the fresh water part.

▬ December 1986, ▭ May 1988

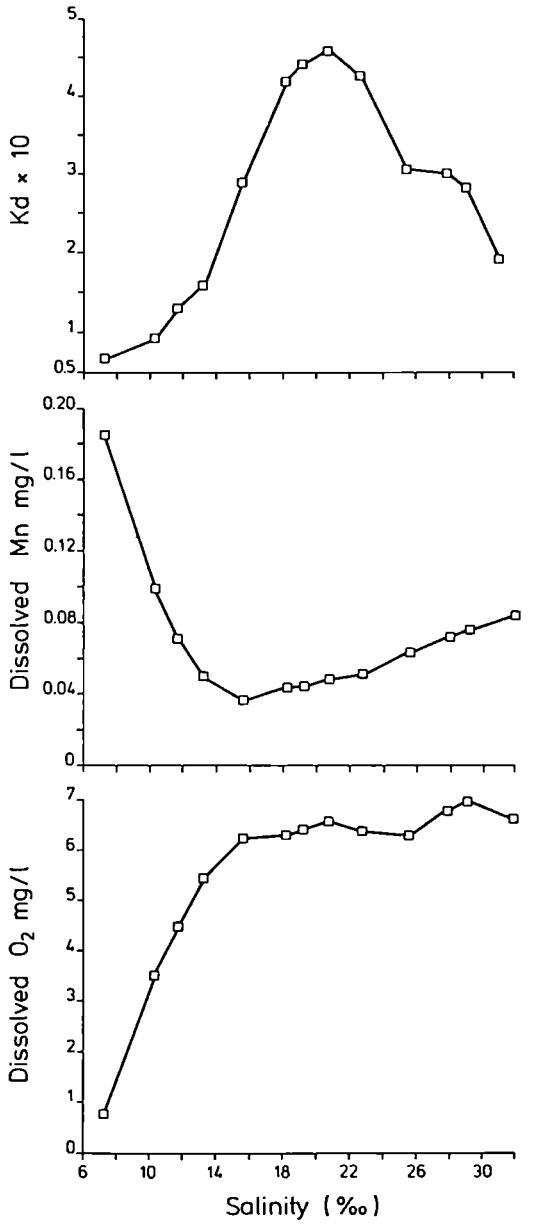

Fig. 10 Evolution of the distribution coefficient of Co-60 with salinity in the Scheldt estuary compared with dissolved oxygen and manganese, obtained in August 1988.

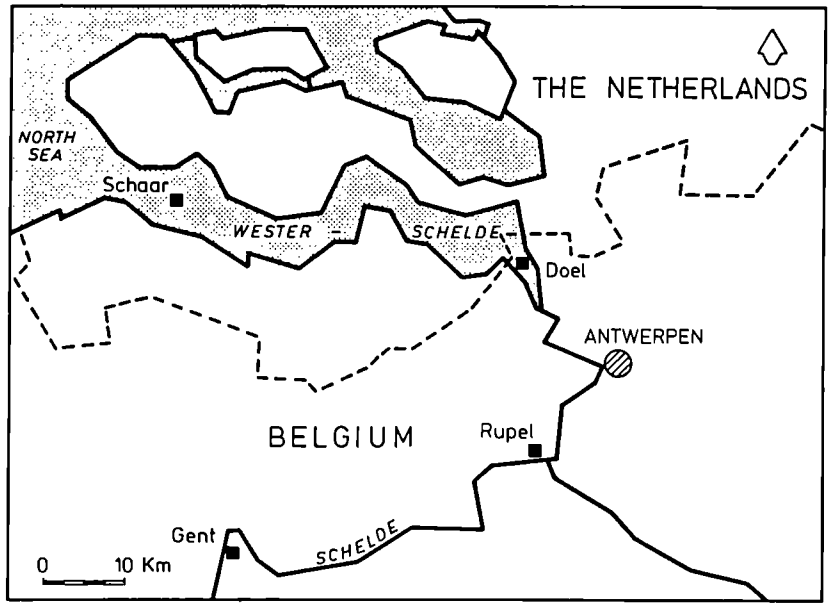

Fig.11 Map of the area

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Ministry of Agriculture,
Fisheries and Food
Directorate of Fisheries Research
Fisheries Laboratory
GB- Lowestoft, Suffolk NR33 OHT

Contract no.: BI6-B-200-UK

Head(s) of research team(s) [name(s) and address(es)]:

Mr. R.J. Pentreath
Fisheries Laboratory
Ministry of Agriculture,
Fisheries and Food
GB- Lowestoft, Suffolk NR33 OHT

Telephone number: (0502)562244

Title of the research contract:

Studies of the geochemical behaviour of artificial and natural radionuclides in coastal waters.

List of projects:

1. Studies of the geochemical behaviour of artificial and natural radionuclides in coastal waters.

Title of the project no.: Bi6 - B - 200 UK

1. Studies of the geochemical behaviour of artificial and natural radionuclides in coastal waters.

Head(s) of project:

Dr D S Woodhead

Scientific staff:

Dr P J Kershaw, Dr D J Swift, Dr S J Malcolm
Mr B R Harvey, Mr M B Lovett, Dr M McCartney, Mrs S J Boggis

I. Objectives of the project:

The main objective of the project was to obtain a detailed understanding of the interactions of a variety of radionuclides with suspended and settled sediments in coastal waters so that the long term behaviour and distribution of certain long-lived radionuclides could be predicted under varying environmental conditions. The detailed behaviour and distributions of natural and artificial radionuclides in marine sediments were determined and the chemical, biological and physical processes controlling the distributions have been investigated.

II. Objectives for the reporting period:

As above

III. Progress achieved:

Introduction

The coastal waters of the northeast Irish Sea have received a substantial input of long-lived artificial radionuclides from the authorised discharges of liquid effluents from the reprocessing plant operated by British Nuclear Fuels plc, at Sellafield, Cumbria. Discharges began in 1952. Relatively soluble radionuclides such as ^{137}Cs behave conservatively in seawater and can be traced readily in the waters of the NW European continental shelf. Many radionuclides are particle reactive, ie. they show a high affinity for particle surfaces. Much effort has been expended in examining the interactions of such nuclides with suspended particulate and seabed sediment, and the key physical, chemical and biological processes controlling their distribution and behaviour. This has allowed assessments to be made of the radiological consequences of past and predicted future discharges - in terms of dose to critical groups - following the development of an appropriate mathematical model (MIRMAID).

Distribution of artificial radionuclides in Irish Sea sediments

The regional distribution of a number of long-lived α-emitting (^{238}Pu, $^{239,240}Pu$, ^{241}Am, ^{244}Cm) and γ-emitting (^{134}Cs, ^{137}Cs, ^{106}Ru) radionuclides has been examined, both using fresh sediment samples and data collected prior to the reporting period. The distribution is dominated by the point-source Sellafield discharge, but is modified by the local hydrographic regime, water depth and sediment grain size (Woodhead, 1988) (Fig 1). A major survey of radionuclide distributions in subtidal sediments was conducted in 1988. This was followed up in 1989 by a survey of intertidal and nearshore sediments using divers. This will provide a much improved inventory of α- and γ-emitting radionuclides, once the data are fully worked up. Long time-series datasets of sediment radionuclide concentrations have been re-examined. Their use is limited somewhat because of changes in the sampling methodology, but examination of isotope ratios has confirmed the persistence of a preferential northerly transport of radionuclides following discharge, independent of grain size effects.

Sediment history

It has been confirmed (Pentreath, 1987; Swift & Kershaw, 1986) that a principal cause of radionuclide mobility in seabed sediments is the bioturbating activity of certain macrofauna. Their effects have been observed in the field and in laboratory tank experiments and attempts have been made to model the complex processes involved (Gurbutt & Kershaw, 1987), incorporating both 'biodiffusive' and 'conveyor-belt' mixing. Bioturbation can result in the penetration of recently contaminated surface sediment to depths of tens of centimetres. In contrast, ^{14}C dating of Turritella

communis shells (Fig 2) has been used to show an overall slow rate of sediment accumulation in this region (~0.1 mm y^{-1}, Kershaw et al, 1988(b)).

In an attempt to remove the effects of bioturbation from the sediment history of Sellafield discharges, cores were collected from a disused dock at Maryport, about 35 km north of the discharge point. The dredged dock had rapidly infilled, with relatively little bioturbation evident. A suite of α-, γ- and β-emitting, artificial and naturally-occurring radionuclides was determined, allowing a detailed chronology to be developed. This in turn allowed an estimate to be made of the total quantities of $^{239,240}Pu$ and ^{238}Pu discharged prior to 1978 (Fig 3), when only the total Pu alpha content was reported. The observed environmental inventory of $^{239,240}Pu$, reported by Pentreath et al (1986), was re-calculated and adjusted from 65% to 74% of the quantity discharged, (Kershaw et al, in press).

Seabed chemistry

Progress has been made in understanding the principal chemical processes within the upper layers of the seabed. Technical developments have demonstrated the need to exercise care in handling pore-waters, particularly to prevent oxidation, to obtain information approximating to the in-situ conditions (Malcolm et al, 1990(a)). Pu and Am do not appear to be closely linked to the cycling of Fe and Mn (Malcolm et al, 1990(b)) - counter to what had been previously suggested. The shape of pore-water profiles appears to be linked to the general bioturbation regime at each site. For example, appreciable quantities of the oxidised form of Pu are found only in areas with high densities of the burrowing ophiuroid Amphiura filiformis, resulting in enhanced bio-irrigation, but the precise causal relationships remain unclear (Kershaw, et al., 1986).

Experiments with a laboratory 'fluidised-bed' sediment column, in which the pore-water chemistry can be controlled, have indicated that Cs may be released under mildly reducing conditions but may become more firmly bound following the release of Fe and Mn (Malcolm, pers comm.).

Systematic variations in the apparent Kd of Pu and Am (Kd = radionuclide concentration on sediment, Bq kg^{-1}/radionuclide concentration in filtrate, Bq l^{-1}) suggest that the sediment/pore water system is not at equilibrium (Fig 4) (Malcolm et al., 1990(a).).

Naturally-occurring radionuclides

Extensive use has been made of the radionuclides of the ^{238}U decay-series to identify and quantity key biogeochemical processes. $^{210}Pb/^{226}Ra$ and $^{234}Th/^{238}U$ have been used to examine sediment mixing rates (Kershaw et al., 1988(c); Swift & Kershaw, 1986) and accumulation rates (Kershaw et al., in press). $^{234}Th/^{238}U$ disequilibrium measurements have provided a means of assessing the controls on scavenging by suspended particulate and seabed sediment, (Kershaw & Young, 1988), and of incorporating such processes in a compartment model of the Irish Sea (Fig 5) (Kershaw et al., 1988(a)).

An interesting development has been the identification of enhanced levels of ^{238}U decay-series radionuclides (but not ^{232}Th decay-series) in seawater, sediments and

biota in NE Irish Sea. The enhancement has been traced to an anthropogenic input from a phosphogypsum plant (McCartney et al., in press).

Water column

The controls on sorption and desorption of radionuclides on suspended particulate have been examined. The speciation of transuranium elements has been studied and the role of colloidal material assessed. The latter is beset with technical difficulties, often ignored in similar studies, but progress has been made using ultrafiltration techniques. It is clear that lower MW cut offs are required for marine waters compared with freshwaters. The size distributions of both artificial and naturally-occurring radionuclides have been determined.

A large scale survey of seawater concentrations in UK waters was conducted in 1985, confirming the continuing decrease in radionuclide concentrations in response to the decrease in Sellafield discharges.

Cohesive sediment dynamics

The fate of a large fraction of the seabed inventory of artificial radionuclides is linked to that of the sediments. Long-term current meter moorings have been combined with profiles of near-bed current velocities and suspended particulate to improve our understanding of the controls on sediment resuspension and transport.

Modelling

A box model (MIRMAID) of the Irish Sea has been developed to predict the distribution of artificial radionuclides released from Sellafield. The box structure is based on the observed distribution of radionuclides, sediment type and extent of bioturbation. Vertical and horizontal exchanges have been derived from field observations of residual flows, suspended loads, resuspension and bioturbation. Sediment processes have been incorporated and tested using $^{234}Th/^{238}U$ disequilibria data and the model development and predictions of radionuclide behaviour, and the resulting doses to the critical group via seafood consumption (Fig 6), have been described in a series of publications (Gurbutt et al., 1988; Kershaw et al, 1988(d); Pentreath et al, 1989(a); Pentreath et al.,1989(b); Gurbutt & Kershaw, 1989). A notable finding is that the model predicts a net flux of ^{137}Cs out of the seabed, in a nearshore zone along the Cumbrian coast, from 1983 onwards, a prediction which supports field data on seawater concentrations as a function of the unit discharged. (Hunt & Kershaw, 1990; Kershaw et al., 1990.)

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IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

V. Publications:

Figure Legends

Figure 1. Inventory of $^{239,240}Pu$ (MBq m^{-2}) in the seabed of the NE Irish Sea (from: Woodhead, 1988).

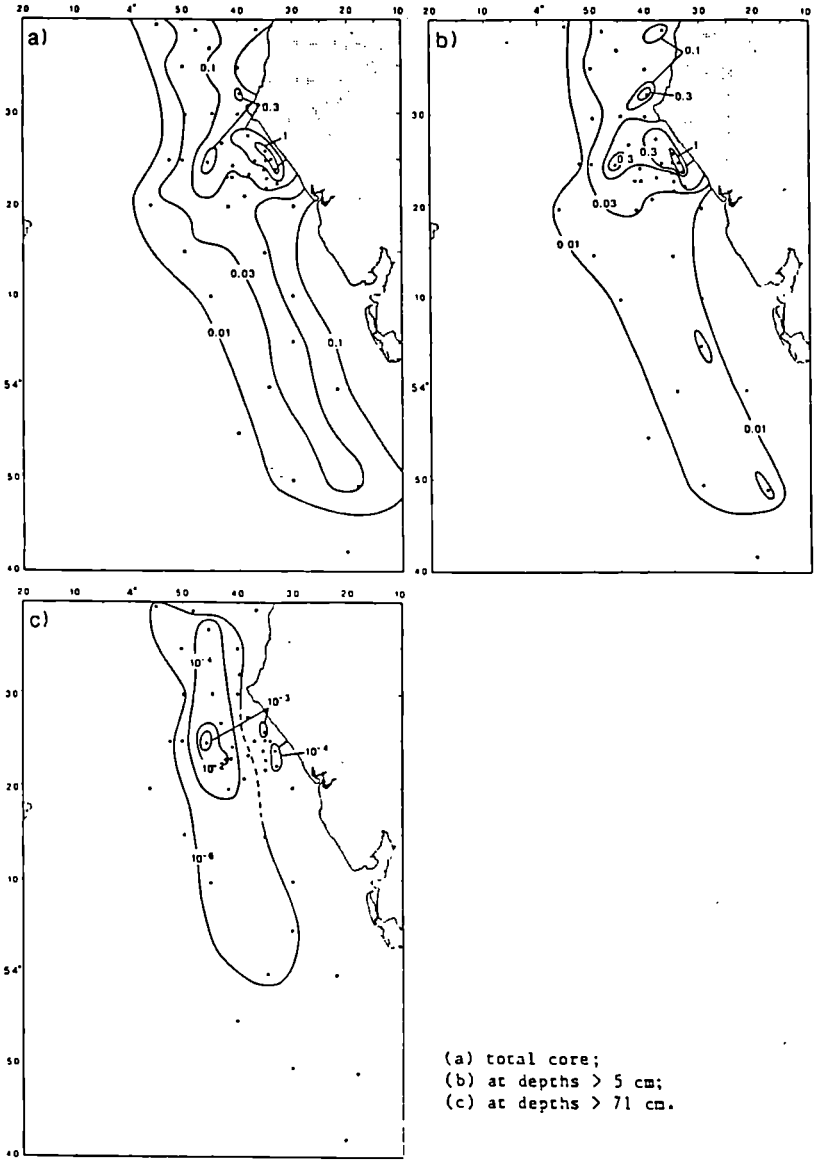
Figure 2. Reservoir-corrected ^{14}C ages of T. communis shells with corresponding conventional ^{14}C ages of the sediment, from the NE Irish Sea (from: Kershaw et al., 1988 (a)).

Figure 3. Actual and predicted discharges of ^{238}Pu (TBq y^{-1}) from BNFL Sellafield, 1959 to 1986, using a dated sediment core (from: Kershaw et al., in press).

Figure 4. Profiles of: a) reduced $^{239,240}Pu$ Kd, and b) reduced ^{241}Am Kd, in Irish Sea sediments from near the discharge point (from: Malcolm et al., 1990).

Figure 5. Comparison of field observations with MIRMAID model predictions of the inventory of excess ^{234}Th in the seabed (expressed as a percentage of the overlying water column production from the decay of ^{238}U; from: Kershaw et al., 1988(a)).

Figure 6. Calculated dose rates, using the MIRMAID model, received by a critical group from seafood consumption: a) for the period 1952 to 2050; b) compared with estimated dose rates from monitoring data, for the period 1980 to 1987 (from: Pentreath et al., 1989).



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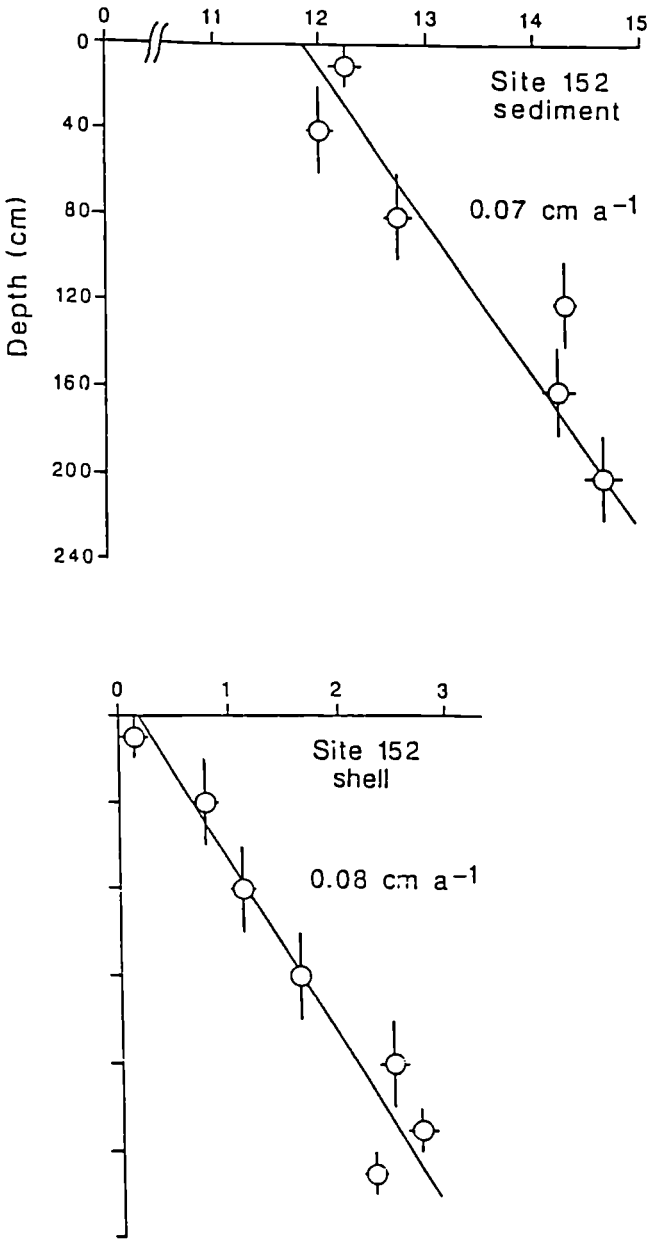

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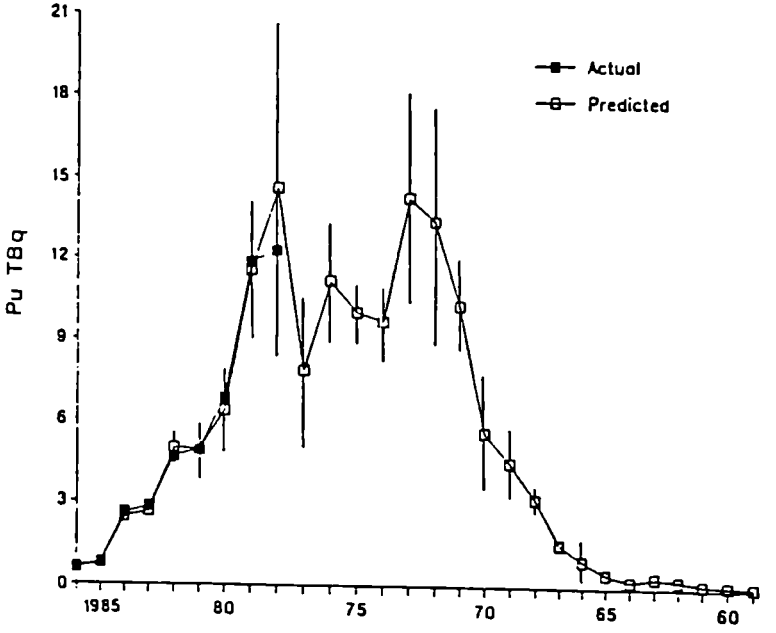

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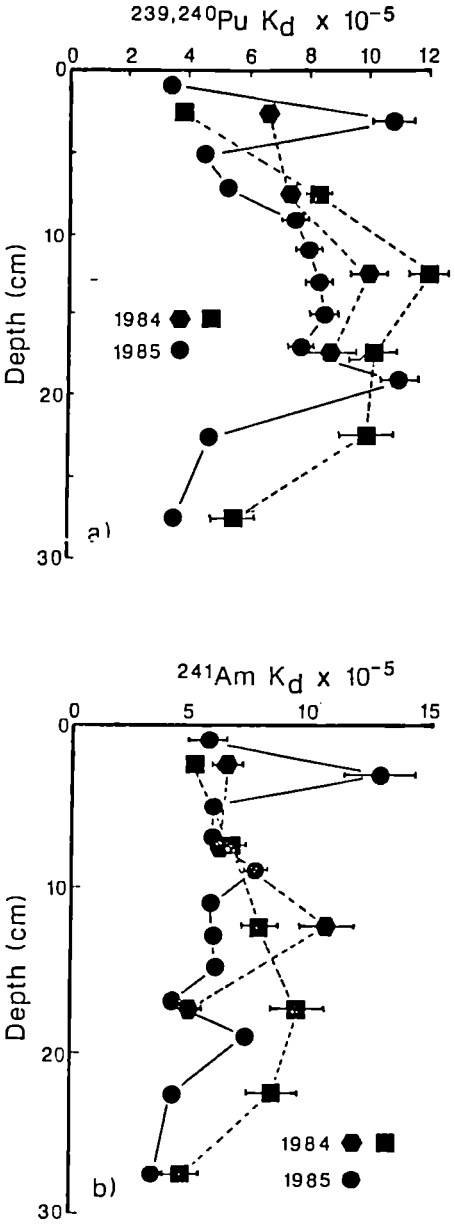

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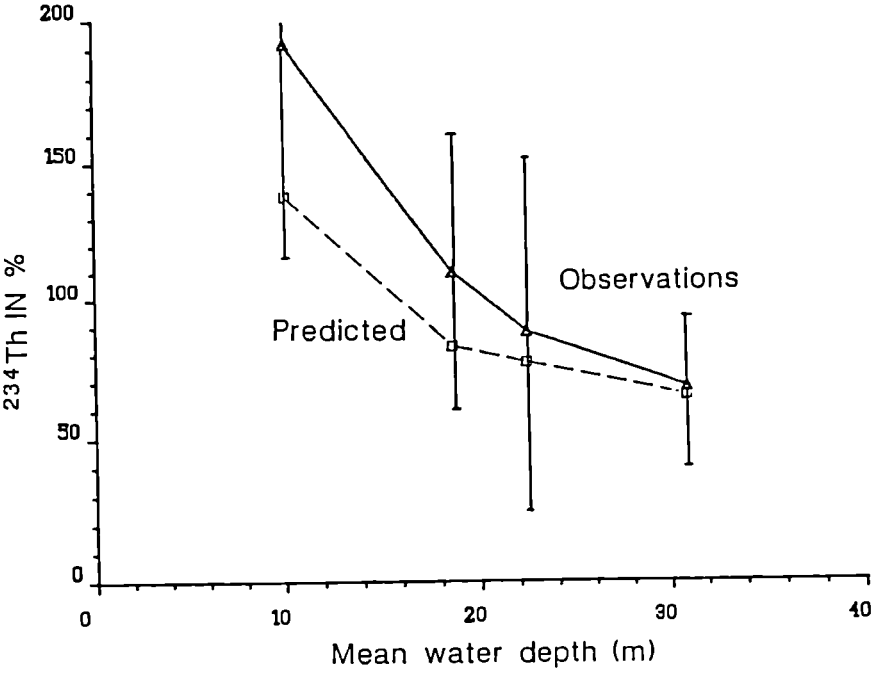

Figure 5. Comparison of field observations with MIRMAID model predictions of the inventory of excess ^{234}Th in the seabed (expressed as a percentage of the overlying water column production from the decay of ^{238}U; from: Kershaw et al., 1988(a)).

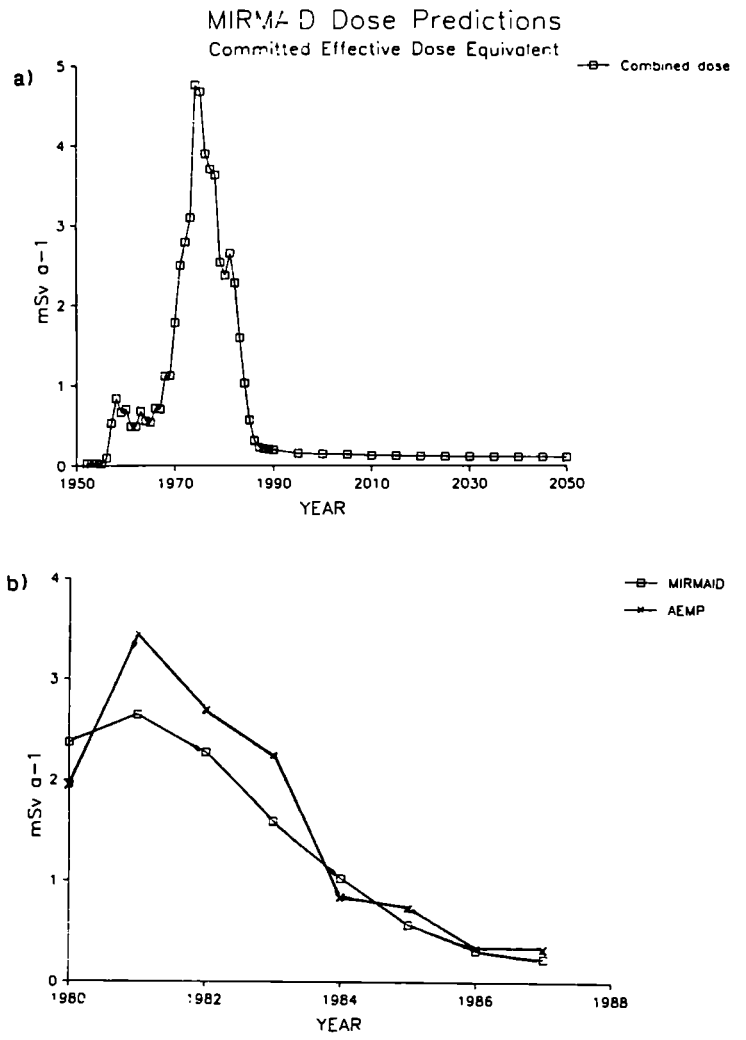

Figure 6. Calculated dose rates, using the MIRMAID model, received by a critical group from seafood consumption: a) for the period 1952 to 2050; b) compared with estimated dose rates from monitoring data, for the period 1980 to 1987 (from: Pentreath et al., 1989).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-B-234-F

Institut de Biogéochimie Marine
Ecole Normale Supérieure
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Title of the research contract:

Artificial radionuclides transfer from the Rhone Delta to the Mediterranean

List of projects:

1. Artificial radionuclides transfer from the Rhone delta to the Mediterranean

I - INTRODUCTION

The Rhône river suspended matter represents one of the major source of material to the Gulf of Lion however, the surface sediment plume is usually limited to the nearshore area, i.e. to less than 20 km from the river mouth. In most cases artificial radionuclides are strongly attached to S.M.. In order to specify the actual significance of the Rhone river to the central Mediterranean Sea, it is necessary to understand how the Rhône river SM with it associated radionuclides behaves during estuarine mixing.

To gain more insight into these processes, it has been decided to use as tracers the artificial radionuclides discharged to the Rhône river by various nuclear power plants and by the reprocessing plant at Marcoule located 120 km upstream from the river mouth. However, the inventories and concentrations of these radionuclides are still poorly known. Previous studies of particulate radioactivity in the lower Rhône river have considered only deposited sediments ; gamma-emitters and $^{239+240}Pu$ were measured up-stream and downstream of the Marcoule plant (Foulquier & Pally, 1984 ; Foulquier et al. 1987). Ranges of riverine concentrations of dissolved gamma-emitters downstream from Marcoule were also published by Foulquier et al. (1987) and by Lambrechts & Foulquier (1987). The first comprehensive assessment of artificial alpha and gamma radionuclides discharged by the nuclear industry in the Rhone river during the period 1982-1985 was published by Martin & Thomas, 1990. It allowed to establish an estimate of corresponding fluxes to the Mediterranean Sea, in both the particulate and dissolved forms. Radionuclide distributions in the mixing zone were difficult to interpret because of large variations of river reference concentrations, attributed to probable irregular nuclear effluent releases on a short time scale.

The aim of this project were :

1. to carry out a regular survey of the Rhone river discharge of artificial radionuclides.
2. to assess their distributions between the dissolved and particulate phases at the river/sea water interface.

II - SAMPLING AND ANALYSIS

From 1986 to 1989 large volume (200 litres) water samples were collected from Arles (located on the major branch of the delta, 49 km upstream the river mouth), with 30 litre Niskin bottles. Water samples were similarly collected in the surface plume along transects carried out, from the river mouth to 20 km southwards (Cl = 20 g/l). Suspended matter was immediately preconcentrated by continuous flow tangential filtration at 0.45 µm followed by continuous centrifugation at 32000 rpm. The suspended matter samples were freeze-dried for subsequent analysis.

Suspended sediments were ground and homogenized for gamma-counting with a high purity Ge detector (32 % efficiency and 1.80 keV resolution at 1.332 MeV) placed inside a 1 m^3 lead shield 15 cm thick. Efficiency calibrations were checked during IAEA intercalibration exercises and using international NBS standards.

III - RESULTS AND DISCUSSION

III.1 - The Rhone river solid discharge

The quality of flux estimates is strongly dependant on the river solid discharge. Indeed the only data which have been published were obtained in 1963-1966, much before the completion of damming works in the lower course of the Rhône river, and varied from 1.3 to 6.0 Mt/y (Savey & Deléglise, 1967). We tried to update these estimates for the recent years. A synthesis of 120 suspended matter measurements in the Grand Rhone branch from 1982 to 1989 was used to establish the following SM content-water discharge relationship : log (SM, mg/l) = 1.8 log (discharge, m^3/s) - 4.3 ($r = 0.84$)

Taking into account daily discharge measurements at the Vallabrègue dam (CNR data), solid discharges were computed for the period 1978-1989, using daily water discharges and the above relation. They show a simular range of variations from 2.1 to 5.7 Mt/y.

- An exception is the year 1989 where yearly liquid discharge dropped to 1063 m^3/s, i.e. about 60 % of long term averages. As a consequence, solid discharge was exceptionally low : 1.1 Mt/y. Short floods are often responsible for a large fraction of the annual solid discharge. (e.g. 32 % of the annual discharge occurs during 10 days in April 1986).

These results show that our regular survey performed in 1989 is only representative of extreme low water stage conditions.

III .2 - Radionuclides in the river suspended matter

III.2.1. Concentration variations

The most striking observation is the overall large variability of gamma emitters concentrations. For instance, Mn-54 varies from 30 to 24000 fCi/g, whereas Cs-137 is less variable (2000 to 20000 fCi/g). As previously shown, activity ratios are more constant ; the Cs-134/Cs-137 ratio is 0.12 ± 0.03 ($n = 25$) from 1982 to 1989, confirming the potential usefulness of this ratio for tracing the Rhone influence at sea (Martin & Thomas, 1990). The time scale of these variations may be very short ; repeated measurements have shown that fluctuations reaching a factor of 10 may be observed during the same week (July 1987). This extreme case corresponded to the end of a flood (Mouchel et al., 1989). Although a cyclic trend at the week scale of nuclear discharges by the Marcoule plant may be sometimes observed (SCPRI, 1984), some natural impact of hydrology on trace element concentrations is also likely.

We therefore tried to correlate the radionuclide concentrations with the solid discharge at the time of sampling (fig 1) :

- negative correlations were found for all the radionuclides, except Cs isotopes.

- although significant correlation coefficients were found, data are more scattered than expected from analytical accuracy ;

- the slope of the regression line is for certain radionuclides (especially Mn-54, also Ru-106, Ag-110m and Co isotopes), but weak and nearly unsignificant for others (especially Cs isotopes). An intermediate situation is found for Ce-144 and Sb-125.

III.2.2. - Interpretations

The large scattering of the data indicates that the relations with hydrology cannot account alone for concentration variations. Indeed fluctuations of the rates of nuclear effluents releases must influence the data, but this point cannot beassessed in the absence of sufficient information, especially on the short term. Even if we assume a constant effluent discharge, concentration decrease with solid discharge may result from a number of processes. It is likly to be mainly ascribed to two different processes : a mixing with an higher proportion of "inactive" calcite and a decrease of the relative abundance of organic matter to which a significant fraction of the artificial radinuclides is associated.

The behaviour of Cs isotopes supports this interpretation since their slope of decrease is much smaller than in the case of the other elements, and since the affinity of Cs for organic matter is generally the smallest, as shown by the weak enrichment factors for plankton. The very small decrease of Cs isotope concentrations with solid discharge would thus only represent the effect of mixing with "inactive" components such as calcite.If this interpretation is true, the higher the slope of concentration decrease, the higher the fraction of radionuclide associated with the organic matter. Comparison of Cs-137 and Co-60 (fig 1) suggests that a large fraction of the Co isotopes (and Mn-54, Ru-106, Ag-110 m) is bound to river Particulate Organic Matter (POM).

III.2.3 - Particulate radionuclide fluxes to the Mediterranean

We shall compare the results obtained in 1989 with previous estimates based on the 1982-1985 data and an average solid discharge of 4.3 mt/y (Martin & Thomas, 1990). It has been assumed that concentrations measured once a month could be extrapolated to the entire month. This simplification may introduce some bias during monthes when important hydrological variations occured. However, when daily solid discharges are considered it seems that this simplification should not be a source of important errors since during most of the year discharge variations were not strong. The only exception was April 1989 when about 50 % of the annual solid discharge occured ; but fortunately the monthly sample was collected during the period of high flow and is therefore representative of the particulate flux. Concentrations have been multiplied by the monthly solid discharge calculated from the turbidity - flow relationship mentionned above, and not from sample SM content. Results are presented in table 1.

As expected, particulate radionuclide flux is small as compared to normal hydrological conditions (1982-1985).If a rather constant rate of nuclear effluent release is assumed, this low river discharge would imply some increased trapping of artificial radionuclides in the river system, followed by a probable flushing of this excess during later flood events in 1990. Such trapping/flushing alternance provides a mechanism able to amplify the natural irregularity and pulse-like hydrological behaviour of the Rhône.

This behaviour tends however to be counterbalanced by a concentration increase of radionuclides presenting a strong affinity for the POM. It can be seen table 1 that, whereas the 1989/1982 - 1985 flux ratio is low for Ce-144, Cs isotopes and Sb-125, it is much higher for Co isotopes and Ru-106. Mn-54 is an extreme case but the higher flux in 1989 may also imply a recent increase of its discharge with nuclear effluents.

In conclusion, the discharge of particulate artificial radionuclide to the Mediterranean Sea is characterized by the alternance of periods of low flux or retention separated by strong pulse-like inputs. This process seems partly compensated for certain elements (e.g. Co) by POM variations, but this regulating mechanism is probably negligible (in term of fluxes to the estuarine zone) as compared to the solid discharge fluctuations. It may however account for a large part of the frequent variations of particulate trace element concentrations observed in the Rhone river.

III.3 - Fractionation of selected radionuclides between the dissolved and particulate phases in the Rhône river estuary.

III.3.1 - Quasi in situ sorption experiments

Few hours after sampling water samples were spiked with radiotracers (^{109}Cd, ^{60}Co, ^{54}Mn, ^{65}Zn, and ^{134}Cs) - 10 nCi per sample and 10^{-9} to 10^{-8} moles of stable metal added with the tracer, and kept in dark and adiabatic bottles. After 3 to 6 hours in July 1987 (high discharge), December 1988 (high discharge) and June 1989 (low discharge, Sandrine Survey) water was filtrated through 0.4 µm polycarbonate filters (Nuclepore). Then both filter and filtrate were counted on a high sensitivity Germanium detector linked with a 8192 channels analyzer (Enertec-Intertechnique). Results are expressed as logs (FDC), Fast Distribution Coefficients in l/kg.

III.3.2 - Results

Cadmium and Zinc

Riverine FDC's are similar during December 88 and "Sandrine" survey and lower during July 1987 survey, this may be explained by the evolution of riverine suspended matter properties, expressed in terms of Ammonium Saturation Index or Heat of Immersion in water, which were lower during July 87 survey (Garnier et al. 1990). Both December 1988 data and July 1987 mixing experiment (Mouchel et al. 1989) show a marked and regular FDC's decrease from river water to sea water (a factor 25 and 10 for Cadmium and Zinc, respectively). For standard FDC's determinations during July 1987 survey and during "Sandrine" survey, FDC's decrease is much less important, almost zero for Zinc, all "Sandrine" brackish FDC's are shifted up compared to July 1987.

Comans and Van Dijk (1988) have recently demonstrated that sorption of cadmium on natural (Rhine river) suspended matter was totally reversible in estuarine conditons and that complexation of cadmium by chloride could totally explain the observed desorption of particulate cadmium after mixing with different fractions of sea water. Accordingly, most estuaries in the world (Boyle et al. 1982, Elbaz et al. 1987) exhibit a non conservative dissolved cadmium distribution, generally explained by desorption from suspended matter.

December 1988 data and all mixing experiments data precisely agree with Comans and Van Dijk's findings (figure 2) and with the formation of chlorocomplexes. We may therefore conclude that such an FDC's decrease is the normal situation when river water mixes with sea water. Then, during "Sandrine" survey and also during July 87 survey, where the sampling is much poorer and the hydrological situation induces some complications, the reactivity of suspended matter must dramatically increase to overcome chloride complexation, this can be due to mixing with more reactive suspended matter and/or to a modification of riverine suspended matter surface properties as they mix with seawater. This phenomenon is enhanced during "Sandrine" survey

because of the low riverine suspended matter concentration, and perhaps by a higher marine particles/colloids production during this period of the year.

Zinc behaviour is so analogous to that of cadmium that we could assume a decrease by a factor of 10 of FDC's to be the normal situation. According to Turner et al. (1981), chloro-complexes cannot explain such a distribution for zinc. However, using iron hydroxides as a model solid, Baliestrieri and Murray (1983) also obtained approximately a factor 10 decrease of distribution coefficient from $NaNO_3$, 0,1 M to sea water, by successive addition of various salts they demonstrated that magnesium controled the evolution of distribution coefficients of zinc from fresh water to sea water probably by competing on sorption sites. The evidence is not as strong as for cadmium because independant model computations cannot confirm the influence of magnesium (or calcium possibly), but we may consider, from experimental evidences, that the decrease of FDC's observed in December 1988 and during the mixing experiments is the normal evolution of distribution coefficients from river water to sea water. Then, as for cadmium, the reactivity of suspended matter strongly changed from river to sea water during "Sandrine" survey.

Cesium

It is well established that the uptake of cesium by sediments or suspended matter is depressed by salinity ; according to Stanners and Aston (1981), dissolved potassium can account for more than 90 % of the observed influence of salinity, that is a factor 80 decrease from fresh water to sea water.

As for cadmium and zinc, December 1988 survey and mixing experiments are in good agreement with former laboratory results. During "Sandrine" survey, the decrease seems to be a little lower, because some samples show a highly enhanced affinity for cesium by the sea end member (factor 3 to 6). This point is still unexplained, but the SEM examination of suspended matter should help to understand these innexpected phenomena.

Unlike cadmium and zinc, the distribution of riverine cesium FDC's does not follow the observed evolution of surface properties ; former studies (Duursma and Eisma, 1973) suggest that clay mineralogy could be an important factor controlling cesium distribution.

Manganese and Cobalt

The distribution of these elements is significantly different from that of cadmium, zinc or cesium. Unlike cesium, and like cadmium and zinc, they have globally more affinity for suspended matter during Sandrine survey. Unlike cadmium and zinc, freshwater FDC's during Sandrine survey are much higher than fresh water FDC's obtained during former surveys. Another striking feature is the very high variability of manganese FDC's, the ratio between the higher and the lower value is higher than 3000 (excluding kinetic experiments).

The actual concentration of manganese in estuaries, as well as in most aquatic systems, results from several highly variable processes : oxidation - which can be biologically mediated -, photoreduction and microbial reduction in sediments followed by diffusion from (resuspended or not) sediments. Pure sorption (ion exchange or surface complexation of Mn (II) ions) is certainly the less variable process, it can be completely

dominated by the others.

After sampling, during the incubation period, surface exchange or oxidation are likely the main processes in addition to biogical uptake, reduction of manganese oxides is not expected because i) samples are kept dark, no photoreduction could occur, ii) Rhône estuary surface waters are well-oxygenated, and then oxygen consumption is not sufficient to cause strong deficits during the incubation period. Morris et al. (1982) have shown that the dissolved manganese concentration could dramatically decrease during the few days following sampling and that the process was more important in fresh water than in slightly brackish waters. The regular increase of FDC's we could observe for freshwater samples is in agreement with thesse findings. It is expected that the kinetics of surface exchange or surface complexation of Mn(II) ions is similar to that of Cd(II) or Zn(II) ions. Therefore slow kinetics, which are not observable for cadmium or zinc, must be attributed to another process. The very high effect of NaN_3 addition on manganese uptake suggst the existence of biologically mediated process.

It was very clearly established (Diem and Stumm 1984) that manganese oxydation is a catalytic process, possible catalysts being manganese oxides themselves (autocatalytic), other oxide surfaces or specialized bacteria. All catalytic processes will have similar kinetics, even the autocatalytic one since the increase of MnO_x due to fresh precipitation will not much modify the total concentration of oxides, therefore, we will not be able to determine the main process from the analysis of kinetic data only. Moreover, intracellular incorporation of Mn should also not be excluded, but, given the pseudo-equilibrium which could be attained by zinc FDC's, we expect that trace metals incorporation by living growing cells was not significant. Whatever the catalyst, a catalytic oxidation should show a linear increase of log (FDC)'s which can be observed during kinetic experiments in fresh water.

The observed fast kinetic of manganese transfer to suspended matter can be used to compute a time scale related to the process : given the measured rate, all manganese would disappear from the water column within few hours. Since dissolved manganese is measurable in the Rhône river, another opposite process has to occur simultaneously. Unlike, classical surface exchange processes these two (or more) processes are not directly equilibrating as the two components of a reversible reaction, on the contrary their intensity may depend in various ways on various external forcings (light, biological activity...). In biogeochemical situations similar to that of "Sandrine" survey, a high variability of dissolved manganese should be expected, this point should be considered when attempting to monitor the fluxes of manganese out of the Rhône river.

Cobalt is known to be have similarly manganese, coprecipitation of cobalt with manganese oxides has been suggested as an explanation (Murray 1975). On the whole, the results we could obtain on the Rhône river and estuary confirm the existence of a relation between the adsorptive behaviour of both components, this relation is confirmed again by the kinetic experiments and by laboratory sorption experiments on lyophilized Rhône suspended matter despite significantly lower FCD's for manganese and cobalt. However, the recent "Sandrine" survey data showed that the distribution of cobalt FDC's could be regular while the Mn-FDC's distribution was not, therefore,if the overall relation between manganese and cobalt sorption remains valid under a wide range of experimental conditons, other regulating processes have to be searched for cobalt.

III.3.3 - Conclusions

This series of quasi insitu determinaton of the partition of selected radionuclides in the Rhône river estuary under various seasonal and hydrological conditions lead to several types of important conclusions :

1) Fresh water FDC's of zinc and cadmium seem to be related to suspended matter surface properties as determined by Garnier et al. 1990. On the contrary, cesium FDC's evolution is totally in opposition. We do not think that this opposition has much sense in itself. It is likely that a common (hydrological, sedimentary, biological ?) process in the Rhône basin controls both surface properties - and related sorption affinities for zinc and cadmium - and changes in the mineralogical composition of Rhône river suspended matter which influence on cesium sorption.

2) In the brackish part of the system, we could point out different types of situations. For cesium, we always observed an important decrease of FDC's from freshwater to seawater. The normal situation is a factor 60 decrease, with some additional scatter most likely due to a mixing of suspended matter from various origins. For the other metals (cadmium, zinc, cobalt and manganese) the December 1988 survey may be considered as a reference because of the high amount of suspended matter brought by the river Rhône and because of the low biological activity in winter. The observed decreases of FDC's (a factor 25 for cadmium, a factor about 10 for manganese and zinc) are in reasonable agreement with laboratory sorption experiments on model or natural suspended solids described in the litterature. During the other surveys, and for some metals, the observed behaviour can be very different from the reference. This is the case of cadmium and zinc during "Sandrine" survey (June 1989) which have a very high affinity for suspended matter in the brackish part of the system. Our hypothesis is that this is due to a mixing of riverine suspended matter with marine suspended matter or colloids, with a much higher affinity for these metals. This is also the case of manganese which affinity for suspended matter is much higher during "Sandrine" survey, probably through a biological process as shown by the strong negative influence of NaN_3, addition on manganese sorption.

3) The very strong relation between cobalt and manganese FDC's which had been deduced from former surveys in the Rhône delta, is only partly confirmed by the last survey ("Sandrine") survey. Manganese oxides certainly exert some important control on cobalt fixation, but further studies are needed.

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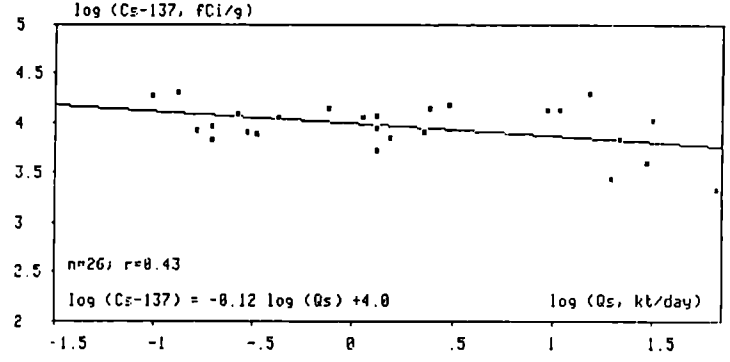
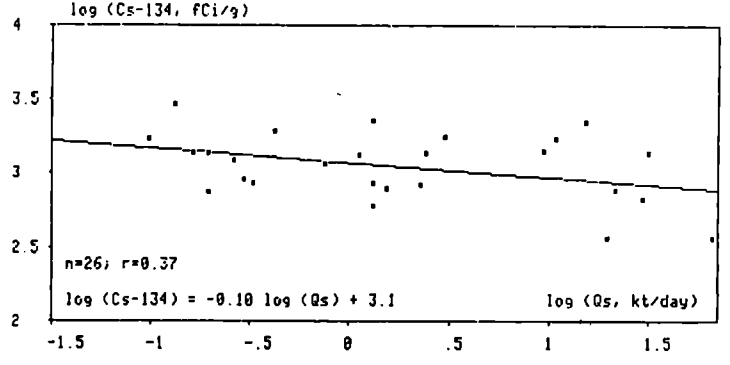
LIST OF FIGURES

Fig. 1 (a-d). Relation between radionuclide concentration in suspended water and the solid discharge (r = correlation coefficient).

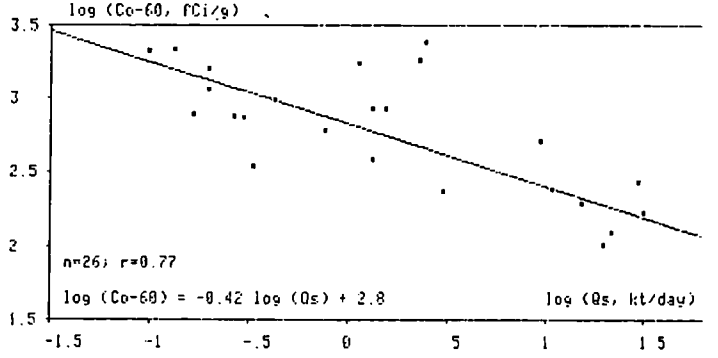
Fig. 2 (a-e). Standard FDC's during all surveys (July 1987, December 1988 and June/July 1989 "Sandrine" survey).


log (Cs-137, fCi/g)
n=26; r=0.43
log (Cs-137) = -0.12 log (Qs) +4.0
log (Qs, kt/day)

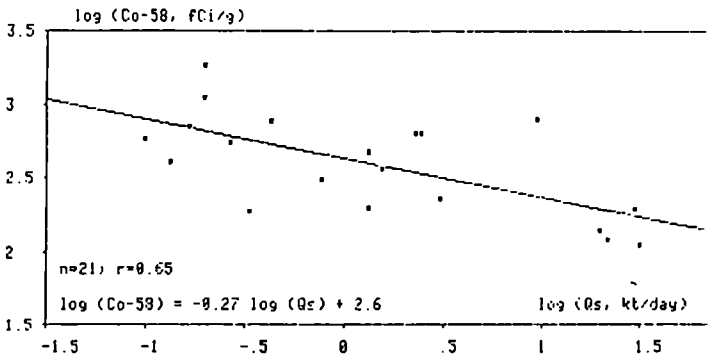
1 a


log (Cs-134, fCi/g)
n=26; r=0.37
log (Cs-134) = -0.10 log (Qs) + 3.1
log (Qs, kt/day)

1 b


log (Co-60, fCi/g)
n=26; r=0.77
log (Co-60) = -0.42 log (Qs) + 2.8
log (Qs, kt/day)

1 c


log (Co-58, fCi/g)
n=21; r=0.65
log (Co-58) = -0.27 log (Qs) + 2.6
log (Qs, kt/day)

1 d

Table 1.

Mois	SM mg/l	Qs Kt/month	Ag-110m Ci/y	Ce-144 Ci/y	Co-58 Ci/y	CO-60 Ci/y	Cs-134 Ci/y	Cs-137 Ci/y	Mn-54 Ci/y	Ru-106 Ci/y	Sb-125 Ci/y
Jan	5.10	22.40	0.01	0.01	0.00	0.01	0.02	0.18	0.13	0.10	0.00
Feb	3.50	49.99	0.11	0.04	0.09	0.08	0.04	0.34	0.65	0.55	0.01
Mar	10.80	146.30	0.04	0.26	0.07	0.12	0.12	1.27	2.10	1.36	0.06
Apr	29.70	524.20	0.04	0.34	0.10	0.20	0.31	2.82	3.93	5.24	0.07
May	12.40	177.70	0.04	0.41	0.07	0.15	0.14	1.29	2.45	4.63	0.04
Jun	4.30	17.79	0.01	0.03	0.02	0.02	0.03	0.17	0.34	1.10	0.01
Jul	4.80	18.12	0.01	0.04	0.00	0.01	0.02	0.15	0.12	0.43	0.00
Aug	4.90	9.27	0.00	0.03	0.01	0.01	0.01	0.11	0.06	0.16	0.00
Sep	17.30	5.32	0.00	0.01	0.00	0.00	0.01	0.07	0.02	0.12	0.00
Oct	3.00	3.70	0.00	0.01	0.00	0.01	0.01	0.07	0.02	0.11	0.00
Nov	29.50	47.50	0.01	0.11	0.03	0.09	0.04	0.39	0.47	0.47	0.01
Dec	17.60	52.27	0.04	0.19	0.03	0.13	0.07	0.74	0.46	1.09	0.05
Total 1989		1074	0.31	1.48	0.43	0.83	0.81	7.60	10.77	15.36	0.25
average 82-85		4300	<2.3	15.10	2.19	1.41	10.00	65.10	6.22	31.90	1.90
1989/1982-85 %			>14	9.82	19.51	58.93	8.10	11.68	173.21	48.16	13.39

Table 1. Rhone discharge of artificial gamma emitters : Comparison of 1989 data with previous estimates (S.M. = suspended matter ; Qs = solid discharge).

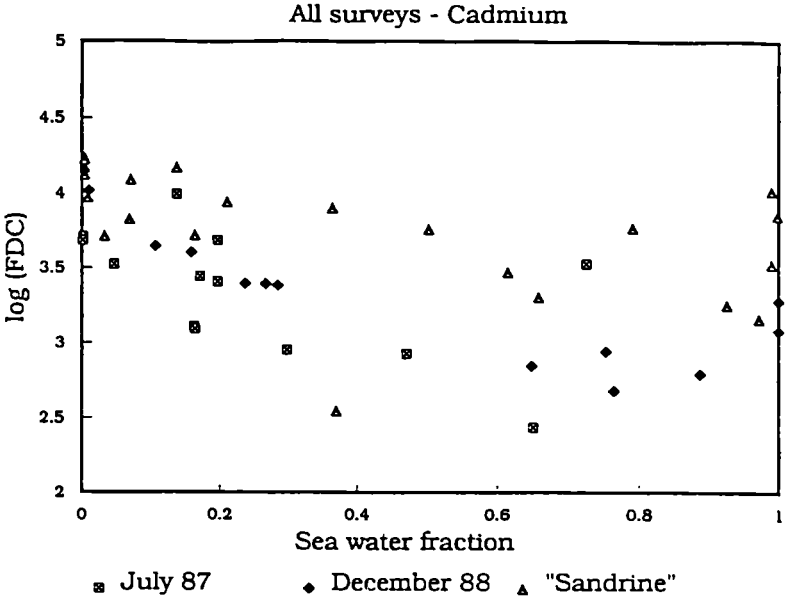


Figure 2a

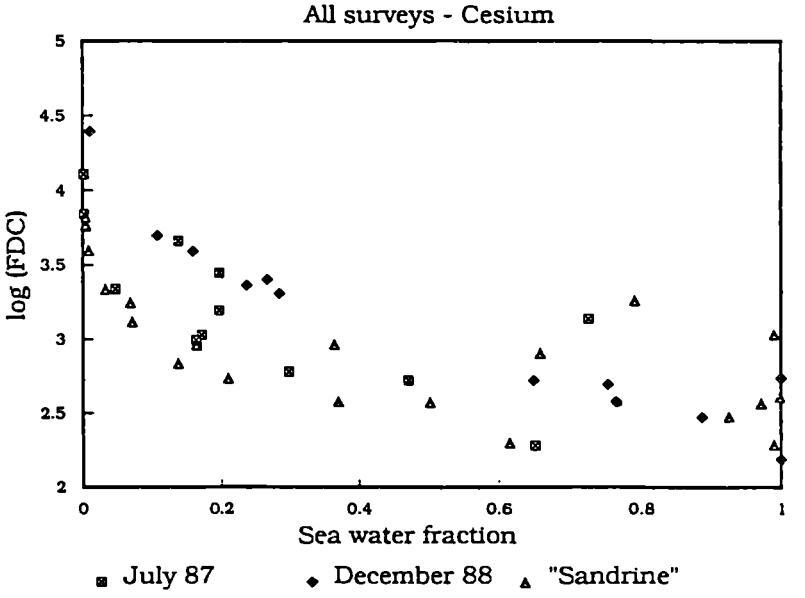


Figure 2.b.

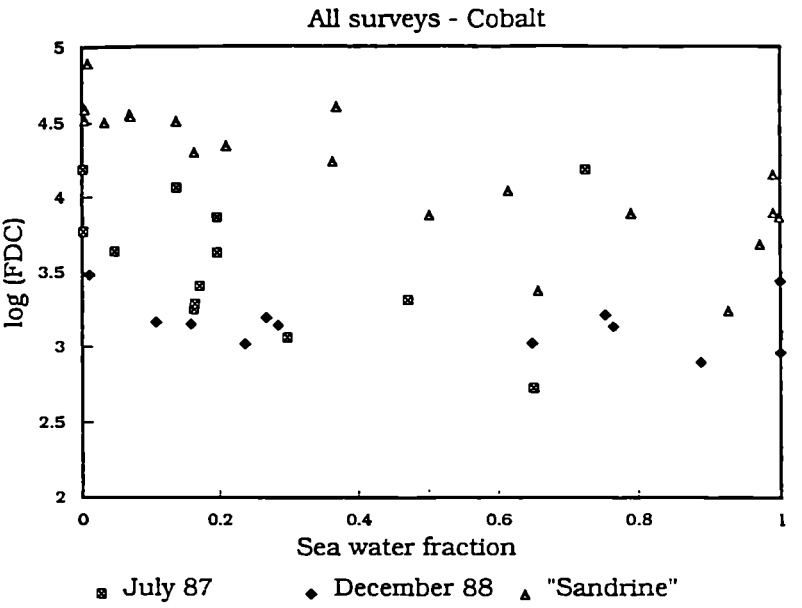


Figure 2.c.

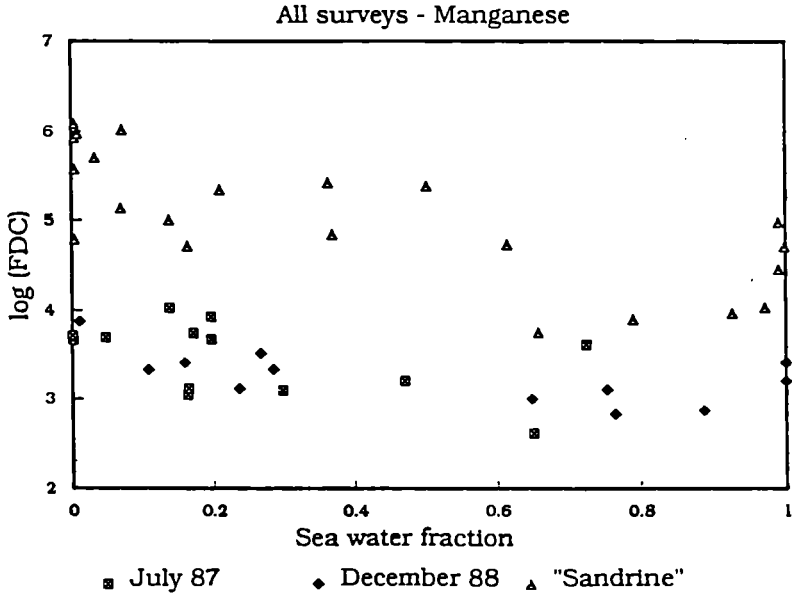


Figure 2.d.

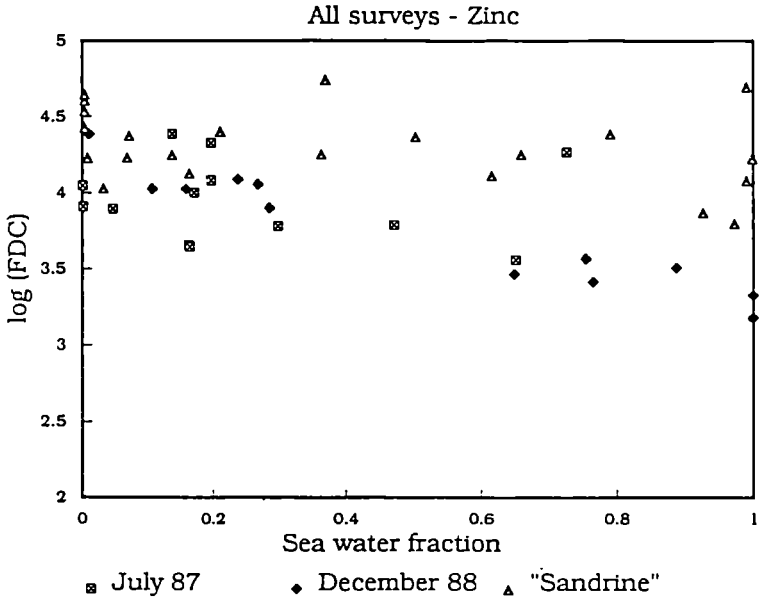

All surveys - Zinc
log (FDC)
5
4.5
4
3.5
3
2.5
2
0
0.2
0.4
0.6
0.8
1
Sea water fraction
July 87
December 88
"Sandrine"

Figure 2.e.

PROGRESS REPORT

Contract: Bi7-008 Sector: A21

Title: Modelling the transport of radionuclides through the freshwater environment

1	Hilton	NERC
2	Galvào	LNETI
3	Cremers	Univ. Leuven (KUL)
4	Foulquier	CEA - Cadarache
5	Pieri	Univ. Nantes
6	Belli	ENEA
7	Vanderbourght	Univ. Antwerpen
8	Serrano	Univ. Malaga
9	Hambuckers - Berhin	Univ. Liège

I. Summary of Project and Global Objectives

The main objectives of the project are:

i) to identify areas where the present generation of models are breaking down;

and ii) to improve our fundamental knowledge in these areas so that more easily transportable (generic) models can be developed. The main areas of work were identified in previous studies, namely, K_d , CF and catchment to lake transport.

Preliminary studies on the importance of bacteria in the food chain have also been included.

Significant advances are now being made in the fundamental understanding of Cs adsorption/ desorption. The frayed edge site hypothesis, particularly with respect to micaceous clays, is beginning to explain much of the observed remobilisation behaviour of Cs in the presence of NH_4^+ ions and/ or high concentrations of K^+. The K_d of several sediments/ soils have been predicted from some sediment characteristics and water chemistry. Results can be within a factor of 2 of measured values. However some estimates are still only within a factor of 5x. A further source of variability in K_d estimation is caused by the slow migration of Cs into the clay mineral lattice, where it becomes effectively immobilised. The development of better measurement techniques should considerably assist in the production of kinetic models of Cs immobilisation/desorption rate changes.

The importance of the chemical form of any radionuclide of interest has now been established, both for the measurement of K_d and CF. A major part of the variability of reported CF values in algae and higher plants could well be explained by differences in the membrane potential in the cells, which can vary from plant species to plant species and with water chemistry. Further work is planned to explore this possibility further. Understanding of the direct transfer of radionuclides into higher food organisms (brine shrimp and water flea) of fish is yielding to a three step model; adsorption onto the cell wall; translocation of the metals across the solution - body interface into the cells followed by transfer to other parts of the body. More experimental work is required in order to verify this approach. Within higher organisms (eel) Cs has been shown to be mainly in the ionic form in both the liver and muscle. The AS lysosome/ AS cytosol fraction appears to give a good measure of the

mobility of the radionuclide in the animal. Experimental trophic chain sequences have highlighted the importance of food intake, compared to direct uptake from the water, in the transfer of Co and Ag to fish. The sedimentary sink appears to be an important source of Ag-110m for several fish species. In trout and perch, much of the variability in Cs concentrations measured in the field has been accounted for by variations in fish size.

The transfer of large quantities of Cs from the catchment has been observed in detail in one lake. Several hypotheses have been proposed to explain the process and further work is required to elucidate the problem. Speciation of radionuclides down a river system, in conjunction with frayed edge site models, is beginning to throw light on the transport processes operating in rivers. Two main bacterial groups have been identified from rivers and sediments. Initial studies have looked at the uptake and loss of Co-60 and Cs-134 from water to suspended bacteria. Further development is now awaited in order to incorporate these data into a system model.

Several areas of model limitation have been identified and potential causes have been hypothesised. Steady progress is being made towards the verification of these hypotheses and the ultimate goal of a generic model of radionuclide transport in the aquatic environment.

Head of Project 1: Dr Hilton.

II. Objectives for the reporting period

Sub project 1: Processes of Cs transport throughout aquatic systems.

To identify the most important variables which control the transfer of radioactivity in solution from catchment soils to lakes and rivers.

Sub project 2: Ecological influences on the uptake of radioactivity by fish.

To identify the major environmental causes of gross variability in Cs concentrations in fresh water fish after Chernobyl.

III. Objectives for the next period

Sub project 1: To monitor the chemical properties of settling particulate material in a lake and to correlate them with Cs adsorption levels.

Sub project 2: To extend the present size/ Cs Activity analysis to other lakes in the UK.

Progress achieved including publications

Sub project 1:

A single catchment, chosen from a group identified as having high levels of secondary input, has been studied in detail. Data on the change with time of Cs activity in both the lake and several contributory streams have been obtained. A statistical analysis has shown that two, out of six, streams have significant relationships with flow and time from the initial fallout. These two streams also contain much higher activity levels than the other four. A detailed study of the stream subcatchments has shown that the two statistically different streams emanate from saturated peat catchments, whereas the other streams run off from either ranker or thin podsolic soils. An analysis of the lake sediments shows that the catchment contains low quantities of clay minerals, particularly illite. Five mechanisms of Cs release have been suggested:

1) A lack of illite in the catchment removes any stable, long term store for Cs.

2) Flooding of the peat soils increases the ease of washing out of caesium.

3) Humic compounds in the peat soils chelate the Cs and hold it in solution.

4) Low concentrations of ammonia in rainfall displace Cs from frayed edge sites on the small quantity of illite particles in the catchment.

5) Low molecular weight humic acids, formed by oxidation of humic material, deform the frayed edge sites on the small quantities of illite particles in the catchment, releasing the Cs.

Further studies are now being constructed in order to try and elucidate the mechanism of this secondary input.

Sub project 2:

A large number of data on fish size and Cs activity have been obtained from several lakes in the UK by colleagues at MAFF. These data show considerable scatter so that, in the most extreme cases, it is not possible to observe any trend in the data after Chernobyl. Statistical analysis has shown that a power relationship exists between activity per unit mass in the flesh and fish size (either weight or length). Data points which did not fit this relationship were identified, from field observations made when the fish were caught, as fish introduced into the lakes in order to enhance the fishing. When the original data corrected for these effects good relationships with time were observed which could be matched with events in the catchments.

Publications

Elliott, JM., J.Hilton, E. Rigg, PA. Tullett, D. Swift, P. Leonard Radioactivity in fish from two Cumbrian lakes after Chernobyl:sources of variability within the data. in press J. Appl. Ecol.

McDougall, S., J. Hilton and A. Jenkins. (1991) a Dynamic model of caesium transport in lakes and their catchments. Water Res. in press.

Hilton, J., W. Davison, J. Hamilton-Taylor, M. Kelly, F. Livens, E. Riggand D.L. Singleton. Some observations on the behaviour of Chernobyl derived Ru-103 and Ru-106 in two freshwater lakes. Submitted for publication.

Hilton, J., W. Davison, J. Hamilton-Taylor, F. Livens and M. Kelly Measurement, interpretation and modelling of Cs-137 transport through two freshwater lakes after Chernobyl. Submitted for publication.

Hilton, J., R. S. Cambray, and N. Green. Chemical fractionation of radioactive caesium in airborne particles containing bomb fallout, Chernobyl fallout and atmospheric material from the Sellafield site J. Environ. Radioactivity. In press.

Head of Project 2: Dr Galvào. (Maria Carolina Vaz Carreiro)

II Objectives for the report period

Sub-Project 1: Physico-chemical behaviour of radionuclides in freshwater sediments

Characterization of Tejo River sediments in terms of specific sites, selectivity pattern of cesium to K^+, NH_4^+, Ca^{2+}, Mg^{2+} and site heterogeneity. Study of sorption kinetics.

Sub-Project 2: Study of radionuclides transfer in a freshwater food chain

Contamination through the water of the three trophic levels with Co-60. Study of the uptake and retention kinetics.

III Objectives for next period

Sub-Project 1:

Study of radiocesium desorption kinetics and quantification of the so-called ageing effects.

Study of the radiostrontium behaviour in Tejo River sediments. Sorption and desorption based on methods as applied to radiocesium.

Sub-Project 2:

Contamination through the trophic chain. Study of the uptake and retention kinetics.

IV Progress achieved including publications

Sub-Project 1:

M.J. Madruga, working at the University of Leuven with Prof. Cremers, carried out a study concerning the radiocesium sorption behaviour in freshwater sediments (1). The characterization of Tejo River sediments was done through the determination of: cation exchange capacity and exchangeable ions, number of specific sites, selectivity pattern of cesium towards potassium, ammonium, calcium and magnesium, and site

heterogeneity.

For the adsorption experiments two granulometries were used and several conditions representative of water composition in the Tejo River. It was concluded that the experimental K_d values of radiocesium agreed quite well with the prediction based on sediment characterization and water composition. Those values also show that radiocesium sorption is governed by micaceous minerals and the potassium and ammonium concentrations in the liquid phase.

Sub-Project 2:

The experimental study concerns a simplified trophic chain: a planktonic microalgae, Selenastrum capricornutum Printz (Chlorophyceae), a small filter Crustacean, Daphnia magna Straus (Cladocera), and two fishes, Tinca tinca Linnaeus (omnivorous) and Chondrostoma polylepis polylepis Steindacher (omnivorous, but feeding mainly on macro and microalgae), both fish are Teleostei Cyprinidae. The water used is from Fratel dam (in the Tejo River) where the physico-chemical characteristics of the water are known.

The ^{60}Co transfer from water to the microalgae was undertaken, but we consider that the results need to be confirmed.

The direct ^{60}Co transfer from water to D. magna, carried out over three weeks, leads to an equilibrium at about 11 days, for an initial radioactivity in water of 39 Bq ml^{-1}. The evaluated concentration factor, referred to wet weight, is CF = 53 $\pm$ 6. The contamination experiment may be repeated along with the retention time experiment, as the first retention experiment was not conclusive.

For the fish Tinca tinca, the uptake experiment from ^{60}Co labelled water was carried out with fishes weighing on average 1.7 $\pm$ 0.3 g; in water with an initial radioactivity of 38 Bq ml^{-1}. The equilibrium was reached at about 43 days and the evaluated

concentration factor, referred to wet weight, is CF = 4.2 $\pm$ 0.5.

The retention experiment showed that data fit an exponential model with two rate functions: $R_{(t)} = 22\ e^{-0.512\ t} + 78\ e^{-0.009\ t}$ and the following half-lives, $TB_1 = 1.4$ days and $TB_2 = 77$ days.

For the other fish species Chondrostoma polylepis polylepis, the experiment was undertaken with fishes whose mean weight was 3.1 $\pm$ 0.3 g, with an initial water radioactivity of 25 Bq ml^{-1}. The evaluated concentration factor is CF = 3.6 $\pm$ 0.4, referred to wet weight, the equilibrium also being reached at about 43 days.

In this case the retention experiment data fit an exponential model with only one rate function: $R_{(t)} = 100\ e^{-0.013\ t}$, showing therefore only one half-life, Tb = 53 days.

The ^{60}Co concentration in organs and tissues is being studied, in both cases, and will be helpful to interpret these data.

^{60}Co physico-chemical forms in water have been studied through ionic exchange resins and have shown, in all experiments, that the cationic forms are prevalent, always above 80%; some anionic forms are also found, but neutral forms are merely traces.

Head of Project 3: Prof. Cremers

II. Objectives for the report period

Research efforts concentrated on short-term and long-term effects of radiocesium behaviour in sediments. Short term effects relate to the quantitative prediction of radiocesium sorption and its variability in a variety of sedimentary materials. The long-term effects relate to the potential desorption of radiocesium and its remobilization in freshwater sediments or at the freshwater-marine interface. Particular emphasis was to be given to ageing processes and the kinetic aspects of the sorption desorption process of radiocesium in sediments.

III. Objectives for the next period

Research will be focused on two major objectives: (a) the optimization of a methodology for determining the extent of immobilization of radiocesium in sediments and the development of a routine procedure for monitoring the ageing process; (b) the measurement of rate constants of radiocesium sorption-desorption and laboratory validation of the kinetics model which has been developed. The objectives are to be carried out on the basis of measurements on a range of materials including sediments from Esthwaite Water, studied by IFE, Ketelmeer and Hollands Diep, studied by ECN, and adriatic sea lagune sediments, studied by ENEA

IV. Progress achieved

The working hypothesis underlying our approach is the following: the sink responsible for radiocesium sorption is the pool of "Frayed Edge Sites" (FES) located at the edges of micaceous clay particles. These sites exhibit a high sorption selectivity for poorly hydrated cations (Cs, NH_4, K). Subsequent to the interception in these sites, radiocesium can migrate, most likely by a process of solid-state diffusion, into the dehydrated interlayer zone, thus becoming irreversibly trapped and no longer able

to participate in solid/liquid partitioning.

1. Short-term effects

The short-term sorption of radiocesium, defined as K_D, established after a contact time of 24 hours can be described by the equation:

$$K_D(Cs) = \frac{[K_D.m_K]}{m_K + m_N.K_c(N/K)} \quad (1)$$

in which $[K_D.m_K]$ refers to the radiocesium interception potential of the sediment, m_K and m_N to the concentrations of K and NH_4 in the liquid phase and $K_C(N/K)$ to the NH_4 to K selectivity coefficient in the specific sites. $[K_D.m_K]$ equals the plateau value of the $K_D(cs).m_K$ products obtained in the presence of 0.015M silver-thiourea which is used as a masking agent for the non specific sites. $[K_Dm._K]$ identifies with the product of [FES].K_C(Cs/K). $K_C(N/K)$ is obtained as the ratio of $[K_D.m_N]$ and $[K_D.m_N]$ values.

In general $[K_D.m_K]$ values for freshwater sediments are in the range of 1-10 meq/g. For very sandy sediments, they may be as low as 0.1 meq/g. For all sediments examined, $K_C(N/K)$ values are invariably in the range of 4 to 7 (a set of values found for reference micaceous clays) i.e. NH_4 is 4 to 7 times more competitive than K in radiocesium sorption.

Eqn (1) has been tested for a broad range of riverine and lacustrine sediments and K-NH_4 scenarious. It was found that K_D values can in general be predicted within a factor of 2. However, in a number of cases and at m_K in the range of 10^{-4}M, predictions may fall short of experimental values by a factor of up to 5. So far, the reasons for this are not understood.

In order to improve predictive capability, a number of other masking agents - also

characterized by very high selectivity for reversible ion exchange sites- were tested. Among these, silver-ethylenethiorea (AgENTU) and bistrimethylammoniumhexane (BTM-6) were submitted to a series of tests on various sediments. It was found that $[K_D.m_K]$ values increased by a factor of about 1.5 (AgENTU) to 2 (BTM-6). The AgENTU complex appears to be the most practical choice in future studies.

2. Long-term effects

The long-term behaviour of radiocesium is relevant to three interrelated processes: (a) the kinetics of sorption-desorption: (b) the remobilisation of radiocesium, as induced by a change in environmental scenario such as the increase in NH_4 levels in the interstitial fluid of anoxic sediments of the 100-fold increase in K-concentration upon the deposition of freshwater sediments in a marine environment: (c) the interlattice immobilization of radiocesium in micaceous minerals. These processes are discussed in some detail below.

a) Kinetic aspects

A kinetic three-box model, based on first-order rate assumptions, has been developed for describing the solid-liquid sorption-desorption dynamics in sediments. the three boxes are: the liquid phase (L), the Frayed edge phase (F) and the interlattice phase (I). The rate equations for the sorption-desorption processes are:

$$\frac{d\,Cs(L)}{dt} = -k_1.Cs(L) + k_{-1}.Cs(F) \qquad (2)$$

$$\frac{d\,CS(I)}{dt} = k_2.Cs(F) - k_{-2}.Cs(I) \qquad (3)$$

These rate equations have been solved and an analytical solution has been obtained using Laplace transform. Several preliminary tests of this model have been carried

out and the sorption-desorption kinetics can be described reasonably well. The main drawback of this approach is that the rate of the immobilization process in the lattice has to be obtained rather indirectly on the basis of radiocesium measurements in the liquid phase. Conditions could be improved considerably by using direct measurements on the solid phase for assessing the time-dependence of this immobilization process. This aspect is dealt within detail in section b. It appears furthermore that the rate limiting factor for the desorption of radiocesium from the FES is the built-up of the radiocesium levels in the liquid phase. This is directly related to the well-known observation on the discrepancy between sorption and desorption K_D values, which lead to the conclusion of irreversible sorption. Evidently, the extent of the built-up of radiocesium levels in the liquid phase is directly related to the S/L ratio used in the batch desorption experiments and the conclusions regarding the extent of the fixation process are somewhat arbitrary.

b) Remobilization and lattice fixation: a new method

The traditional method for obtaining the non-exchangeable fraction of radiocesium in geochemical substrates is based upon dispersion in a concentrated solution (1M) of NH_4 or K at some particular S/L ratio. Such procedure is based upon common knowledge that in general, ion exchange reactions in clays are rapid, if not instantaneous processes. For reasons described above, such views are not applicable to radiocesium desorption. This is well corroborated by findings showing that desorption yields improve by increasing either the number of extractions or the L/S ratio or equilibration time. The kinetic aspects are illustrated in Figure 1 for Tejo river sediments dispersed in 1M solutions of NH_4 and K or synthetic seawater. It is seen that the fraction of radiocesium desorbed in seawater is rather small (about 10%). However, such a batch procedure is quite different from the field scenario for which

freshwater sediments are deposited in an "infinite bath" characterized by near-zero levels in the liquid phase. The conclusions regarding the marine radiological impact of radiocesium-contaminated freshwater sediments, as based on such laboratory test, may therefore be entirely erroneous.

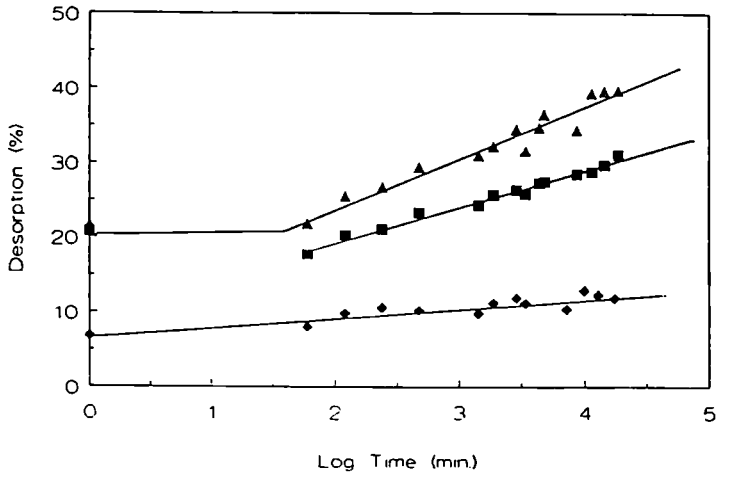


Figure 1. Time dependence of radiocesium desorption yield in Tejo river sediments (fraction < 500 μm; clay content: 2.85g/l). Total desorption time: 2 weeks: (▲) 1M KCl; (■) 1M NH4Cl: (♦) synthetic seawater.

A new laboratory procedure has been developed for which such boundary conditions are established. The principle of the method is as follows: radiocesium-contaminated sediments are equilibrated with high capacity ion exchangers (resins, zeolites) in the K or NH_4 form (depending on the experimental scenario). By choosing an appropriate excess of these solids - characterized by high K_D-Cs values - conditions can be generated for which the radiocesium levels in the liquid phase of the sediment-water

systems can be readily reduced by one or two orders of magnitude. The disruption of the S/L equilibrium brings about a Cs-desorption flux which is continuously intercepted by the resin. Two versions of the method have been developed: a membrane version in which the sediment is confined to a dialysis tubing and for which the desorption process is followed by monitoring the Cs-activity collected on the resin (outside the membrane); a suspension version in which the resin is mixed in a suspension of fine-grained sediment particles and in which the process is monitored by activity measurements of the sediment, after the rapid settling of the resin. The conditions for carrying out are extremely mild, usually 10^{-3}M in K or NH_4. The retardation function of the membrane has been quantified and corresponds - as expected - to a purely exponential function characterized by a half-life of about 2 minutes.

Figure 2 shows some examples of desorption curves on various systems: illite clay, Tejo river sediment and a highly weathered ceramic rooftile (containing mica residues). It is seen that, in the case of the suspension version, desorption rates are quite high: 85% desorption in illite after 15 minutes using 10^{-3}M NH_4. It is apparent that the desorption rate when using the membrane version is significantly lowered. It is furthermore apparent that the desorption rate in the case of an NH, scenario is significantly higher than in the case of K.

This procedure appears to be a promising tool in quantifying the time dependence of the radiocesium immobilization process. It is seen that in the case of the Tejo river sediment, measurable differences in desorption yields can be demonstrated for increasing ageing times. Some preliminary tests have been carried out on desorption yields obtained after 2 months on various sediments; the results are 40-50% immobilized (two Po-sediments), 35% immobilized (one Meuse sediment), 20-25%

immobilized (three Loire sediments).

These data indicate that significant differences in ageing behaviour occur among different sediments. Future studies will concentrate on these ageing processes the results of which should be of considerable practical importance in that they allow the prediction of the time period after which radiocesium may no longer be remobilized.

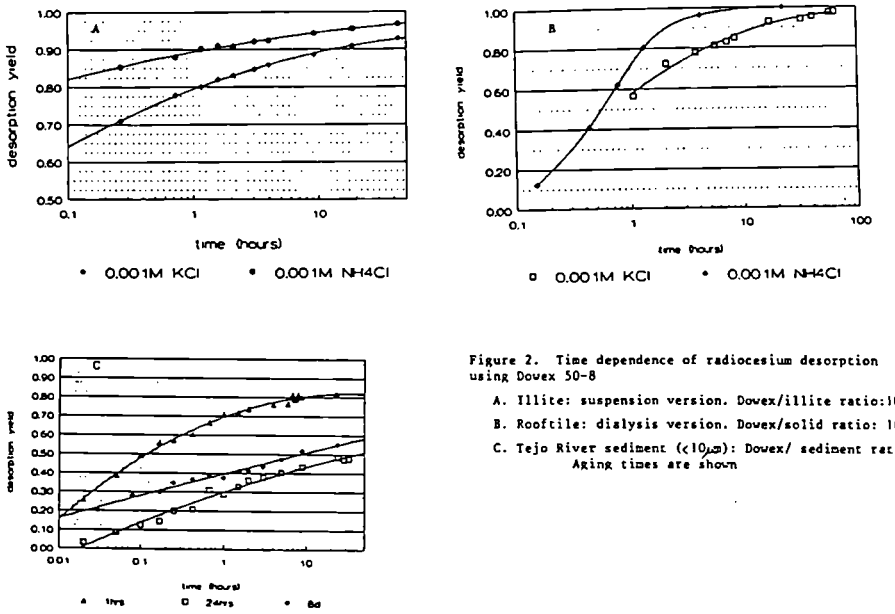


Figure 2. Time dependence of radiocesium desorption using Dowex 50-8

A. Illite: suspension version. Dowex/illite ratio:100

B. Rooftile: dialysis version. Dowex/solid ratio: 16

C. Tejo River sediment (<10μm): Dowex/ sediment ratio:100
Aging times are shown

Head of Project 4: Foulquier L.

II objectives for the reporting period

To obtain an initial appreciation of two subjects:

1 A comparison of the impact on the River Rhone of liquid effulents from nuclear installations with fall out from the Chernobyl accident.

2 To carry out an experimental study of the transfer mechanisms of silver 110m in a simplified freshwater trophic chain.

III Objectives for the next period

Our knowledge of the transfer (accumulation and elimination) of silver-110m will be extended by a study of a larger experimental ecosystem. It will be necessary to obtain values for the six following transfer coefficients:

Water - Scenedesmus Obluquus.

Water - Daphnia Magna.

Water - Salmo trutta.

Scenedesmus Obluquus - Daphnia Magna

Daphnia Magna - Cyprinus carpio

Cyprinus carpio - Salmo trutta.

At the end of this experimental phase, a final report will be presented. It will describe the principals and application of a mathematical model for simulating the distribution of Ag-110m

throughout a freshwater ecosystem.

IV Progress achieved including publications

i) A comparative study of the impact of liquid effulents from nuclear installations with fallout from the Chernobyl accident.

Our laboratory studies the radioecology of the River Rhone which, over its 300 km length, has, on its banks 17, nuclear power stations and a reprocessing factory for spent fuel.

National legislation requires us to collect, both upstream and downstream of nuclear installations, samples of water, sediment, aquatic vegetation and fish which are treated before making measurements of radioactivity by gamma spectromatry or by radio chemical methods (for alpha or beta emmiters). The results of these measurements are stored in a computer data base.

Natural radioactivity (due to K-40, Be-7, and to elements of the uranium and thorium families) is constant over the whole river with mean values in the environment of:

1 $Bq.l^{-1}$ in water,

2.300 $Bq.kg^{-1}$ dry weight in the sediment,

1.780 $Bq.Kg^{-1}$ dry weight in emergent macrophytes,

2.270 $Bq.kg^{-1}$ dry weight in emergent bryophytes,

110 $Bq.kg^{-1}$ wet weight in fish.

At Creys-Malville below the power station at Tricastin, before the Chernobyl accident, 12 artificial radionuclides were observed (table 1). Aquatic vegetation is a good indicator of radioactivity. Particularly the bryophytes which respond quickly to fluctuations of radioactivity in the water. Levels of radioactivity in fish, although lower in concentration, still reflect the composition of the power station effluent.

Table 1 Artificial radioactivity from different compartments of the Rhône, at Creys, above Tricastin (before the Chernobyl accident).

Nuclides	Water Bq/l	Sediments Bq/Kg	Emergent Higher plants	Bryo-phytes Bq/kg	Fish Bq/kg
Ag 110m		3,0 ± 1,8 (6)*	3,5 (1)		0,1 - 0,8 (4)
Co 57			8,5 ± 19,5 (6)		0,1 - 0.4 (2)
Co 58	<0,004 (2)	5,4 ± 4,0 (5)	165 ± 207 (24)	48-296 (2)	16 ± 15 (35)
Co 60	<0,007 (2)	4,1 ± 2,9 (13)	35,7± 41,9 (23)	16-22 (2)	2,3 ± 1.9 (57)
Cs 134		1,6 ± 1,0 (5)	1,7(1)	8,8 (1)	0.4 ± 0.1 (78)
Cs 137	<0,008 (2)	15,2 ± 6,9 (16)	5,1 ± 2,0 (25)	12-24 (2)	1,1 ± 1 (326)
Mn 54			42 ± 49 (14)	10-15 (2)	2 ± 2 (19)
Ru+Rh106		95 (1)	71 (1)		1-8 (3)
Zn 65					0,4-20 (13)
Zr 95			10-62 (3)		0,1-1 (3)
Sr 90	<0,04 (2)		5,4 ± 5,3 (4)		1,6 ± 0,5 (45)
H3(Bq/l) in water of combustion		5960 ± 3400 (11)	85 ± 76 (4)		20 – 1320 (12)

* (number of measurable samples)

Artificial radioactivity (mainly tritium) in compartments of the Rhône are 10 to 50 times lower than natural radioactivity levels. They are characterised by the presence of fission products (Cs -134,137 and Sr-90) and products from either activation or corrosion of materials within the reactors (Co-58, 60, Ag-110m, Mn-54, Zn-65, Zr-95...).

The accident at Chernobyl occurred on 26 April 1986. The radioactive cloud passed over the east of France during the first week of May 1986. The radionuclides were deposited essentially as wet deposition, either during rain or by the washing out of aerosols. 19 radionuclides have been detected in the fallout but only 5 (after the rapid disappearance of I-131 and Te-132) were observed to be significant in the compartments of the Rhone during the month of May (Table 2).

Table 2. Artificial radioactivity in different compartments of the Rhone between Creys-Malville and Tricastin in May 1986.

Nuclides	Sediments Bq/kg sec	emerges something or other	fish Bq/kg
Cs 134	90 ± 68 (7)	122 ± 30 (7)	4,2 ± 1,1 (44)
Cs 137	178 ± 132 (7)	363 ± 221 (7)	9,2 ± 2,4 (44)
Ru 103	257 ± 229 (7)	1964 ± 671 (7)	3,0 ± 1,2 (44)
Ru+Rh 106	152 ± 133 (7)	1154 ± 355 (7)	
Ag 110m	6,1 ± 4,2 (7)	17 ± 11 (7)	1,1 ± 3,4 (3)

After May 1986, a decrease in the radioactivity was observed in all compartments of the river. The speed of the reduction depended in part on the radiological half-life of the radionuclide and also on the properties of the compartment (biological half-life of organisms for example).

Table 3 shows, for the stretch of the Rhone Crey-Malville and upstream of Tricastin, the speed of reduction of different radionuclides.

Table 3 Times required for compartments of the Rhone, between and Crey-Malville and upstream of Tricastin, to lose half the radioactivity (days).

radionuclides	sediments	bryophytes	phanerogamous	fish
Cs134	344	210	140	245
Cs137	580	280	180	355
Ru103	33	40	10	-
Ru106	170	120	70	-

By the end of 1988 the levels of radioactivity were the same order of magnitude as those measured before the accident.

The chernobyl accident had a significant effect on all trophic compartments for a period of 2 years.

II Mechanisms of transfer of Ag-110m in a simplified freshwater food chain.

Radioecological field studies showed the presence of Ag-110m in the principal compartments of freshwater ecosystems receiving liquid effulents from nuclear installations. The presence of the radio element in the natural environment results principally from liquid effluent from pressurised water reactors which are functioning normally. Since May 1986, the frequency of detection has increased as a result of the atmospheric fallout following the

Chernobyl accident. As a result of these two source terms, on the River Rhone for example, Ag-110m was measured in a number of field samples, particularly in sediments and fish (Table 4). In all cases, field studies have shown that sediments integrate Ag-110m pollution, forming in the ecosystem the most concentrated physical reservoir which represents a source of secondary contamination for food-chains.

A series of experiments was made in the laboratory on organisms which spend greater or lesser amounts of time in the sediments in order to quantify the possible accumulation and retention of this radio element, and to evaluate the relative contribution of each vector of accumulation (water, sediment, food) in their contamination. The experimental ecosystem proposed comprised two physical compartments, water and sediment, and a two stage trophic chain. This was composed of a second order consumer, Cyprinus carpio and two food organisms, first order consumers which were chosen because of their way of life, strictly benthic for Gammarus pulex and larvae of Chironomus sp.. These prey species constitute the major part of the food for freshwater fish. The permutations for transfer, which are possible for Ag-110m between the five compartments studied were divided into two groups, the direct transfer from water or sediment into the three chosen organisms, and the trophic transfers observed with carp, for the latter the source of silver was in the food (Gammurus or Chironomid larvae previously treated with Ag-110m). Each test comprise two phases, one accumulation phase during the course of which the organism was in the presence of the radio element and an elimination phase when it was moved to an inactive medium.

For the treatment of results, the analytical approach adopted required each transfer coefficient to be obtained via a mathematical equation, the basis of which was a theoretical analysis of the system (blackbox model). This approach allowed a calculation of parameters for these

Table 4. Concentration of Ag110 m in sediment (Bq.kg^{-1} dry weight) and in fish (Bq.kg^{-1} wet weight) of the River Rhone between Creys-Malville and Tricastin. Mean and 95% confidence intervals were obtained from significant values.

Years	Sediments	Fish
1984	(0/11)	(0/15)
1985	3,2±1,6 (7/17)	(0/82)
1986*	6,2±3,9 (19/22)	0,8±1,6 (5/97)
1987	4,1±2,7 (13/13)	0,9±0,7 (4/178)
1988	9,8±7,9 (16/17)	0,2±0,1 (4/164)
1989	3,3±1,8 (5/14)	0,8±0,8 (9/140)

* Measurement taken on samples collected after the accident occurred in one of the reactors of the Chernobyl power station (26 April 1986). Number of significant values/number of measurements.

Table 5. Maximum values of the radioecological parameters characterising the accumulations of Ag110 m by chosen organisms and biological half lives (days).

Organism	Accumulation vector	Maximum value of radioecological parameter of accumulation	Biological half life (day)	
			T_b1	T_b2
Gammarus pulex	water	$CF^{(1)}=1,1.10^3$	15	-
	sediment	$TF^{(2)}=1,9$	0.02	24
Chironomus sp.	water	$CF=1,1.10^3$	0.02	11
	sediment	$TF=2.10^{-1}$	0.4	4.5
Cyprinus carpio	water	CF=106	0.5	30
	sediment	$TF=2.10^{-2}$	0.3	15
	Gammarus pulex contaminated by water	$TTF^{(3)}=2,3.10^{-2}$	23	-
	Chironomus sp. contaminated by sediment.	$TTF=1,35.10^{-1}$	1	87

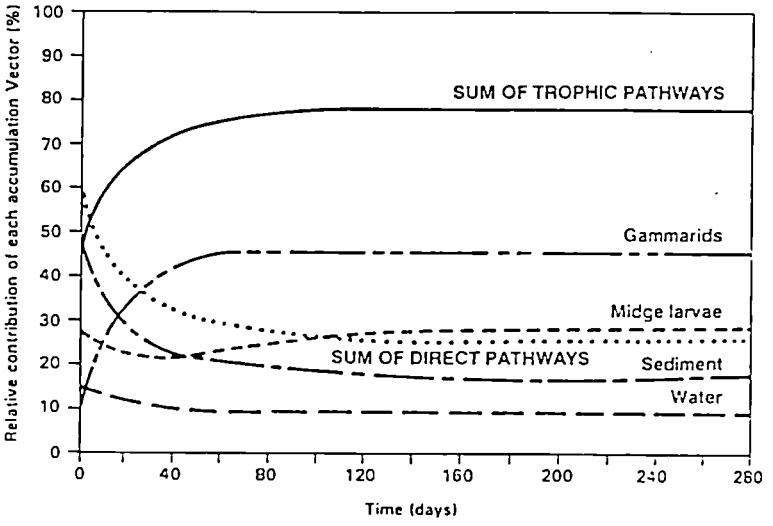
1)CF=concentration factor = (concentration in organism (Bq.kg^{-1} wet weight)) / (concentration in water (Bq.l^{-1}))

2)TF=transfer factor = (concentration in organism (Bq.kg^{-1} wet weight)) / (concentration in sediment (Bq.kg^{-1} wet weight))

3)TTF=trophic transfer factor = (concentration in the prey (Bq.kg^{-1} wet weight)) / (concentration in the consumer (Bq.kg^{-1} wet weight))

equations for each part of the studied trophic chain. The maximum values of concentration factors (transfer from water) transfer factor (transfer from sediments) and trophic transfer factors (transfer from food) are tabulated in table 5, along with a description of the corresponding elimination phase (biological period). These results characterise the accumulation of Ag110 m by the three chosen organisms and underline the importance of this radio element because the values of the calculated radio ecological parameters are very high compared to those characterising other radionuclides such as Cs-137 or Co-60.

By the using the accumulation equations calculated from these tests, it is possible to evaluate the relative contribution of each transfer vector during an annual cycle of feeding for the carp (275 days are spent during the winter rest period (Fig 1)). The direct route (water and sediment) is only greater than the trophic route for a period of less than 6 days. Very rapidly, the trophic route becomes more important and reaches 80% within 60 days of contamination in the conditions of nutrition used in the tests.



Similarly in a situation where the sediment is grossly contaminated, the transfer of Ag110 m from this compartment via a second order consumer is rapid, and results principally from the trophic route. It seems that the clearest influence on the contamination of second order consumers is the presence of prey organisms lying in sediments which have a significant quantitative effect on fish which are feeding optimally.

In conclusion it seems clear that the sediments play a major role in the distribution of Ag110 m in the aquatic environment, acting as a persistent source of pollution. In order to understand their global significance the first laboratory results of the transfer mechanisms of Ag110 m in freshwater ecosystems were integrated into a large experiment model, comprising a representative of primary production (Scenedesmus obliquus) and a top level predator (Salmo trutta). This experimental model is made up of a trophic chain with four levels and two physical compartments (water and sediment) and will make up the lowest level of conception for deterministic mathematical model for the simulation of the distribution of radio elements in a trophic chain as a function of the mode of contamination (chronic or pulse). This model will take into account the essential ecological parameters (seasonal cycles of prey species, seasonal cycling of feeding for fish, rate of growth and feed utilisation). The final objective of our programme of research is to explain and predict the distribution of Ag110 m throughout a freshwater ecosystem in the case of a pulse or chronic contamination of the environment.

V. Publications

FOULQUIER L., DESCAMPS B., LAMBRECHTS A., PALLY M. (1991).

GARNIER-LAPLACE J., BAUDIN J.P., FOULQUIER L. (1991). Analyse et évolution de l'impact de l'accident de Tchernobyl sur le fleuve Rhône. Verh. Internat. Verein. Limnol. Stuttgart 1991. (Sous presse).

GARNIER-LAPLACE J., BAUDIN J.P., FOULQUIER L. (1991). Experimental study of ^{110m}Ag transfer from sediment to biota in a simplified freshwater ecosystem. *Proceed. of the 5th International Symposium on the interactions between sediments and water. Uppsala, 6, 9 August 1990.* Soumis au comité de lecture de la revue HYDROBIOLOGIA.

Head of Project 5: Prof. Pieri

II. Objectives for the reporting period

Determination at the molecular level of Cs-137 in eel. This freshwater fish is studied for its economic impact (food).

III. Objectives for next period

- Determination, at the molecular level, of parameters which are responsible for the transfer factors of cesium, and which correlate, with the diffusion of this radionuclide into the edible parts of the animal.
- Connect the factors that we have defined with the global decontamination time of the animal.
- Comparison with the behaviour of other radionuclides (Am-241, Tc-99) in aquatic animals.
- Localization of the radiocesium with other metals at the molecular level. Influence of other metals on the behaviour of radiocesium and inihibition if any.

IV. Progress achieved including publications

Subcellular fractionation is done on the eel liver and eel muscle, according to the methodology used previously (Galey et al., 1986, Goudard et al., 1991).

The results are given in the table 1.

	Nuclei	Lysosomes Mitochondria	Light Mito. Plasma Memb. Light Lyso.	Microsomes	Cytosol
Liver % cpm	4.6	4.3	1.9	1.9	87.3
Muscle % cpm	16.7	2.1	1.3	1.1	78.8

Table 1 - Distribution of Cs-137 in the subcellular fractions of the liver and muscle of the eel.

The main radioactivity is found in the soluble fraction in the liver (87%) and in the muscle (79%).

The fraction of the cytosolic compartment is done on Sephacryl S300 (Fig. 1 and 2).

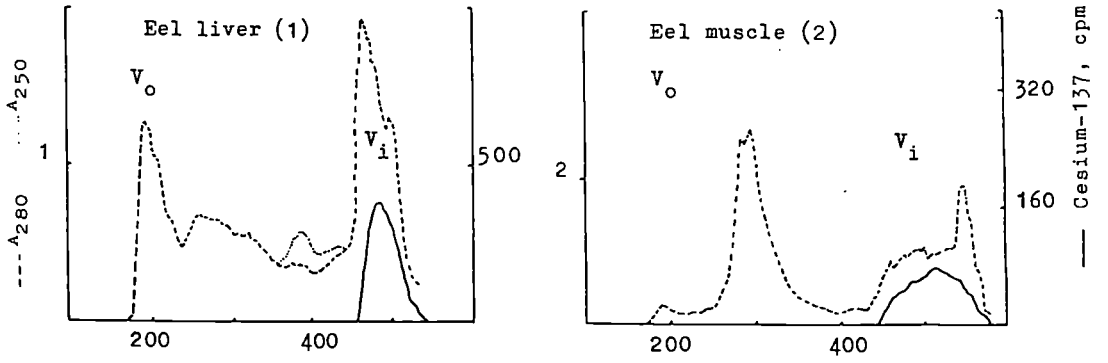


Fig. 1 and 2 - Sephacryl S300 gel permeation chromatography of cytosols from Cs-137 contaminated liver (1) and muscle (2) cells in the eel, Anguilla anguilla.

The radiocesium is found in the Vi of the chromatogram without any binding to proteins.

	EEL	
	Liver	Muscle
	Cs-137	
- Metabolic transfer coefficient = AS lysosomes/AS cytosol close to:	0.34	0.24
- Lysosomal decontamination ratio = cpm lysosomes/cpm cytosol	0.05	0.03
- Metabolic activity ratio = cpm of the soluble proteins, excluding the Vi in the chromatogram/cpm of the organ homogenate	0.003	0.054
- Metabolic localization ratio = cpm of the soluble proteins, excluding the Vi in the chromatogram/cpm in the Vi	0.003	0.056

TABLE 2. Metabolic coefficients and ratios

In the Table 2, if we consider the lysosomes as a decontamination organite, we can see that the transfer of cytosol to lysosomes is 12 times higher than for americium in the hepatopancreas of the lobster.

The metabolic activity ratio is higher for technetium in the hepatopancreas of the lobster and we know now that Tc can be bound to metallothioneins.

Knowing that all the small molecules and ions have a free diffusion into stationary phase, Vi is a total bed volume. The ratio between the activity in the Vi and the activity in the chromatographic zone where macromolecules (proteins) have a partitioning coefficient can be considered as a metabolic localization ration.

This ratio is rather representative of ionic diffusion of the radionuclide in the

contaminated animal organism.

It is very low in the eel liver for the cesium showing the very limited trend of the radiocesium to be fixed by the cytosolic proteins.

In comparison, the ratio is 500 times higher for technetium-95m and 2500 times higher for americium-241 in the lobster hepatopancreas.

In other words, two radionuclides can show the same activity in the cytosol of the liver but should have a completely different behaviour according to this ratio.

The contamination of the edible parts of animals will follow to a certain extent this ratio scale.

All the coefficients and ratios must be interpreted with care and considered with the physiology of the animal and their phylogenetic level.

Publications

Galey, J., Goudard, F., Pieri, J., Germain, P. & George, S.G. ^{241}Am binding components in the digestive gland cells of the marine prosobranch Littorina littorea. Comparative Biochemistry and Physiology 85A, 333-340 (1986).

Goudard, F., Durand, J.P., Galey, J., Pieri, J., Masson, M. & George, S.G. Subcellular localization and identification of ^{95m}Tc and ^{214}Am binding ligands in the hepatopancreas of the lobster Homarus gammarus. Marine Biology, accepted for publication 24 August 1990.

Head of Project 6: Dr Belli

II Objectives for the reporting period

The main objectives for this period are:

- identification of water and suspended particles sampling points on the basis of the hydrodynamic and chemico-physical characteristics of the Stella river (River of the north-eastern part of Italy flowing into the Marano and Grado Lagoons);

- adaptation of a Modular Selective Water Sampler, developed by ENEA-DISP to examine the radionuclide distribution between water and suspended particles, for use in brackish water.

III Objective for the next period

The main objectives for the next period are:

- Characterization of suspended particles and the relationships with Caesium adsorption/desorption in a river of the Friuli-Venezia Giulia Region as a function of the different salinity values in the river water

  Samples of water and suspended solids will be collected along the river, with 2 mobile systems, each containing a series of different mesh filters and also a system of ionic exchange resins. With these mobile systems, the volume of treated water for each sampling point, will be more than 600 litres per hour. The collected samples will be analyzed by direct gamma spectrometry.

III Progress achieved including publications

Materials and Methods

In 1990 a field survey was carried out to define the characteristics of the Stella river in order to determine the area affected by the saline wedge.

During a complete tide cycle (from 8 am to 8 pm), salinity and temperature were measured every hour at different depths along the water column in four transects located in the terminal part of the river (7 km).

Furthermore in a transect located near the mouth of the river into the Marano and Grado Lagoons, the flow rate was measured on 5 profiles using 3 current meters.

Taking into account the hydrologic features, sediment samples were collected at the interface of the river-lagoons systems by a piston corer of 7 cm diameter. Sampled cores were 5 cm long and were submitted for sedimentologic and radiometric analysis.

During a field programme, a molecular selective water sampler developed by ENEA-DISP to examine the radionuclide distribution between water and suspended particles, was adapted for use in brackish water.

Results and discussions

The saline wedge coming from the lagoons influences the Stella river at distances less than 7 km from the mouth. In fact in the last transect the salinity values found during all the tide cycles were lower than 1 g/l.

The salinity and temperature data collected in the other transects show that the river water is stratified in three well distinguishable layers in the time period around the maximum tidal excursion (9.30 am). The salinity values recorded from 8.00 to 10.30 am in the water column show the following 3 layers:

- from 0 to 1.5 m from the bottom of the river the values range between 30 and

20 g/l;

- from 3 to 5.5 m from the bottom of the river the values range between 5 to 1 g/l;
- from 1.5 to 3 m from the bottom of the river there is a transition layer.

The temperature values recorded showed the same behaviour.

Cs-137 concentrations, Organic matter (O.M.), Sand, Silt and Clay contents in the sediments collected at the interface of the river-lagoons system, are reported in the following table:

Sampling area	Cs-137 Bq/kg	O.M. %	Sand %	Silt %	Clay %
Stella River	130	2.7	28	50	22
Lagoons	60	2.8	3	67	30
Lagoons	50	1.9	0	85	15
Lagoons	59	1.9	0	75	25

These data show that the lagoons are the deposition area for the suspended particles carried by the river flow and at the same time the Lagoons contribute to the dilution of the radioactivity transported by the river.

The data collected in the first year of activity show that it will be possible to characterize the radionuclide distribution in water and in suspended particles as a function of the different salinity values of the Stella river.

Other research groups collaborating actively on this project

F. De Guarrini, Local Health Board (USL 1), Trieste (Italy)

R. Giacomelli, ENEA-AMB, Saluggia, Vercelli (Italy)

M. Marinaro, Local Health Board (USL 1), Trieste (Italy)

G. Mattassi, Local Health Board (USL 8), Latisana, Udine (Italy)

P. Spezzano, ENEA-AMB, Saluggia, Vercelli (Italy)

Publications

1. Belli, M., Blasi, M., De Guarrini, F., Franchi, M., Giacomelli, R., Marinaro, M., Mattassi, G., Nocente, M., Sansone, U., Spezzano, P., Ventura, G., 1989, Risultati di 2 anni di indagini radioecologiche nelle Lagune di Marano e Grado, Sicurezza e Protezione, suppl. al Notiziario ENEA, 21, 1989, 77-88.

2 Poggi, M., 1989, Unità Modulare per il Campionamento delle Acque e del Particolato in sospensione, Sicurezza e Protezione, suppl. al Notiziario ENEA, 21, 1989, 121-126.

Head of project 7: Prof. Vanderborght

Objectives for the reporting period.

Theoretical developments:

1) Development of a mathematical model for the physical and chemical speciation of radionuclides in salt- and freshwater environments.
2) Development of a mathematical model for the biological availability of radionuclides in salt- and freshwater environments.

Experimental developments:

1) Uptake of radionuclides in brine shrimp and water flea over time in physically and chemically defined conditions (temperature, pH, salinity).
2) Effect of complexation with inorganic and organic ligands on the availability of radionuclides.

Objectives for the next period.

Theoretical developments:

1) Integration of the models for the speciation and availability of radionuclides in salt- and freshwater environments.

Experimental developments:

1) Physical and chemical speciation of radionuclides in defined and natural environments of varying complexity.
2) Availability of radionuclides in brine shrimp and water flea in defined and natural environments of varying complexity.

Progress achieved during the reporting period.

The metals selected for this study are caesium (class A), cobalt (borderline) and silver, (class B). There is a marked difference between the types of binding sites preferred by class A and class B metal ions. Class A ions (e.g. caesium) seek out oxygen-binding sites while class B ions prefer nitrogen and/or sulphur centres. The borderline metals (e.g. cobalt) form an intermediate group which is ambivalent in behaviour.

The organisms used for the assessment of radionuclide availability are the water flea (freshwater) and the brine shrimp (saltwater). Both of these organisms have a high capacity to adapt to different kinds of environments which makes it possible to perform experiments over the widest range of conditions, (salinity, temperature, hydrogen concentration).

1) Physical and chemical speciation of radionuclides in salt- and freshwater environments.

We have developed a model that considers the speciation of an element in a particular aqueous environment as the result of a variety of both physical and chemical processes which act in concert. Acid-base, oxidation-reduction, association-dissociation, adsorption-desorption

and precipitation-dissolution processes are the regulatory factors which control the species of an element in an aqueous solution. Based upon the concepts of chemical kinetics and equilibrium a theoretical construction has been built that describes the principal physical and chemical processes that take place in such a system. Our speciation model can thus be defined as a mathematical description of the interactions that occur in an aqueous environment. That is (i) calculate the aqueous speciation of the major and minor elements among both inorganic and organic ligands, (ii) account for adsorption-desorption and dissolution-precipitation processes and (iii) consider chemical reaction kinetics.

The model uses the ion-association concept which invokes the existance of molecular speices like the free metal ion and metal-ligand complexes. A semi-empirical relation is used to describe the conditional stability of each complex species formed with ionic strength

The major problem in the building of such a speciation model is the compilation of a data base that includes the information needed or a quantitative description of the processes considered. In aqueous systems the number of interactions and species that have to be considered can be considerable depending on the complexity of the system . Although a larger number of protonation constants complex stability contants and solubility products have been measured, independent measurements for the same reaction may vary considerably. Our compilation of the information shows that in the best of all cases, measured values can be considered accurate to about one-tenth of a log unit, but values for constants of the same reaction that differ over an order of magnitude are also encountered. For many complexes formation constants have not been measured and estimates must be made by comparison with values obtained for other metal ions with the same ligand, or similar ligands with the same metal ion.

The selection of the values for the constants that describe the reactions which are considered ie the most critical step in the development of a speciation model. Although the methods we have developed for the compliation of a reactions data base has considerably improved the model it is not error free. The most important sources of error are the inaccuracies in the values of the constants which decribe the reactions. To measure the effect of these errors on the results, the model considers these variations in its analysis. The results of the speciation model are the concentration of the different species considered in the model including uncertainty limits determined by the errors in the values of the constant used.

The model has been used to model the speciation of metals and radionuclides in defined systems of different composition. It has proved of great value in the design and analysis of experiments concerning the availability and uptake of metals in defined salt-and freshwater systems.

2) Biological availability of radionuclides in salt-and freshwater environment:

The process of radionuclide uptake by aquatic organisms is rather complex and involves different steps which up to now have not been integrated into one single model. Briefly, the first step involves the fixation of the element on the cellular surface of the solution-body interface. This initial phase of metal uptake is assumed to be a simple adsorption process, with the cell walls of the living cells perceived as providing surface sites for physical and chemical adsorption. The second step involves the translocation of the metals across the solution-body interface into the cells. This process is facilitated and analogous to enzyme-substrate interactions. The third step involves the transfer of the radionuclide to other parts of the body including excretory system.

The aim of our experimental work concerning the biological availability of radionuclides is to determine the quantitative role of these different steps. Within this framework it is important to know to what extent chemical speciation processes are important in determining the availability of the radionuclides to the organisms, both during short and long periods of exposure. Pilot studies caried out with the borderline element cobalt show that the availability of the radionuclide is largely controlled by adsorption and complexation proceses. The stability of the interaction is not important in determing the availability of the radionuclide (e.g. complexation with either weak or strong ligands has the same effect). Uptake of the metals is linear in time with no evidence of saturation over the first 24 hours of incubation. Regulatory processes which may be important in controlling the uptake and accumulation of the metals during exposure over prolonged periods will be studied in the future. The results of uptake and elimination experiments show that both the first fixation step and the second translocation step are fast proceses. The form of the metal in the environment, (free or bound) together with the hydrogen concentration in the solution are the two most important factors that determine the availability of the metals in an aquatic environment.

Publications for the reporting period:

Blust, R. (1990) Modelling the transport of metals across biological interfaces. Belgian Journal of Zoology. 120: 8-9.

Blust, R., Baillieul, M. and Decleir, W. Metal complex structure-stability relations in metal availability to the brine shrimp. Aquatic Toxicology. In Press.

Blust, R. and M. Baillieul. Modelling the speciation of metals in the aquatic environment. The science of the Total Environment Submitted.

Head of Project 8: Dr Serrano

II. Objectives for the reporting period

During the period already elapsed, we have done a review of the main physiological variables that influence the accumulation of radionuclides in freshwater plants (Higher plants and algae), in order to explain the high degree of variance in the presence available data on concentration factors (CF) in different physiological types of organisms studied so far. Furthermore these variables have been suggested as "control variables" for future experiments, in order to obtain more reproducible data.

III. Objectives for next period

For the next period some experiments on the permeability of Cs^+, relative to K^+, will be carried out, in the aquatic liverwort Riccia fluitans, under different conditions in order to modify the main characteristics of the membrane of this species (in particular the membrane potential). The aim of these experiments is to investigate the influence of relative permeability and membrane potential (Em) on the concentration factors (CF) for Cs 134.

IV. Progress achieved including publications

During the period already elapsed, we have done a critical review of, both physico-chemical and physiological variables affecting the accumulation of radionuclides in the lowest trophic levels (algae and freshwater higher plants), in order to explain the high degree of variation in the existing literature data on concentration factor (CF) in these organisms. We have proposed that these "control variables" be controlled in future experiments both in the field and in the laboratory.

There are two main processes affecting the accumulation of radionuclides in autotrophic freshwater organisms:

1. Absorption
2. Adsorption

Absorption is the process of take up and storage of radionuclides inside the cell, and it is affected by a large number of variables. The absorption of a given radionuclide depends on: a) the available fraction in the external medium, b) the electrochemical driving force working on the available fraction, and c) growth rate.

The environmental variables that influence the process of absorption are as many as those affecting the physiological processes pointed out above. Since not all of these variables are independent, it is possible to choose some of them, of critical importance, to standardize the experimental conditions and, if possible, the data collections, in order to handle comparable data. So Temperature and pH are the main variables affecting the chemical speciation and, hence the available fraction.

The variables affecting the electrochemical driving force are those which modify the membrane potential (Em) and the internal concentration of ions, i.e. respiration rate and external concentration of K^+.

Growth rate, in contrast, is itself a good control variable because it is not difficult to measure in many organisms, and can be also controlled in unicellular species.

Adsorption is largely a physical process, and dependson Temperature and the number of "sites" available in the external cell surface (the periplasmic space). This area is different from species to species and could be estimated by means of surface/volume measurements.

The ideas summarized here have been explained in detail in a manuscript entitled "Radionuclide accumulation and transfer in freshwater organisms. Current concepts and perspectives", that is being revised by the members of the group.

Head of Project 9: Dr Hambukers-Berhin

II Objectives for the reporting period

- to determine the main characteristics and compare biochemical profiles of aerobic bacteria isolated from the sediment and from the water column of the Meuse river (Belgium).
- to study the uptake of Co^{60} and Cs^{134} by the free bacterial community of the water column.

  to study the release of Co^{60} and Cs^{134} by the bacterial community in relation to environmental parameters such as the temperature and the pH of the water column.

III Objectives for the next period

- to study the Co^{60} and Cs^{134} uptake and release by the following systems: bacteria-organic particles (algal debris) and bacteria-inorganic particles (clay).
- to determine the effects of environmental parameters such as the temperature, the pH and the redox potential Eh on the decontamination of the bacterial communities.

  The influence of Eh could be important in the sediments.

IV Progress report

1. The aerobic communities colonizing the sediments and the water column of the Meuse river (Belgium) were isolated. Each strain was examined for morphology, gram staining and various biochemical tests.

   Strain similarities were estimated by the matching coefficients of **Sokel and Michener** (1958) S_{SM}, and of **Jaccard** S_J (**Sneath and Sokal**, 1973). Cluster analysis was

carried out using the average linkage method (SAS, 1985).

The cluster analysis showed that all the strains were recovered in two main clusters. 70% of all the strains constituted the cluster 1 where 63% of the strains were isolated from the water column and 37% from the sediments.

The strains belonging to cluster 2 displayed other metabolic features.

All of the strains of cluster 2 but one were isolated from the sediments.

The uptake of Co^{60} and Cs^{134} by the bacterial community collected from the Meuse river was investigated in presence of increasing radiocontamination (up to 2000 Bq ml^{-1}.

The data were plotted according to the reciprocal form of the Michaelis-Menten equation: $1/V = (1/V_{max}) + (K_m/V_{max}).\ (1/[S])$ where V was the rate of uptake ($Bq.g^{-1}.h^{-1}$), [S] was the activity of medium ($Bq.ml^{-1}$), V_{max} was the maximal rate and K_m was the Michaelis constant.

The uptake of Co^{60} could be described by two kinetics depending on the range of the water contamination, from 24 to 90 $Bq.ml^{-1}$ and from 90 to 2000 $Bq.ml^{-1}$. These levels determined respectively the maximal rates V_{max1} and V_{max2} and the Michaelis constants K_{m1} and K_{m2} (table 1).

The level of radiocontamination respectively explained 58% and 98% of the variation of the contamination rates.

The Cs^{134} uptake only showed one kinetic. 73% of the variation of the contamination rate were explained by the level of radiocontamination of the water column.

During the radiocontamination, 33% and 24% of the variations of the concentration factor could be explained by the contact duration between the biomass and the radionuclides for the Co^{60} (from 22 to 2000 Bq Co^{60}, ml^{-1}) and Cs^{134} (from 16 to 300

Bq $Cs^{134}.ml^{-1}$) respectively. At the end of the uptake experiment, the activity remaining in the water column ranked between 25% and 40% of the initial activity in the case of Co^{60} (initial activity: 0 to 2000 $Bq.ml^{-1}$) and between 45 and 95% in the case of Cs^{134} (initial activity: 0 to 300 $Bq.ml^{-1}$).

Table 1: Kinetics of Co^{60} and Cs^{134} uptake by the bacterial community

	[S]	K_m/V_{max}	$1/V_{max}$	r	V_{max}	K_m
Co^{60}	24→90	$23.8.10^{-3}$	$0.4.10^{-3}$	0.7595	2500	63.70
	90→2000	$61.3.10^{-3}$	$53.5.10^{-3}$	0.9924	18692	1145.79
Cs^{134}	22→295	$186.7.10^{-3}$	$0.8.10^{-3}$	0.8570	1286	240

3. The decontamination of the bacterial community loaded with Co^{60} and Cs^{134} was investigated in relation to the environmental parameters, temperature and pH. When the temperature of the water column was maintained at 20°C, the kinetic of the decontamination of the bacterial community was described by the following mathematical relation:

$y = m\,e^{-ax} + n\,e^{-bx}$, where y was the Co^{60} or Cs^{134} content of bacteria ($Bq.g^{-1}$ d.w.) and x was the time (h); a and b were parameters depending on the desorption rate and are considered as an estimation of the biological half-times Tb_1 and Tb_2 respectively.

For both radionuclides, the biological half-times Tb_1 were found be extremely low, of the order of few seconds to few minutes whereas the biological half-times Tb_2 were higher (15h to 461h for Co^{60} and 39h to 8,976h for Cs^{134}).

When the temperature of the water column was maintained at 13°C (average

temperature of the Meuse river), the results showed that radionuclides fixed by bacteria were not released. At 20°C, the decontamination of the bacterial community was followed as the pH increased from 6.5 to 9. The chosen criteria was the increase of radioactivity in the water column. The data were fitted by using the mathematical relation: $y = m(1-e^{ax}) + n(1-e^{bx})$, where y was the Co^{60} or the Cs^{134} content of the water column ($Bq.ml^{-1}$) and x was the time (h); a and b parameters were the biological half-times. Two values of pH of the water column were critical for the release of Co^{60} and Cs^{134} by bacterial community; the rate of desorption was the lowest at pH 8.0 and the highest at pH 7.0.

From these results, it could be concluded that, when the average temperature and pH of the Meuse river (pH 8.0, 13°C) were changed, a loss of the radionuclides immobilized by the bacterial biomass occurred and more radioactivity was consequently recovered in the water column.

Progress Report

Contract: Bi7-042 Sector: A21
Title: Radioecology of transuranics in the marine environment.

1	Mitchell	University College Dublin
2	Iranzo	CIEMAT
3	Guegueniat	CEA - Cherbourg
4	Damiani	ENEA

I. Summary of Project and Global Objectives

The overall objective of this project, which is a cooperative study between four European laboratories, is to improve and refine our understanding of the behaviour of plutonium, americium and other long-lived radionuclides in the marine environment. The study embraces five distinct marine zones namely, the Irish Sea, The Channel and the Seine Estuary, the Almanzora river bed/mouth and adjacent shelf, the Gulf of Taranto and the Ligurian Sea. Although these domains differ widely in their physical oceanography, many of the fundamental processes governing the behaviour of transuranics and other long-lived nuclides are common to them all and, as such, are the main focus of the work now being undertaken by the participating laboratories.

A feature of the collaboration is the application of compatible and, in some cases, identical analytical techniques to the study of the physical and chemical speciation, dispersion, sediment transport and organic complexation of transuranium nuclides under a wide range of marine (and riverine) conditions. The programmes of the participating laboratories have been closely coordinated with the aim of enhancing the value and universality of the results obtained. To this end, the group has met twice in the course of the present reporting period, to discuss the technicalities of coordination and the progress of researches carried out by each laboratory. Additional, bi-lateral meetings have also been held. A number of marine research cruises have been undertaken and the results from some of these are now to hand. Of particular interest is the forthcoming research cruise in the Western Mediterranean aboard the R.V. Bannock (18 July - 4 August, 1991), in which each of the collaborating institutions will be participating either directly or indirectly.

A number of project-related research publications have been published by the individual laboratories or are in press. Further publications, covering both the present reporting period and the next, are anticipated. It has also been agreed to prepare a joint paper on appropriate features of the overall project for publication in the international literature by the end of the second reporting period.

Head of Project 1: Dr. Mitchell

II Objectives for the reporting period

(1) To test at the UCD laboratory a dual isotopic tracer technique to separate the oxidation states of plutonium in sea water.
(2) To examine the distributions of plutonium and americium between filtrate and suspended solids in near-surface and near-bottom water throughout the Irish Sea and to determine Kd coefficients for both elements.
(3) To compare the oxidation state distribution of plutonium in filtered surface and bottom waters in both the Eastern and Western Irish Sea.

III Objectives for next period

(1) To complete the analyses of samples collected in the open waters of the Irish Sea during the first reporting period, with particular emphasis on Pu oxidation state distribution measurements.
(2) To examine the chemical speciation of Pu and Am in estuarine waters (Carlingford Lough) containing elevated concentrations of dissolved organic carbon.
(3) To develop new techniques for the determination of colloidally bound Pu and Am in marine and estuarine waters and apply them to both the Irish and the Mediterranean environments.

IV Progress achieved including publications

It is of fundamental importance when assessing the long-term impact of transuranium nuclides on the environment to have a clear understanding of the species which are formed under different conditions together with the related biogeochemical cycles. Although, over the short term, the behaviour of these elements may be dominated by source-dependent species, releases from Sellafield being a case in point, the concentrations of such species will diminish with time and, ultimately, behaviour will be controlled by source-independent forms.

In the programme presently underway at University College Dublin, the speciation of plutonium and americium released from Sellafield has been examined at a number of stations in both the Eastern and Western Irish Sea. Specifically, the oxidation state groups of plutonium (Pu III+IV and Pu V+VI) have been studied in near-surface and near-bottom waters in order to determine whether any differences were apparent in the deeper waters of the Western Irish Sea. Of interest also was the determination of representative Kd coefficients for the more particle-reactive reduced species in the Western Irish Sea under equilibrium conditions - there is evidence that plutonium and americium concentrations in both the sea water and surface sediment compartments in the Western Irish Sea are relatively constant at the present time.

Other objectives included a careful examination of the Pu-238/Pu-239,240 and Am-241/Pu-239,240 quotients in filtered water, suspended matter and surface sediments and the use of the Pu-

241/Pu-239,240 ratio to deduce an effective 'hold-up' time for plutonium in the sedimentary deposits close to the Sellafield outfall.

Sampling and Analysis

Samples of filtered sea water, suspended particulate and surface sediment, collected at six stations in the Western Irish Sea in the course of a cruise aboard the R.V. Lough Beltra, were analysed for plutonium and americium using standard radiochemical procedures based on co-precipitation with ferric hydroxide, followed by separation of plutonium from americium by solvent extraction and/or anion and cation exchange, prior to electrodeposition and alpha spectrometry.

In a separate operation, near-surface (3 m) and near-bottom (3 m above seabed) water samples were collected from the R.R.S. Challenger in December 1989 at six stations in the Eastern and Western Irish Sea and analysed for plutonium and americium as above. Duplicate samples, taken at the same stations, were processed on board ship to separate the two oxidation state groups of plutonium using the neodymium fluoride coprecipitation technique of Lovett and Nelson. The latter was repeatedly tested in our laboratory prior to use under 'field' conditions. Carry-over between species was invariably small and was corrected for by assessing the cross-over between the Pu-236(VI) and Pu-242(IV) tracers added immediately upon sampling. Good agreement was observed between the sum of the oxidized and reduced plutonium measured in the filtrate using the latter technique and that determined in duplicate samples using the technique of co-precipitation with ferric hydroxide. The oxidation states of plutonium associated with suspended particulate in the Western Irish Sea were also examined and it was confirmed that the plutonium was almost completely in a reduced form (>97%), in excellent agreement with previous studies carried out elsewhere.

Results

A preliminary analysis of our data suggests that for the transuranics near-equilibrium conditions may presently prevail in the Western Irish Sea, in contrast, for example, to the case of radiocaesium, where the concentrations continue to decline. As transuranic discharges from Sellafield have been reduced to very low levels, it now appears thet the dominant source term for the Western Irish Sea has become remobilised plutonium and americium from the sedimentary deposits in the north-eastern Irish Sea close to the outfall, where it has been established that a significant 'hold-up' of transuranics occurs.

Measured Pu-238/Pu-239,240 quotients in filtered sea water, suspended particulate and surface sediment in the Western Irish Sea are clearly indicative of releases from Sellafield, being in good accord with those observed throughout the North-Eastern Irish Sea.

As expected, there is a clear difference between tha Am-241/Pu-239,240 quotient in filtered sea water and that for the suspended load, reflecting the stronger particle reactivity of americium. The Pu-241/Pu-239,240 ratios in filtered sea water and sediment were found to be identical, namely 18 +- 2. This value is almost half that reported for the source term during the period of peak discharges from Sellafield, i.e., the mid-1970's, and implies that there is a 'hold-up' of plutonium between Sellafield and the Western Irish Sea (mud bank) of about 15 years.

It is evident from the data that proportionately more of the plutonium and americium in the water column close to the outfall is associated with suspended solid. The mean percentages of Pu-239,240 and Am-241 on suspended particulate in the Eastern Irish Sea were 64 +- 19% and 92 +- 2% compared to 28 +- 8% and 64 +- 17% respectively, in the Western Irish Sea. Differences in the suspended load in the Eastern and Western Irish Sea cannot account for the significant variation in these percentages as one moves away from Sellafield and suggests that other factors must be considered.

In contrast to the systematic variation with increasing distance from Sellafield in the partition between suspended particulate and filtrate fractions, the percentage of Pu(V,VI) in the latter was esentially constant. No distinction between surface and bottom waters was detected, nor was there any difference between Eastern and Western Irish Sea stations. In fact, the agreement between these stations is remarkable, with little scatter in the data, and shows that throughout the open waters of the Irish Sea the percentage of Pu-239,240(V,VI) in filtered water lies in the range 77 - 94% with an overall mean (+- 1 S.D.) of 85 +- 6%. The equivalent figures for Pu-238(V,VI) are 70-95% and 84 +- 7%, respectively.

No systematic variation of Kd with depth at a given location was observed. However, a clear difference between the mean Kd values for plutonium and americium in the eastern and western zones was evident from our data. In the western zone, mean Kds for plutonium and americium of (8 +- 2) x 1E4 and (4 +- 2) x 1E5 respectively, were determined, whereas in the eastern zone the corresponding values were (4 +- 2) x 1E5 and (3 +- 1) x 1E6. The latter are almost identical to the mean Kds obtained from samples taken within the Eastern Irish Sea (mainly in the eastern zone) in 1977-79 by Pentreath et al., namely (3.2 +- 0.5) x 1E5 and (2.2 +- 0.3) x 1E6, respectively.

The Kd for Pu-239,240(III,IV) has also been determined and, again, a similar difference between the two zones is evident from our data. The mean Kd for Pu-239,240 (III,IV) in the eastern zone was found to be (3 +- 2) x 1E6, in excellent agreement with the mean of (3.5 +- 0.8) x 1E6 reported by Nelson and Lovett for samples collected within 20 km offshore of Sellafield. The Kd for Pu-239,240(V,VI) at (2.5 +- 0.5) x 1E3, was some two orders of magnitude smaller than the mean value for the reduced species, namely (6 +- 4) x 1E5.

Factors which could, conceivably, influence a Kd determination include suspended load particle-size distribution, the presence of significant concentrations of dissolved organic carbon, excessive silicon concentrations, the chemical speciation of the element in the receiving water body, the presence/absence of hot particles and experimental artifacts. As our results demonstrate, the oxidation state distribution of plutonium is identical in both the Eastern and Western Irish Sea zones and cannot account for the observed difference in the Kds between the two zones. Organic carbon concentrations throughout the open waters of the Irish Sea are known to be much lower than the levels (>1 mg/l) required to alter measured Kds. Further, although Irish coastal waters are enriched in silicon, while eastern waters are enriched with anthropogenically derived nitrogen and phosphorous, these differences are small and very unlikely to give rise to a measurable perturbation in Kd. Moreover, we do not consider that the particle-size distribution or the composition of the suspended load is significantly different in the Eastern and Western Irish Sea, given the degree of turbulent mixing which takes place in these waters; nor do we believe that our data suffer from a systematic shift caused by an experimental artifact given that measurements made in the Western Irish Sea on three separate occasions are in good agreement with one another.

We are, therefore, led to the conclusion that the variation with increasing distance from the source in the percentages of plutonium and americium on suspended particulate, together with the differences between the Kds for both elements in the eastern and western zones are related to the nature of the discharges themselves. It is known that only about 1% of the plutonium released from Sellafield is in an oxidized form, while all of the americium is in a reduced form. Thus, the great preponderance of the particulate fraction in the effluent provides a ready explanation for the elevated percentages on suspended particulate close to the outlet. 'Hot' particles have also been identified in the effluent and are known to persist in the environment for months before dissolving. Moreover, it has been established by other workers that about 10% of the plutonium in surface sediments close to Sellafield is in the form of 'hot' particles.

The presence of these particles in the effluent from Sellafield may be one of the main reasons why Kds for plutonium and americium in the Eastern Irish Sea appear to be significantly higher than in the Western Irish Sea, where 'hot' particles are less likely to be present in the same relative abundance and may, indeed, have become fully solubilised over the period of time taken to reach this zone.

Publications

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Processes, 9-14 September 1991, Norwich, U.K., In Press.

2. Mitchell, P.I., Vives Batlle, J., Ryan, T.P., McEnri, C., Long, S., O'Colmain, M., Cunningham, J.D., Caulfield, J.J., Larmour, R.A. and Ledgerwood, F.K. Plutonium, Americium and Radiocaesium in Sea Water, Sediments and Coastal Soils in Carlingford Lough. Ibid.

3. Mitchell, P.I., Vives Batlle, J., O'Grady, J., Sanchez-Cabeza, J.A. and Vidal-Quadras, A. Critical Group Doses Arising from the Consumption of Fish and Shellfish from the Western Irish Sea. In New Developments in Fundamental and Applied Radiobiology: Proc. 23rd Annual Meeting of the European Society for Radiation Biology, Dublin, 23-26 September 1990 (Eds. C.B. Seymour and Carmel Mothersill), Publ. Taylor and Francis, 1991, 380-91.

4. Crowley, M., Mitchell, P.I., O'Grady, J., Vives, J., Sanchez-Cabeza, J.A., Vidal-Quadras, A. and Ryan, T.P. Radiocaesium and Plutonium Concentrations in Mytilus edulis (L.) and Potential Dose Implications for Irish Critical Groups. Ocean and Shoreline Management, 1990, 13, 149-61.

Head of Project 2: Dr. GASCÓ

II Objectives for the reporting period

- Sampling of surface sediments (5 cm depth) in river bed and beach (Almanzora river mouth)
- Sampling of river sediment cores (50 cm depth)
- Sampling of river trenchs (30 cm depth)
- Sampling of biota
- Pretreatment : Freeze-dried,ball-milled,sieved 1x1 mm
- Analysis of plutonium and cesium-137 in river surface sediments and beach-sand
- Grain sized and geochemical composition

III Objectives for next period

- Sampling of sea sediment in Continental Shelf and Almanzora canyon (Cruise Bannock)
- Sampling of sea-water
- Sediment pretreatment
- Analysis of river cores and trenches
- Discussion of results obtained into the river bed

IV Progress achieved including publications

(Methodology, Results, Discussion)

Sampling

Five sampling lines were marked out onto the river bed and three stations were selected on each line. The sampling network is observed in Figure 1.

Three areas were traced on the beach in order to obtain representative samples. Five cluster were selected. Forty four surface points 100x100x5mm were sampled as representative background.

Sediment cores (50 cm depth) and trenches (30 cm depth) were picked up.

Pretreatment

Two Kg of sediment were dried until constant weight at 70°C during 5 days. The samples were sieved through 1x1 mm mesh size. The gravels and bubbles were rejected. The weight per unit area (g/m^2) were determined in each sample.

Analysis

The plutonium was separated from sediments using the Wong's procedure with an extra column to eliminate the thorium traces.The measurements were carried out by alpha spectrometry of the plutonium electroplated onto stainless-steel discs according to Talvitie's

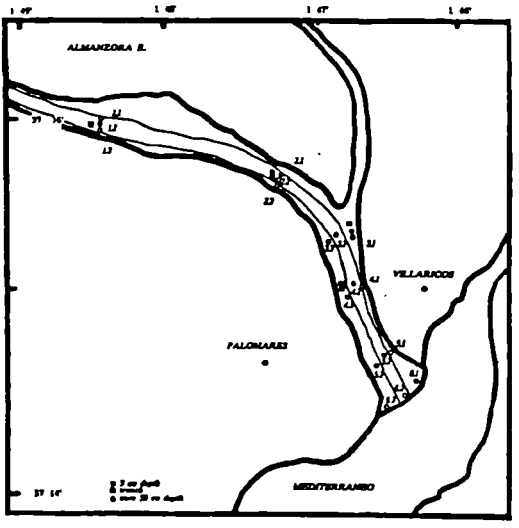


Figure 1 : Sampling stations

procedure.Radiochemical yield was obtained using ^{242}Pu as tracer.The ^{137}Cs were determined by gamma espectrometry using a hyper-pure coaxial type n-germanium detector. The accuracy of the measurements was checked with standard samples of sediments. Intercalibration exercises confirms the reability of the data.

Inventories

The inventory was calculated by the following equation:

Inventory = $a_i Dw_i / s_i$

ai = activity concentration of radionuclides
Dwi = Dry weight
si = Section

Results and Discussion

The results obtained are shown in Table 1 and represented in Figure 2.

Table 1 : Inventories and Concentrations activities in Almanzora river sediments

Stations	Activities Bq/Kg+2σ		Inventories Bq/m²	
	$^{239+240}Pu$	^{137}Cs	$^{239+240}Pu$	^{137}Cs
1.1	0.079 ± 0.018	5.2 ± 0.6	0.93	61.1
1.2	0.081 ± 0.016	1.4 ± 0.4	1.53	25.5
1.3	0.111 ± 0.028	4.9 ± 0.8	0.64	28.1
2.1	0.041 ± 0.016	1.4 ± 0.8	0.41	13.8
2.2	0.055 ± 0.016	3.4 ± 0.7	0.66	40.5
2.3	0.080 ± 0.022	5.5 ± 0.9	1.08	73.7
3.1	0.057 ± 0.012	1.5 ± 1.0	0.52	13.6
3.2	0.064 ± 0.020	2.7 ± 1.3	0.70	29.8
3.3	0.073 ± 0.026	1.5 ± 1.0	0.93	19.3
4.1	0.046 ± 0.026	3.2 ± 1.4	0.72	50.5
4.2	0.053 ± 0.020	2.0 ± 1.5	0.60	22.6
4.3	0.232 ± 0.060	1.5 ± 1.0	1.80	11.4
5.1	0.110 ± 0.026	2.6 ± 1.0	0.65	15.6
5.2	0.055 ± 0.014	1.3 ± 0.9	1.07	24.9
5.3	0.074 ± 0.030	1.6 ± 0.5	1.35	30.0
6.1	0.189 ± 0.040	0.23±0.06	1.09	1.3
6.2	0.192 ± 0.080	0.19±0.06	1.15	1.1
6.3	0.156 ± 0.040	0.21±0.06	0.90	1.2

*5 cm depth

There are few data from ^{137}Cs and $^{239+240}Pu$ fallout in Mediterranean river sediments. The concentration ratios between river and marine sediment can give us an idea of the magnitude of tranfer processes.These ratios are ranged from 10 to 20 in Palomares, and are equal than obtained in Italian coast between Magra and Vara river and Mediterranean sedimerts from Continental Shelf. The plutonium and cesium concentration level is similar to that from fallout and can be compared with those published in the literature on the river sediment concentrations in other areas[1,2].

The low values of inventories can be explained by the mean rainfall in Almería (220 L/m²). This rainfall decreases in Palomares like corresponding to a semidesert-ecosystem (40-70 L/m² per year). Other fact that can be mentioned is the decreasing of cesium concentration close to the Almanzora mouth. The ratio between these radionuclides changes significantly in this area possibly

due to the ^{137}Cs penetration through the sediment profile is more extensive in these sediments than in the others. These results will be discussed after analysis of river cores and published in due course.

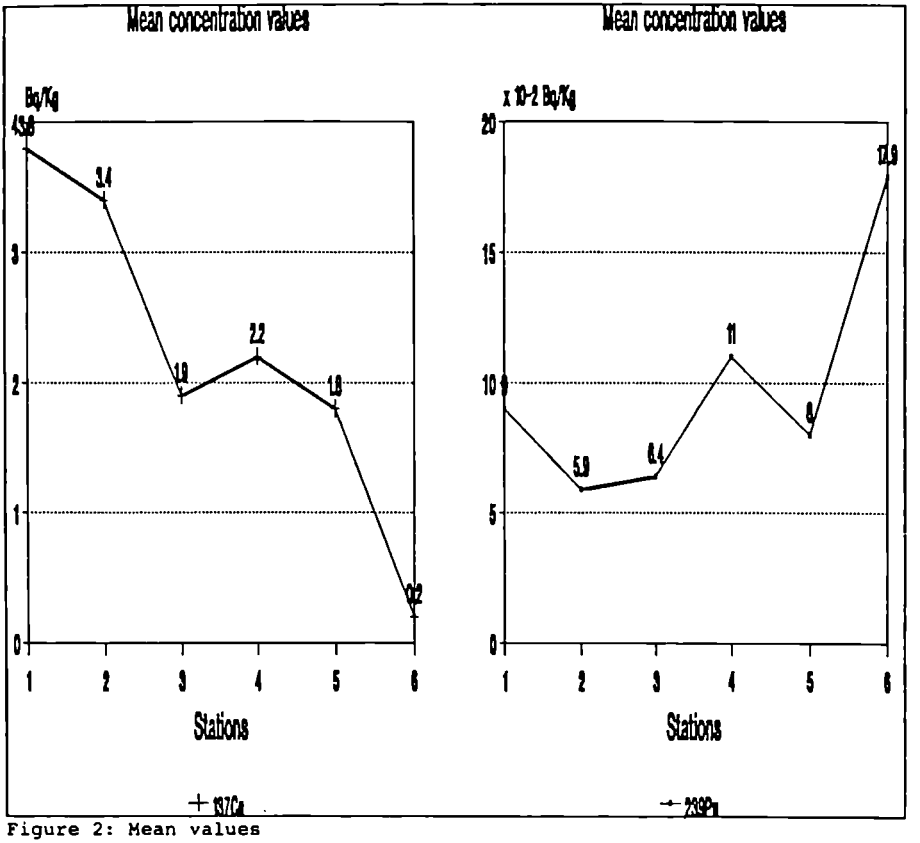


Figure 2: Mean values

The grain size composition manifests the changeable sedimentation onto the river bed.

(1) Papucci,C.;Delfanti,R.;Jennings,D.J.Environ.Radioactivity 2(1985)293-310
(2) Simpson,H.J.;Trier,R.M.Annual Technical Report 1981.COO-2529-19.
(3) Coughtrey,P.J;Thorne,M.C.Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems (A critical review of Data)1983.V.1.V.4.

Publications

(1) Informe sobre las actividades realizadas para el proyecto de la CEE C.Gascó,L.Romero. CIEMAT/PRYMA/UGIA/M5A07/05/90

Head of Project 3: Dr. Guegueniat

II Objectives for the reporting period

1. Study of the gamma-emitters (including americium-241) in slowly accumulated subtidal sediment over the past 30 years.

2. Complex formation of americium with organic matter in river estuaries with reference to americium of marine origin.

III Objectives for next period

1. Study of Pu isotopes in slowly accumulated subtidal sediment over the past 30 years.

2. Complex formation of americium with organic matter in estuaries with reference to americium of fluvial origin.

3. Complex formation of some rare earths with organic matter in estuaries. Comparisons with americium.

IV Progress achieved including publications

1. STUDY OF THE GAMMA-EMITTERS (INCLUDING ^{241}Am) IN SLOWLY ACCUMULATED SUBTIDAL SEDIMENT OVER THE PAST 30 YEARS.

D.BOUST

After their controlled release from the reprocessing plant of La Hague into the marine environment, radionuclides achieved a partition between soluble and particulate phases which depends on the physico-chemical properties of the encountered water-masses, on the surface characteristics of suspended particles, or on the interactions with the marine biota.

The general circulation of water-masses throughout the Channel brings the dissolved species towards the Dover Straights. The particulate species, that is to say the particulate-bound radionuclides are likely to follow the same path. Nevertheless, this is achieved over much longer time-scales, to such an extent that a certain proportion of them have not yet reached (and perhaps will never reach) the exit of the system (of the Channel, in the present case). This is due to the fact that particles have a specific dynamic behaviour and undergo numerous sedimentation-resuspension cycles before raising a final sink.

The key-point for the study of the long-term behaviour of particulate radionuclides is to find sediment cores liable to give raise to a good record of the radionuclide transits. This is indeed difficult because the Channel is a highly energetic environment. In these conditions, only harbours can give shelter to continuous fine-grained sedimentation in the vicinity of the point of release. Unfortunately, most of them undergo active dredging operations. Despite of all these limitations, we succeeded in finding out a good site within the roads of Cherbourg.

We then validated the site to ensure that the radionuclide record is continuous and not affected by any further post-depositional processes:

- the sedimentation rate determined by ^{210}Pb excess is fairly constant at 2.9 $\pm$ 0.1 cm a^{-1} so that our 80 cm long core gives raise to a nearly 30 year long recording period;
- the sediment characteristics are constant troughout the core: grain-size distribution (less than 4 μm fraction nears 50%), carbonate content (around 35%), major elements;
- as far as they can be detected by chemical leaching of Mn and Fe in carbonate phase and mineral coatings, no diagenetic processes are liable to alter the vertical distribution of radionuclides after their incorporation to the sediment column.

The preliminary results are surprising but promising:

- the vertical profiles of short-lived radionuclides, such as ^{106}Ru and ^{125}Sb, cannot be clearly interpreted because those radionuclides are mainly transferred by water-masses; in some cases, the time of transit between La Hague and Cherbourg can be of the order of magnitude of 1 year; this will have to be confirmed by statistical analysis;
- the vertical profiles of long-lived radionuclides, such as ^{137}Cs, ^{154}Eu, ^{241}Am, are reminiscent neither of the instantaneous released activity versus time, neither the cumulated released activity versus time; in other words, their activities increase towards the sediment-water interface as the released amounts decrease; moreover, the interelement ratios are rather constant: $^{137}Cs/^{241}Am = 2.5 \pm 0.5$, for example.

Among the various interpretations (influence of the conditions and/or the chemical characteristics of the release, transits from the Sellafield plant which are not supported by any evidence, *to our present knowledge*), we have developed the following one: the signal recorded in the core is strongly modulated by natural processes. This has been evidenced by Fourier analysis which yields two significant frequencies when applied to carbonate content, major element concentration, and both natural and artificial long-lived radionuclides, 18 and 4 years, which correspond to well-known cyclicities of the lunar orbit (that is to say, tide coefficient or energy of the medium). As a consequence, my working hypothesis is that different sediment provinces undergoing more or less energetic tide currents are liable to induce specific particulate flux from the Golfe Normand-Breton towards the eastern Channel. The sheltered area of Cherbourg acts as a natural integrating sampler of the eastwards migrating particles mentioned above.

2. COMPLEX FORMATION OF AMERICIUM WITH ORGANIC MATTER IN ESTUARINE ENVIRONMENTS IN THE CASE OF AMERICIUM OF MARINE ORIGIN.

F. Paquet, P. Guegueniat, P. Germain, R. Gandon

In an EC programme (MAST) it has been shown that the waters of the Channel, contaminated by the waste from the fuel reprocessing plant at La Hague, flow along the coasts of Belgium, Holland, Germany and Denmark and mix very slightly with the central waters of the North Sea, contaminated by Sellafield waste. These observations are based on a study of antimony-125 carried in the water flow. It follows from this that the input from the major rivers (Seine, Rhine, Escaut, Meuse, Elbe, Weser...) are only slightly dispersed in the bulk of the Channel and the North Sea. The probability of complex formation between trace elements carried in the water and organic matter or other complexing agents derived from river sources, is therefore high. Before considering a medium scale study of the dispersion of transuranic elements, it would be desirable to determine experimentally, by means of americium, if complex formation would have to be taken into consideration as an important parameter in future studies. By way of comparison the transport of cobalt-60 will also be studied.

A first series of studies compared the complexing properties of the estuarine waters of the Seine, Rhine, Escaut and of the Vire (the western area of the Seine Bay). The following values of organic carbon were found respectively: 8.5, 4.5, 4.3 and 3.2 mg/l. The technique used was to determine by means of cationic resin chromatography the degree of complex formation as indicated by a change in chemical composition (Guegueniat, 1971. Rapport CEA-R4125; Marchand, 1974. J. Cons Int. Explor. Mer 130/142). The americium-241 and the cobalt-60 were introduced into waters of variable salinity but possessing a constant concentration of organic ligands. The salinity was adjusted by the addition of salt before the addition of the radioactive tracers so as to simulate a marine environment. If it was desibable to simulate a river environment for the same tracers, these would have to be introduced before the addition of the salt. After a contact time of seven days, the complex formation (soluble anionic or neutral form) in a salinity of 15% showed the following percentages:

Cobalt-60: Seine (12%), Escaut (4%), Vire (3.5%), Rhine (2%)
Americium-241: Vire (50%), Escaut (35%), Seine (32%), Rhine (13%).

The complex formation represents, therefore, a factor which must be taken into consideration at least within the estuaries of large rivers. The significance of this complex formation may ve very variable according to the particular case studied and to the particular radionuclides used. In this respect the most interesting waters to study are those of the Seine (for cobalt-60) and the Vire (for americium-241). Complex formation with americium-241 (30 to 60%) is generally superior to that of cobalt-60 (3 to 12%). The rhine, which has twice the level of organic carbon compared with the other rivers studied, has a relatively weak complex forming capacity, both for cobalt-60 and for americium-241. The development of complex formation as a function of salinity and time has been studied for americium and for cobalt.

Head of Project 4: Dr. Damiani

II Objectives for the reporting period

- Study of the time evolution of radioactivity levels in the North Adriatic Sea. Chernobyl radionuclides as tracers of sedimentation and erosion processes.

- Studies on the distribution of natural radionuclides in seawater and suspended particulte matter: scavenging of particle reactive nuclides in selected ecosystems.

III Objectives for next period

- Studies on the vertical distribution of natural and artificial radionuclides in the water column and in sediments of mediterranean deep-sea environments.

- Transport of radionuclides from the continental shelf to deep-sea in uncontaminated (Gulf of Taranto - Ionian Sea) and potentially contaminated (Palomares - Balearic Sea) areas.

IV Progress achieved including publications

1. Radioactivity levels in North Adriatic Sea

Following the Chernobyl accident, a multiannual research programme was planned in the North Adriatic Sea, to study the evolution of the levels of radioactivity in different compartments of the marine environment for a better understanding of radionuclides dynamics in the natural environment.

The N-Adriatic is a shallow water area, strongly influenced by the input of freshwater and particulate matter exported by the major Italian rivers, which drain the Alps basin, where Chernobyl fallout was highest in Italy.

The evolution of the concentrations of Cs-137 and Sr-90 in seawater has been studied in the period June 86 to September 90. The levels of the two radionuclides are presently quite uniform in the area extending from the Gulf of Trieste to the Po river delta. In September 1990 the average concentration of Sr-90 (2.5 mBq/l) was very similar to pre-Chernobyl values, while Cs-137 levels were still slightly higher (average concentration: 6.02 mBq/l). Due to the much higher Chernobyl deposition of Cs-137 with respect to Sr-90, the ratio Cs-137/Sr-90 raised to 12 in June 86 and regularly decreased to the present value of 2.4. The time evolution of this ratio may be used for the characterization of the dense waters forming during the winter in the N-Adriatic area.

The concentrations and inventories of Cs-134 and 137 in sediments were highest in the pelitic areas and in the terminal tract of the major rivers (Isonzo and Tagliamento). Inside these areas, high variability in concentrations and inventories was found. Restricted zones close the the river mouths showed inventories of Cs-134 one order of magnitude higher than the average value for the pelitic area (0.6 kBq/m2). The samples collected in 1989 confirmed this trend, with concentrations of Cs-134 and Cs-137 on the order of 20-30 Bq/kg and 150-200 Bq/kg respectively, in the most contaminated areas.

The sediment cores were characterized by the absence of pre Chernobyl Cs-137 in the area of influence of the Tagliamento river. Erosion processes may be active in this shallow water area (10 m), where relevant meteorological events may lead to resuspension and southward transport of surface sediments.

A similar situation was evidenced near the Po river delta: the highest concentrations of radionulides were found in the pelitic area, where the levels of Cs-134 and Cs-137 were, in 1989 and 1990, on the order of 4 and 30 Bq/kg respectively. However, in a small, protected zone, southwest of the delta, the levels of the two radionuclides were substantially higher (20 Bq/kg of Cs-134 and 100 Bq/kg of Cs-137).

In the sediment cores, Cs-134 was present in the first 4 cm, indicating a maximum sedimentation rate in the area on the order of 1 cm/y.

In 1990 preliminary measurements were carried out on the distribution of natutural radionuclides in the water column, for the study of gas fluxes between sea and atmosphere. The average Ra-226 concentration in N-Adriatic seawater was 2.8 mBq/l. The measurements were carried out during the summer, when the thermocline was well defined. From the Rn-222 deficit in the wind mixed layer the Rn fluxes (5-7 Bq/m2 d) at the air-sea interface and transfer velocities (4-7 m/d) were calculated at two stations. Below the thermocline, increasing Rn-222 concentrations were measured, due to the vertical and lateral transport from bottom sediments and from adjacent shallow water sediments respectively.

Scavenging of particle reactive nuclides in the Gulf of La Spezia (Ligurian Sea).

The disequilibrium U-238/Th-234 has been used to determine the rates of removal processes in the Gulf and inside the harbor of La Spezia, in the framework of a general programme on biogeochemical fluxes in the area.

Large volumes (6-8 m3) of water were filtered and passed through BaSO4 impregnated Al2O3 sorption beds for the quantitative recovery of dissolved thorium and radium isotopes. The distribution coefficients of Th-234, calculated at different stations and different water depths ranged from 2x10E6 to 5x10E6. The mean residence time of Thorium was on the order of 1 day in the dissolved and of 5 days in the particulate phase. The sedimentation rate obtained by the vertical profiles of Th in the water column, 3.5 mm/y, was comparable to that calculated from the vertical profiles of Pu-239,240 and Pb-210 in a sediment core collected in the same area (4.6 mm/y).

Publications

Queirazza G., Roveri M., Delfanti R. and Papucci C. - Gas exchanges at the air-sea interface: a technique for radon measurements in seawater. Proceedings of the international symposium on Radionuclides in the study of marine processes, Norwich, sept. 1991. In press.

Delfanti R., Fiore V., Papucci C., Moretti L., Tesini E., Salvi S., Bortoluzzi S., Nocente M. and Spezzano P. - Chernobyl radionuclides as tracers of sedimentation processes in the northern Adriatic Sea (Italy). Ibid.

Battaglia A., Delfanti R., De Pasquale N., Fiore V. and Papucci C. - Indagine preliminare sui processi di rimozione e sedimentazione del materiale particellato con l'uso di radiotraccianti naturali. In: indagini sulle caratteristiche ambientali delle acque del golfo di La Spezia. Rapporto ENEA-ENEL, Gennaio 1991.

Progress Report

Contract: Bi7-006 **Sector:** A22

Title: Behaviour of Polonium-210 and Lead-210 in European marine environments. Application of bioindicators.

1	Köster	RIVM
2	Guegueniat	CEA - Cherbourg
3	Duursma	NIOZ
4	Galvào	LNETI

I. Summary of Project and Global Objectives

The radiation dose stemming from the marine environment is dominated by the consumption of fishery products. The radionuclide Po-210 (T1/2 138 days), a daughter of Pb-210 (T1/2 21 year), has a dominant part in this. Locally large quantities of these natural radionuclides are released into the coastal environment by non-nuclear industries (e.g. in phosphogypsum effluents of Phosphorous industries).

The study has the general aim to obtain insight into the effects of such industries on the activity levels and distribution of Po-210 and Pb-210 in abiotic components and bioindicators, both in estuaries and in nearby coastal waters. And to apply the obtained insights in the development of a model to predict Po-210 and Pb-210 distribution and levels.

The four participants of the contract cover a wide geographical range. Depending on the situation and expertise each participant directs his research to certain parts of the chain: emission, dissolution/sorption, distribution/ accumulation in the abiotic environment, coupled with modelling and coupled with the study of potential bioindicators. By doing this in a coordinated joint study a better insight will be obtained in the chain from emission to effect and in the geographical differences.

The study encompasses the Westerschelde estuary in the Netherlands, the Seine estuary in France and the Tagus estuary in Portugal. A link will be made to the data of the Rhine estuary executed in 1989 (CEC project Bi6-0328-NL). In each of the above estuaries large quantities of Po-210 and Pb-210 are emitted by local phosphate industries. Natural background levels will be studied in Portugal in the Mira estuary which does not receive any industrial input of Pb-210 and Po-210.

Conclusions of general applicability will be drawn and location specific differences will be identified from the data and from the conclusions of the five estuaries studied. This will give new inputs both to generic and location specific modelling as to the direction of future research.

Head of Project 1: Dr. Köster

II Objectives for the reporting period

1) Sampling and characterisation of effluents of Phosphor producing industries in the Netherlands: two wet process plants near Rotterdam at the Nieuwe Waterweg (approx. Po-210 emission 2 E12 Bq.a^{-1}) and one thermo-process plant near Vlissingen at the Westerschelde (approx. Po-210 emission 1 E11 Bq.a^{-1} via effluents and a fivefold quantity into the atmosphere).
2) Reconnaissance of generic abiotic distribution models for estuaries and an inventory of such models specifically for the Westerschelde.
3) Development/installation and first calculations with an abiotic Westerscheldt model for distribution of Po-210/Pb-210.

III Objectives for the next period

1) Laboratory experiments with effluents samples to study: rate of dissolution into surface water; adsorption on suspended matter in surface water; influence of degree of salinity of surface water; the link to findings in environmental studies.
2) Expansion of simple Westerschelde model with Po-210/Pb-210 disequilibria and atmospheric source term. Calculation of dissolved and particulate Po-210/Pb-210; incorporation of effluent specific parameters; sensitivity analyses and validation; evaluation of the shortcomings/advantages of the simple as compared to a detailed complex DELWAQ-type model.

IV Progress achieved including publications

1. Studies of effluents from phosphor producing industries.

Contacts have been made with Rijkswaterstaat, of the Ministry of Transport and Public Works, to obtain samples of phosphogypsum effluent emitted into the Nieuwe Waterweg by the plants near Rotterdam. Handling of these samples required at all stages special precautions since the gypsum precipitates and hardens when the sample is not in motion. Besides the analyses of the effluent (Table 1), a laboratory study was made of its behaviour upon dilution with artificial seawater without suspended matter (Table 2). Notable results are: similarity of the effluents of the two different fertiliser plants; 25% total solid matter in the effluent; of which an increasing amount dissolves with increasing seawater dilution, 6% remains insoluble at a dilution of 1:2.300. This dilution is about 3 times less than the maximal dilution in the Nieuwe Waterweg. The dissolved Po-210 concentrations found in the Nieuwe Waterweg, 0,4 - 32 Bq.m^{-3} (Berger), show a remarkable similarity with the values found in the laboratory study, 10 - 40 Bq.m^{-3}. The Po-210 activity in the (dry) solid matter increases from 900 in the phosphogypsum to 14.800 Bq.kg^{-1} in the insoluble residue. The latter contains 90% of the Po-210 initially present in the effluent. In some harbour basins near the fertiliser plants Po-210 concentrations of 100 to 3.000 Bq.kg^{-1} have been found in bottom sediments, while in the suspended matter in the Nieuwe Waterweg these concentrations range from 100 to 12.000 Bq.kg^{-1} (Berger). The last value is comparable to the value 14.800 found in the laboratory study. These first results indicate that the laboratory studies provide valuable information for the interpretation of field observations and for the application in distribution modelling.

Contacts have been made also with the industry at Vlissingen to obtain samples of its effluents. In the thermo-process most of the Pb-210 and Po-210 sublimates in the sinterovens. Offgas filter water has been sampled near the sinteroven and close to the point of discharge in the Westerschelde, where large quantities of cooling(sea)water and some waste water from other processes have been added to it. At the point of discharge the offgas filter water contains 4 Bq.kg^{-1}, or 4000 Bq.m^{-3} Po-210 and 0,01

$g.kg^{-1}$ solid matter. Further analyses coupled with laboratory studies of this effluent, as well as the link to field observations and the application of the results in the modelling of the Westerschelde are in progress.

Table 1. Analyses of phosphogypsum effluents (grab samples) from two fertilizer plants near Rotterdam, the Netherlands.

	Vlaardingen		Vondelingenplaat	
	Average	Range	Average	Range
Solid matter (dry) $g.kg^{-1}$	267	259-277	235	233-239
% Insoluble of solid matter (when effl. diluted 2300x)	5,9	5,6-6,3	not analysed	
Pb-210 in effluent $Bq.kg^{-1}$	274	260-291	225	206-238
Po-210 in effluent $Bq.kg^{-1}$	251	240-260	174	166-192

Table 2. Behaviour of Po-210 after dilution of phosphogypsum effluent (Vlaardingen sample) with artificial seawater, laboratory study.

	Ratio seawater/effluent (vol./vol.)			
	0/1	23/1	230/1	2300/1
Po-210 in diluted effluent (total) $Bq.m^{-3}$	297000	12800	1270	128
Po-210 in aqueous phase $Bq.m^{-3}$	450	41	17	10
Po-210 in solid matter $Bq.kg^{-1}$	940	1140[1)]	10700[1)]	16000[1)]

[1)] derived from the activities listed in the same column.

2. Modelinventory.

An inventory of Westerschelde and of generic estuary models has been completed for the most part. The following models were studied in detail:

* *DELWAQ* developed by Delft Hydraulics Laboratory. It can be used to calculate the transport and distribution of heavy metals in the longitudinal, lateral, and vertical direction, in the Westerschelde. It is a rather complex model based on the advection-dispersion-equation, which is solved numerically. It computes the concentrations (total, dissolved, particulate) for each of the compartments distinguished in the model area. The compartments can be linked in all directions. It can perform steady-state as well as dynamic simulations, required to cope with discontinuous waste discharges. DELWAQ is in trust of Delft Hydraulics, and is not freely accessible.

* *"O'Kane - method"* (O'Kane 1980), a simple steady-state model. With this method different compartments, in the longitudinal direction in the model-area can be distinguished. For each compartment a massbalance is written. To obtain the concentration per compartment the system of lineair equations can be solved by matrix manipulation. The exchange between the compartments in the estuary can be calculated from the salinity data. The salinity in the boxes is assumed to depend on advection and dispersion.

* *"Simplified differential equation method"* (Jörgenson, 1988). As in DELWAQ this method solves the advection-diffusion-equation mathematically for the longitudinal direction. Further differences are that it calculates only the concentration for steady-state situations, and that it can cope with only one source-term. The simplified differential equation distinghuishes no compartments, but solves the equation by which a longitudinal gradient for the total estuary is obtained.

3. Model development and calculations

The O'Kane-method has been tested for the Pb-210 distribution in the Westerschelde. The length of this estuary is 70 km from the Belgium/Dutch border till Vlissingen at the North Sea. Five compartments are selected based upon the chloride levels. The inputparameters per compartment are: the geometry (volume, estuary width); the sedimentation rate; the chloride

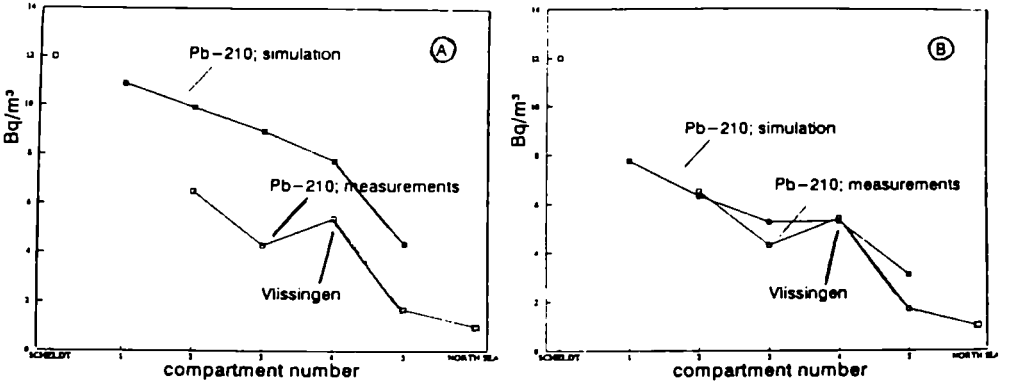
level to express the longitudinal mixing; the suspended matter load; and the parameters listed in Table 3. The flow rate and the Pb-210 activity of the river Scheldt have been used both as source term and as boundary conditions at the Dutch/Belgian border. For the Phosphorous plant at Vlissingen the Pb-210-emission (as specified in its emission permit) has been taken as source term. Two scenario's were defined: scenario A without and scenario B with sedimentationa at a rate of 4,3 mm.a^{-1}.

Table 3. Input-parameters for the calculations of the Pb-210-distribution in the Westerscheldt with the O'Kane model.

Flow rate river Schelde	105	$m^3.s^{-1}$
Pb-210-emission at Vlissingen	1000	$Bq.s^{-1}$
K_d or distribution coefficient of Pb-210	5	$m^3.kg^{-1}$
Pb-210 concentration in the river Schelde	12	$Bq.m^{-3}$
Pb-210 in the North Sea	1	$Bq.m^{-3}$

From the limited comparison it appears, that the measured concentrations do not differ much from the calculations (Figure 1). It can be concluded, that based upon the O'Kane-method a rather satisfactory model has been developped to calculate the steady-state Pb-210 concentrations in the Westerschelde as affected by waste discharges from the phosphate-industry. More detailed conclusions can be drawn after comparison of the calculations with other measurements and after sensitivity analysis of the model.

Fig. 1. The total Pb-210 concentrations (dissolved + particulate) in the 5 compartments in the Westerschelde. Field measurements and results of the model without (A), and with sedimentation (B).



4. Publications, accepted for NRE-V in Salzburg Sept. 1991. Fifth international symposium on the natural radiation environment:
Köster H.W., Marwitz, P.A. (RIVM), Berger G.W. (NIOZ), Weers, A.W. van (ECN), Hagel, P. (RIVO), Nieuwenhuize, J. (DIHO). Po-210, Pb-210 and Ra-226 in aquatic ecosystems, anthropogenic sources, distribution and radiation doses in the Netherlands.
Pennders, R.M.J., Köster H.W., Lembrechts, J.F. Characteristics of Pb-210 and Po-210 in effluents of phosphate producing industries, affecting the distribution of these nuclides upon emission into surface waters.

Head of Project 2: Dr. Guegueniat

II Objectives for the reporting period

In the Seine estuary and the nearby Channel coast a detailed study was made of the Po-210 levels in the mussel Mytilus edulis and in the brown algae Fucus vesiculosus to characterise their potential as bioindicators. Special attention was given to the geographical and seasonal variation in these species.

III Objectives for next period Some striking and unexplicable data were observed. So, the activities of Po-210 will be measured in Mytilus edulis and Fucus vesiculosus as a function of both space and time (Seine estuary and control sites), because of levels of activity are controlled not only by variations of input but also the environmental conditions and certain biological cycles. Po-210 will be measured in sediments where factories have released phosphate gypsum directly into the Seine downstream from Rouen, in order to know the importance of this "source term". Pb-210 will be measured in Mytilus edulis. Studies will be executed to determine the characteristics of Po metabolism by M.edulis.

IV Progress achieved including publications

We have set out to investigate the migration of ^{210}Po in a zone influenced by the discharge of phosphatic gypsum waste which provides a radiolabelled source term, the Seine estuary. In the context of this study, the Marine Radioecology Laboratory developed a technique for the analysis of ^{210}Po, in 1990.

Analytical methods :

Aliquots (0.5-1.0 g) of the powdered biological and suspensions are placed in Teflon bombs along with a ^{208}Po spike (half-life = 2.9 years) used as an isotopic tracer.

The determination of ^{210}Po is performed by the classic method of acid digestion followed by spontaneous deposition onto a silver disc.

As regards the sea-water samples, both filtered and non-filtered sample aliquots (20 l) are acidified before addition of the ^{208}Po spike.

Then, the pH is brought back to values 8-9, by the addition of concentrated ammonia solution. Subsequently, 1600 mg of $KMnO_4$ (in solution) were added along with H_2O_2 in order to provoke the precipitation of MnO_2. After agitation, the supernatant is decanted off and the precipitate recovered then dissolved in 1.2 M HCl containing H_2O_2.

All the samples are taken up into HCl and made up to 0.3 M HCl. Then, the polonium is deposited spontaneously onto a pellet of silver during agitation at 90°C for four hours. Measurements of ^{210}Po activity are performed on an alpha-ray spectrometer equipped with semiconductor detectors (implanted Si passive junction type; 300 mm^2 area) coupled to an analyser. The extraction yields were 80-100 %.

We participed in intercomparison exercice with the sediment sample coded IAEA-368. The result obtained for ^{210}Po is 26.20 ± 0.98 Bq kg^{-1} dry weight (and for ^{210}Pb : 20.7 ± 1.2 Bq kg^{-1} dry weight, gamma spectrometry).

- Geographical zone covered by Polonium-210 studies
In the estuary, severeal station were chosen on the basis of the presence of the indicator species used in this study. In order to better characterize the labelling of indicator species by industrially-derived waste, collection site were selected as baseline controls in various zone located outside the estuary : to the east of the Seine estuary (Fécamp, Wimereux and Gravelines); in the Baie de Seine, west of the estuary (Luc-sur-Mer, Port en Bessin, Pointe de Moulard); west of the Cotentin peninsula (Agon-Coutainville, Pirou, Herquemoulin).

- Nature of industrial releases
Industries discharging phosphatic gypsum have operated for nearly 60 years in the lower Seine valley; over a period of several years, factories near Rouen have released phosphatic gypsum directly into the Seine downstream from Rouen. This type of waste is also released into the Seine estuary by Norsk Hydro Azote (NHA). From 1974, two factories in the Rouen area began to transport their gypsum waste by barge for disposal in the baie de Seine; these activities continued up to 1984 for one factory and 1987 for the other. At present, all the waste produced from these factories is stockpiled on land. The NHA plant continues to release 70,000 tonnes per month of phosphatic gypsum from an outfall into the Seine estuary; about 25 % of the waste produced by this factory is now stored on land.

- Results
In the Seine estuary, at Le Havre, for sea-water filtered at 0.45 µm, activities fluctuate between 0.5 and 1 mBq l^{-1}. There is a tendency for ^{210}Po levels to fall off between June and October 1990. In non-filtered sea-water samples, activities are higher (from 2.5-14 mBq l^{-1}) and are clearly related to the degree of turbidity. ^{210}Po levels are homogeneous in the suspended matter samples (130-180 Bq kg^{-1} dry weight). For *M. edulis*, activity levels lie in the range 90-700 Bq kg^{-1} on a dry weight basis. Samples from stations in the estuarine zone show a marked fall in ^{210}Po activity from March to May/June. At the estuarine stations, values of the order 250-350 Bq kg^{-1}dry weight were measured in March 1990; after a marked decrease in April and May, activities reached fairly constant levels of 120-180 Bq kg^{-1} dry weight during August and the end of the year. This decrease is even more marked at the NHA outlet pipe, from 700 to 140 Bq kg^{-1} dry weight. At control sites, Wimereux-Gravelines, Pointe de Moulard, Agon-Coutainville-Pirou, ^{210}Po levels in mussels show respective values of 180-250, 90-150, 250-340 Bq kg^{-1} dry weight.
For *F. vesiculosus*, ^{210}Po activities are definitely lower than in mussels; values fall in the range 3-22 Bq kg^{-1} dry weight. Fluctuations are apparent. They are different from mussels. In *F. vesiculosus* collected from the estuarine zone, ^{210}Po levels are always higher than samples taken along the Channel coast

except Pirou (Nov. 1990); values obtained range from 12 to 20 Bq kg^{-1} dry weight. Outside the estuary to the west, levels are of the order 4-6 Bq kg^{-1} dry weight, whereas Wimereux in the east shows levels around 10 Bq kg^{-1} dry weight.
The Seine estuary appears as a distinct source-term for ^{210}Po in *F. vesiculosus* at all times, whereas *M. edulis* shows an influence from fluvial input only during the sampling campaign of March 1990. In fact, activities are enhanced in the estuarine zone with respect to control stations along the Channel coast. However, this effect is less clear in the case of mussels collected after March 1990. Although it is true that mussels from the estuarine zone have slightly higher activities than mussels in the Baie de Seine (Pointe de Moulard, Port en Bessin, Luc sur Mer), mussels from other control stations (Agon-Coutainville and Wimereux) have activities which are higher than those recorded in the estuary itself.
The evolution of ^{210}Po levels are difficult to explain since the discharge of phosphatic gypsum from the NHA plant varies only very slightly with time. Thus, to account for the variations in ^{210}Po activity, it may be necessary to invoke other source-terms apart from the NHA outlet, including fluvial inputs from the Seine. Furthermore, we need to improve our understanding of the impact of other ecological factors (e.g flow rates, turbidity, storm effects...) on the ^{210}Po activities observed in mussels.

Publication
An abstract is submitted to "Fifth International symposium on the natural radiation environment". Salzburg, sept. 1991
GERMAIN, P., LECLERC, G., SIMON, S., Distribution of ^{210}Po in Mytilus edulis and Fucus vesiculosus along the Channel french shore : influence of the industrial releases in the Seine river and estuary.

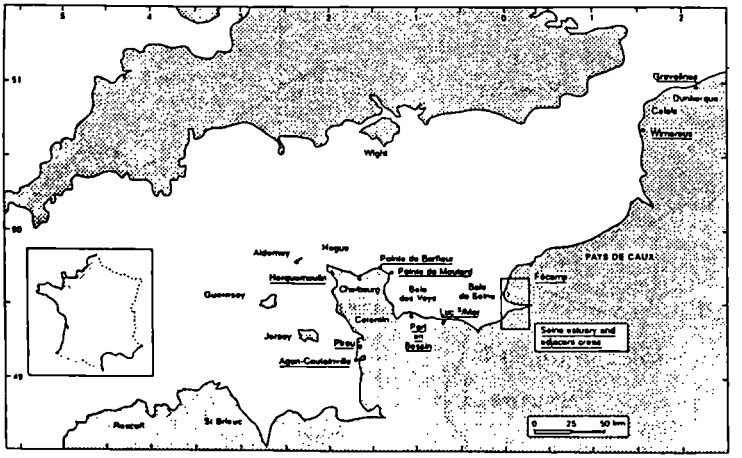


Figure 1 - Sketch map showing location of sampling stations

Head of Project 3: Prof.Dr. Duursma

II. Objectives for the reporting period

The objectives for the reporting period (june 1990 - june 1991) were:
-- to carry out a sampling programme in the Westerscheldt estuary and the coastal zone
-- to analyse the obtained samples in order to get insight in the sources, pathways, sinks and distribution of Po-210 and Pb-210 in this area as a logical continuation of work that has been done by the NIOZ under EC contract B16-0328-NL in the Nieuwe Waterweg in previous years

III. Objectives for next period

Attention will be payed to the analyses of Pb-210 in order to study Po-210/Pb-210 equilibria, to the analyses of Po-210 and Pb-210, dissolved in water in order to estimate K_d values. Will be analyzed in sediment and suspended matter samples so that excess Pb-210 and Po-210 can be estimated. The need for additional sampling in view of the modelling of the data will be evaluated in cooperation with the RIVM, Bilthoven, The Netherlands.

VI. Progress achieved including publications

During the first week of October 1990 a sampling cruise was undertaken in the Westerscheldt. Due to weather conditions sampling in the coastal zone was not possible.
Altogether 58 samples were taken: 37 water and suspended matter samples, 21 sediment samples. Besides, suspended matter concentration, watertemperature and salinity were measured. In the sediment and particulate matter samples Po-210 has been analysed.

Preliminary results

Figure 1 shows, the map of the Scheldt estuary, the sampling locations and the general distribution pattern of Po-210 adsorbed on suspended material.
Po-210 concentrations adsorbed on suspended material are in the range from 70 Bq.kg-1 (coastal water) to 250 Bq.kg-1 (Zelzate, Channel of Sas van Gent naar Terneuzen). Relatively high concentrations are found between Doel and BASF and in the western part of the estuary near Vlissingen.

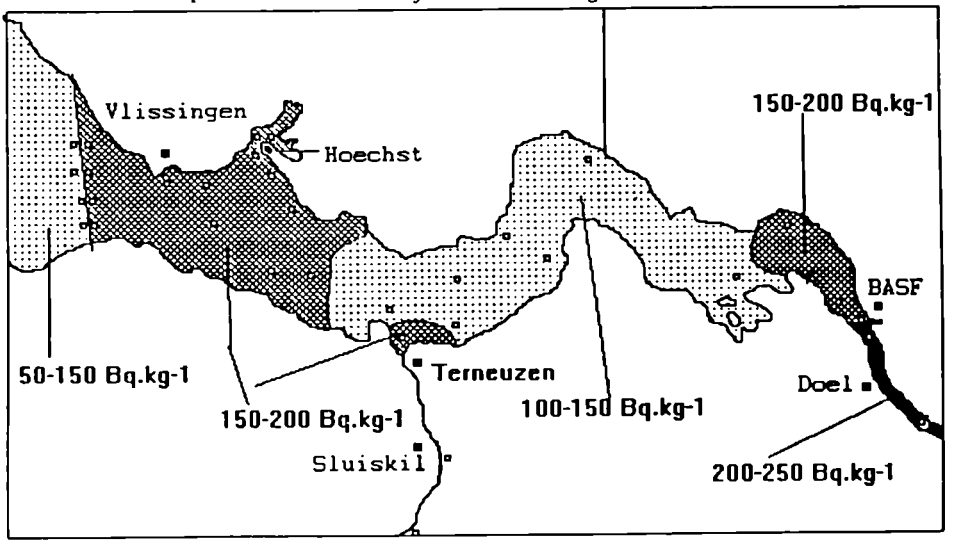


Fig.1 Concentration of Po-210 in suspended material.

Po-210 concentrations in sediments collected in Westerscheldt estuary range from 6 to around 400 Bq.kg-1. The highest concentration is found in the Channel of Sas van Gent naar Terneuzen near the former phosphate processing plant "Zoutchemie". Sediment collected near BASF (concentration 170 Bq. kg-1) seems to be above the natural background, as is the case with a sample taken in the Sloehaven, Vlissingen (148 Bq.kg-1).

Discussion and preliminary conclusion

In the reporting period samples were taken in order to study sources, sinks and the dynamics of the distribution of Po-210 and Pb-210 in the Westerscheldt.
Although not all of the samples taken in the reporting period have been analyzed or interpreted, some preliminary conclusions can be drawn.
Compared with for instance the Nieuwe Waterweg, it seems that the Westerscheldt estuary does not act as a sink for Po-210. The concentrations in the sediment is more or less around the natural background (< 100 Bq.kg-1), with the exception of the Sloehaven and near BASF. This is confirmed by previous Pb-210 measurements in sediments in areas in the Westerscheldt where relatively fine material settles (Berger, G.W. & D. Eisma, 1988b).
This does not hold for Po-210 concentrations as found in suspended matter. The average concentration is around 150 Bq.kg-1, which is higher than concentration as found in the North Sea in previous studies (Berger, G.W. & D. Eisma, 1988; Köster et al., 1990)
The results of this project will be presented at the Radstomp 91 Symposium (Radionuclides in the study of marine processes, 9-14 september 1991, Norwich, UK), title: "Sources, distribution and radiological effects of Po-210, Pb-210, Ra-226 and Th isotopes in Dutch rivers and coastal waters related to the discharges of ore processing plants".

G.W. Berger, Netherlands Institute for Sea Research, Texel.

Literature

Berger, G.W. & D. Eisma, 1988a, Report of Po-210 en Pb-210 measurements in the Dutch coastal waters, rivers and Westerscheldt estuary (in Dutch, VROM project) NIOZ report 1988-2

Berger, G.W. & D. Eisma, 1988b, Dating of sediments in the Westerschelde with the isotopes Cs-134/137 and Pb-210. NIOZ report 1988-12 (RWS project, in Dutch)

Köster, H.W., P.A. Marwitz, G.W. Berger, A.W. van Weers, P.Hagel, J. Nieuwenhuize. RIVM Report 248476004, August 1990 (in Dutch) Po-210 and other natural radionuclides in Dutch aquatic ecosystems; an reconnaissance investigation.

Head of Project 4: Dr. Galvão

II Objectives for the reporting period

In Portugal, a river/estuary, on which no industrial Po-210 emissions take place, will be studied to establish the natural background levels and their variation in this environment. In the Tagus estuary and adjacent coast the focus will be on the distribution of Po-210 and Pb-210 discharged by the phosphorus industry. The major emphasis will be on abiotic materials and to a lesser extent on bioindicators.

III Objectives for next period

The study on the distribution of Po-210 and Pb-210 in the same estuaries during summer conditions: low river flow, higher temperatures and different biological conditions. The whole of this research will alow the comparison between winter and summer conditions in abiotic and biotic components of both estuaries. The comparison of Po-210 and Pb-210 concentration levels in one estuary receiving P-plant effluent discharges and another estuary receiving no industrial discharges, will emmerge from this research.

IV Progress achieved including publications

1. Areas under study

The estuary of the Tagus receives the liquid wastes and undetermined amounts of phosphogypsum from a phosphate fertilizer plant located in the south margin and operating since 1951. The Tagus river drains a catchement area of 86 000 km2 and its average annual flow is of 1.3E10 m3. The estuary has 200 km2 of surface area and receives the industrial and urban effluents from a densely populated region.Its morphology fits the category of tidal lagoon estuaries.

The estuary of the Mira river, located some 200 km south of Lisbon, receives no industrial effluents. The Mira drains a much smaller catchement area, of 1580 km2, and flows across a region with no major towns. Besides the runoff from agricultural lands - agriculture is less intense than in the Tagus valley - it is an acceptable non-modified reference environment. The morphology of this estuary belongs to the funnel-shaped type.

2. Materials and methods

Sampling of phosphate raw materials, phosphogypsum and fertilizers was directly made in the factory and in the adjacent gypsum ponds and liquid wastes outlet.

The sampling of the Tagus and Mira estuaries was performed during high river flow (Jan - March), through the use of an inflatable rubber boat. Sediment samples were collected with a small box - corer and water samples were collected directly into plastic containers and filtered on shore. Biological samples - fish and shrimp - were taken in the Tagus estuary with a trawl net. Intertidal organisms - seaweeds, Balanus and molluscs - were colllected by hand on piers and in soft bottoms.

Po-210 measurements are made by alpha-spectrometry on Ag planchets, with the use of Po-209 as isotopic tracer. Pb-210 is calculated from a second Po-210 plating made 6 months later. A check

of the radioanalytical quality of the results was made through the participation in an intercomparison exercise.

3. Progress and status of the research

3.1. Results of the intercomparison exercise

The intercomparison was made with two IAEA sediment samples (Table I).

Table I - Results of the intercalibration exercise

Sample reference	number of aliquots analysed	Our result ^{210}Po (Bq/kg)	IAEA reference value (Bq/kg)
IAEA-SD-A1	6	71 ± 6	72 ± 2.5
IAEA-368	8	23.4 ± 1.4	24.3 ± 12.6 (a)

(a) Provisional results, ILMR, March 1991

3.2. Phosphorite, phosphogypsum and fertilizers

The source term of phosphatic materials released by the P-plant into the Tagus estuary is being adressed through the analysis of these materials (Table II).

The phosphogypsum collected in the ponds on the river bank of the Tagus estuary (Table II, indicated as A) displays much lower concentrations of uranium than phosphorite. However, the Ra-226 specific activity concentration is nearly the same as in the phosphorite, while Pb-210 and Po-210 have intermediate concentrations. Clearly the uranium series nuclides are fractinated during the ore processing and still important concentrations of Ra-226, Pb-210 and Po-210 remain in the gypsum. Most of the uranium and part of the Pb-210 and P0-210 are incorporated in the phosphoric acid produced. As the phosphoric acid is used in the manufacture of superphophate fertilizers, those nuclides are incorporated in those products. Furthermore, a linear relationship exists between the U contents of the fertilizer and its content in P2O5 (Carvalho, 1991).

3.3. The estuary of the Tagus river

During the sampling trips in January-March of 1991, about 40 surface sediment samples were taken, as well as 18 water plus suspended matter samples, and several biota samples, covering the whole estuary.

The analysis of these samples are being performed and results will became available throughout this year.

Table II - Specific activity concentrations (Bq/Kg) of uranium series radionuclides in phosphatic materials. Each value is the average of n samples. A designates samples from gypsum piles close to Tagus estuary; B designates other fertilizer plant.

	U-238	U-234	Ra-226	Pb-210	Po-210
PHOSPHORITE (n = 4) *	1003	996	1406	1083	954
PHOSPHATE FERTILIZERS:					
18 % P_2O_5 (n = 2)	632	630	862	638	604
30 % P_2O_5 (n = 1)	941	917	608	630	679
46 % P_2O_5 (n = 2)	1867	1863	342	547	382
PHOSPHOGYPSUM					
A (n = 6)	156	156	1043	----	586
B (n = 2)	26	40	950	589	655

* Marocco and Syria

3.4. The estuary of the Mira river

The samples collected during the winter season comprise 18 water samples from the mouth of the estuary up to freswater, covering the entire range of salinity. Suspended matter and bottom sediments from several stations are currently being analysed.

4. Final remarks

The winter sampling programme has been sucessfully performed in Tagus and Mira estuaries. The whole of samples collected covers the abiotic and some biotic components of these ecosystems.

Reliable information about the phosphoric acid production since the start of the P-plant operation and about the disposal of gypsum in the past, was not yet made available by the industrial company. To evaluate the actual gypsum and liquid waste discharged into the Tagus it is foreseen to made repeated analysis of the outlet pipe discharges.

5. Publications

Carvalho, F.P.(1991). Adubos fosfatados e radioactividade natural (Phosphate fertilizers and natural radioactivity). Proceed. of the XII Nat. Symp. of Chemistry, Lisboa, 10-13 March 1991, pp.451-454 (in Portuguese)

Carvalho, F.P. (submitted). Radioactive wastes from industrial phosphate ore processing in the Tagus estuary. 5th Int. Symp. on the Natural Radiation Environment, Salzburg, 1991

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-B-053-D

Kernforschungsanlage Jülich
Postfach 1913
D-5170 Jülich

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. F. Führ
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Title of the research contract:

Simulation of transfer via the soil-plant food chain after accidental release.

List of projects:

1. Simulation of transfer via the soil-plant food chain after accidental release.

Title of the project no.: BI 6-053-D

The soil-plant transfer of the radionuclides Cs-134/137 and Co-57/60 after simulation of a deposition subsequent to a long-term and accidental release from nuclear plants.

Head(s) of project: Dr. W. Steffens

Scientific staff: Dipl. Geogr. M. Bilo, Prof. Dr. F. Führ, J. Klaes, Dipl. Ing. W. Mittelstaedt, Dr. W. Steffens

I. Objectives of the project:

1. Determination of transfer factors describing the uptake of the radionuclides Cs-134/137 and Co-57/60 from soil into plants and food chains after simulation of a long-term contamination of the soil and of a high deposition of these radionuclides on soil and plants subsequent to an accidental release.
2. Investigation of the effects of different element concentrations in the soil on the transfer of Cs-134/137 and Co-57/60.
3. Effect and applicability of various soil cultivation methods (normal or deep plowing) to reduce the soil-plant tranfer of the radionuclides.

II. Objectives for the reporting period:

1. Transfer of Cs-134/137 and Co-57/60 from soil into arable crops, calculation of transfer factors dependent on the element concentration and the time period of these radionuclides in the soil.
2. Applicability of normal plowing and deep placement of the contaminated top layer to reduce the availability and uptake of radiocesium and radocobalt by plant roots and consequently also the contamination of the food chain.

III. Progress achieved:

1. Methodology

The first part of this project, which dealt with the transfer of Cs-137 and Co-60 from soil into plants after chronic deposition and with the effects of various Cs- and Co- concentrations, was started in 1984 and is described in the Progress Report for 1980 - 1984. Chronic deposition was simulated in 1984 by mixing an aqueous solution containing Cs-137 and Co-60 and additional stable Cs and Co as chlorides into the 0-30 cm soil layer of 16 lysimeters filled with undisturbed soil monoliths (70 cm deep) of a clayey silt (orthic luvisol). The radioactivity applied and the element concentrations in the soil after treatment are shown in table 1 and 2.

Table 1: Radioactivity of Cs-134/137 and Co-57/60 after repeated application(1984 and 1986) in MBq/Lysimeter

Lysimeter-Nr.	Cs-134	Cs-137	Co-57	Co-60
1 - 8	24.6	12.7	28.9	15.3
9 -16	24.6	12.4	28.9	15.6

Table 2: Elementconcentration of Cs and Co in the soil after repeated application(1984 and 1986). Natural concentration in the soil: Cs=1.4 mg/kg; Co=5.8 mg/kg.

	Element concentration, mg/kg of soil Mean values of 2 lysimeters each, 0-30 cm layer							
	1+3	2+4	5+7	6+8	9+11	10+12	13+15	14+16
Cs	1.4	1.4	1.4+	6.7	7.9+	10.6	7.9+	15.9
Co	49.6+	74.6	49.6+	67.8	5.8+	19.4	5.8	5.8

+ Additional stable Cs or Co in the original 0-6 cm soil layer placed into 34-40 cm depth

In 1985 the experiment was continued without any new treatment. Spring barley and lettuce were grown. Transfer factors were calculated from the radioactivity values determined in soil and plant samples.

In 1986 the experiment was extended to simulate a deposition of radionuclides on the soil subsequent to an accidental release from a nuclear installation by an additional spray of aqueous solution containing Cs-134, Co-57 and various concentrations of the respective stable element in chloride form on the soil surface of the lysimeters after sowing spring barley. There were one or three treatments at 6 day intervals (table 1 and 2). The radioactivity in soil and plant samples was determined. The data measured for Cs-137 and Co-60 were used to calculate transfer factors. Due to

the method of application, only the radioactivity in the plant dry matter was determined for Cs-134 and Co-57.

In 1987 the soil of a group of 8 lysimeters was tilled 30 cm deep. Thus the Cs-134 and Co-60 applied on the soil surface in 1986 was mixed into the plow layer. The top 6 cm layer of the other group of 8 lysimeters was taken off and after removal of the next 34 cm soil layer the top soil layer was placed at a depth of 34-40 cm and covered with the soil from the 6 - 40 cm layer removed previously. Carrots were grown in all lysimeters. The radioactivity data measured in soil and plant samples were used to calculate transfer factors.

An additional experiment was started in lysimeters ($1m^2$) to investigate the translocation of radiocesium in spring barley after contamination with Cs-134/137 at different developmental stages. An aqueous solution containing Cs-137 chloride (18.5 MBq) was applied on the soil surface after sowing spring barley. The same plants were treated with an aqueous solution containing Cs-134 chloride (1.85 MBq/treatment) at the developmental stages of tillering, at each of tillering, shooting and flag leaf formation and at flag leaf formation only.The radioactivity data measured were calculated as Bq/g dry matter.

In 1988 the lysimeter experiment was continued by growing wheat followed by lettuce and in 1989 potatoes followed by bush beans. The data measured in soil and plant samples were again used to calculate transfer factors.

2. Results

The mean radioactivity values of Cs-134/137 and Co-57/60 measured in soil samples of the different experimental groups are presented in table 3 and 4. For the groups with odd numbers, where the top 6 cm layer containing Cs-137 and Co-60 applied in 1984 and Cs-134 and Co-57 subsequently sprayed on the soil surface in 1986 was placed into the 34-40 cm layer, Cs-134 and Co-57 were found almost exclusively in this layer (table 3). The original uncontaminated 31-40 cm layer was mixed into the remainder of the 30 cm plow layer. Therefore, the radioactivity values of Cs-137 and Co-60 determined in the soil of these groups are clearly lower than the corresponding values of the groups with even numbers (table 4). In these groups the top layer contaminated with Cs-134 and Co-57 was mixed thoroughly into the plow layer.

In all crops grown during the experimental period the mean radioactivity values of Cs-134/137 and Co-57/60 increased by up to 1 - 2 orders of magnitude with increasing Cs- and Co-concentration in the soil by a factor of up to 12 and 13, respectively (table 2, 5 and 6). For all radionuclides higher radioactivity values based on dry matter were found in lettuce than in the other crops. After the application of Cs-134 and Co-57 onto the soil surface the root uptake of these radionuclides by spring barley (1986) and carrots (1987) was significantly higher than the uptake of Cs-137 and Co-60 mixed into the 0-30 cm plow layer. There were smaller differences between Cs-134 and Cs-137 and Co-57 and Co-60 in the crops grown in 1988 and 1989. Deep placement and mixing into the plow layer of the top 6 cm layer contaminated with Cs-134 and Co-57 reduced root uptake only in the experimental groups with low Cs- and Co-concentrations (table 2, 5 and 6).

After application of Cs-137 to the soil surface before the emergence of spring barley and of Cs-134 onto the plants at different developmental stages, a fairly low root uptake of Cs-137 was observed, whereas the uptake of Cs-134 via the areal parts and also the translocation into plant organs not develoing at the time of contamination was higher by several orders of magnitude and increased with the stage of development at the time of contamination (figure 1).

The transfer factors for Cs-134/137 and Co-57/60 calculated from the radioactivity values in soil and plant samples based on dry matter also increased with increasing Cs- and Co- concentration in the soil (table 2, 7 and 8).After the application of Cs-134 and Co-57 to the soil surface (1986) and also in the first vegetation period after mixing them into the 0-30 cm plow layer, the transfer factors in spring barley grains and carrots were distinctly higher than those calculated for Cs-137 and Co-60 in the same crops. In the crops of the following vegetation periods (1988 and 1989) the transfer factors of Cs-134 and Cs-137 and of Co-57 and Co-60 were nearly identical.

3. Discussion

The radioactivity values and the transfer factors for Cs-134/137 and Co-57/60 show, that for all crops grown during the experimental period (1985 - 1989), an increase of the Cs- and Co- concentration in the soil resulted in a higher availability of radiocesium and radiocobalt for root uptake. Apparently the sorption capacity of the clay minerals was not sufficient and the equlibrium disturbed, although these elements were mixed thoroughly into the soil. Due to the use of two of both Cs- and Co- radionuclides it was possible to investigate the transfer from soil into plants after simulation of a low chronic and a single high deposition subsequent to an accidental release from a nuclear installation. After the application of Cs-134 and Co-57 and of additional stable Cs and Co to the soil surface considerable root uptake was observed in the first and second vegetation periods, although these radionuclides were fixed in the upper 2-3 cm layer and the root uptake of nutrients in general takes place in the 5-25 cm soil zone. Also thorough mixing of the contaminated top 6 cm layer into the 0-30 cm plow layer produced only a small reduction. These results lead to the conclusion, that the transfer of radiocesium and radiocobalt from soil into plants will increase in the first and second year after a high deposition due to incomplete sorption and equilibration in the soil.

The results show furthermore, that deep plowing with thorough mixing into the plow layer and deep placement of the radionuclides can be a useful counter measure to reduce the contamination of the food chain via plant root uptake at least for plants like cereals, potatoes and bush beans, the roots of which are mainly concentrated in the upper 25 cm of the plow layer. As compared to root uptake or transfer into storage organs or seeds, the uptake and translocation of radiocesium from contaminated plant leaves into plant organs not yet formed at the time of contamination can be higher by several orders of magnitude.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Institute de Protection et de Suretée Nucléaire (IPSN)
Départment d'Etude et des Recherches sur l'Environment
(SERE), CEN Cadarache, 13115 Saint Paul Lez Durance, France.

V. Publications:

The transfer of cesium-134/137 and cobalt-57/60 from soil into plants after simulation of different types of deposition and effected by various element concentrations and soil cultivation investigated in lysimeters. Presentation at the IVth International Symposium of Radioecology of Cadarache on the Impact of Nuclear Origin Accidents on Environment

Table 3: Radiocesium and radiocobalt measured in the soil after placing the top 6 cm layer additionally contaminated with Cs-134 and Co-57 in 1986 into 34-40 cm depth. Lysimeter No. 1,3,5,7 = group 1, lysimeter No. 9, 11, 13, 15 = group 3

Year	Group No.	Soil layer cm	Mean radioactivity, Bq/g of dry soil Cs-134	Cs-137	Co-57	Co-60
1987	1	0-30	34	100	41	95
	3		42	97	50	107
	1	34-40	1083	127	1427	120
	3		1099	116	1222	119
1988	1	0-30	7	99	8	94
	3		7	99	7	97
	1	34-40	951	120	1075	106
	3		1012	112	1138	101
1989	1	0-30	7	90	7	92
	3		5	94	6	98
	1	34-40	901	114	1015	102
	3		975	106	1068	99

Table 4: Radiocesium and radiocobalt measured in the soil after mixing the top 6 cm layer additionally contaminated with Cs-134 and Co-57 in 1986 into the 0-30 cm plowed layer.
Lysimeter No. 2, 4, 6, 8 = group 2, lysimeter No. 10, 12, 14, 16 = group 4

Year	Group No.	Soil layer cm	Mean radioactivity, Bq/g of dry soil Cs-134	Cs-137	Co-57	Co-60
1987	2	0-30	284	140	355	133
	4		351	134	406	140
	2	30-35	99	42	115	40
	4		75	30	81	34
1988	2	0-30	256	126	302	119
	4		263	124	302	126
	2	30-35	5	2	7	2
	4		19	11	23	9
1989	2	0-30	255	118	289	120
	4		248	115	279	120
	2	30-35	4	2	7	2
	4		3	2	6	1

Table 5: Radioactivity in different plant organs after distribution of Cs-137 and Co-60 (1984) and Cs-134 and Co-57 (1987) in the 0-30 cm soil layer (lysimeters with even numbers) and after placing the top 6 cm soil layer, additionaly contaminated with Cs-134 and Co-57 in 1986, into 34-40 cm depth (1987, lysimeters with odd numbers)

Year	Plant organ	Lysimeter No.	Mean radioactivity, Bq/g dry matter			
			Cs-134	C-137	Co-57	Co-60
1985	Spring barley grains	1- 8		0.41		2.84
		9-16		2.35		0.19
	Lettuce	1- 8		7.48		27.00
		9-16		27.70		8.36
1986	Spring barley grains	1+ 3	10.72	0.88	22.81	5.04
		5+ 7	123.07	3.04	14.86	5.64
		9+11	14.11	3.46	18.95	0.73
		13+15	69.48	6.03	4.53	0.65
1987	Carrot roots	1+ 3	1.87	0.35	20.18	2.24
		2+ 4	3.22	0.54	14.72	2.76
		5+ 7	103.24	2.40	19.25	2.22
		6+ 8	20.67	2.41	9.22	1.96
		9+11	16.24	2.38	17.11	1.97
		10+12	21.13	3.75	6.31	1.14
		13+15	94.56	3.96	1.51	0.70
		14+16	64.89	7.05	2.97	0.91
1988	Spring wheat grains	1+ 3	0.23	0.15	1.12	0.44
		2+ 4	0.52	0.20	4.11	0.88
		5+ 7	4.85	0.51	0.82	0.47
		6+ 8	1.72	0.52	2.83	0.91
		9+11	0.51	0.60	0.73	0.10
		10+12	1.68	0.86	0.68	0.10
		13+15	8.89	1.09	0.14	0.12
		14+16	5.59	2.02	0.29	0.14
	Lettuce	1+ 3	0.79	1.88	19.36	11.08
		2+ 4	4.34	1.85	70.68	14.36
		5+ 7	48.23	4.31	23.65	12.73
		6+ 8	13.91	4.45	49.82	16.08
		9+11	4.89	24.23	13.23	4.28
		10+12	50.10	23.72	18.38	4.06
		13+15	67.06	19.40	3.26	2.89
		14+16	124.15	40.53	7.84	3.07

Table 6: Radioactivity in different plant organs after distribution of Cs-137 and Co-60 (1984) and Cs-134 and Co-57 (1987) in the 0-30 cm soil layer (lysimeters with even numbers) and after placing the top 6 cm soil layer, additionaly contaminated with Cs-134 and Co-57 in 1986, into 34-40 cm depth (1987, lysimeters with odd numbers).

Year	Plant organ	Lysimeter No.	Mean radioactivity, Bq/g dry matter			
			Cs-134	C-137	Co-57	Co-60
1989	Potato tubers	1+ 3	0.15	0.62	1.13	1.75
		2+ 4	2.13	1.03	11.03	2.87
		5+ 7	13.12	1.44	2.66	1.92
		6+ 8	5.38	1.98	7.03	2.56
		9+11	1.06	4.13	1.54	1.11
		10+12	8.55	4.26	2.82	0.98
		13+15	15.28	3.24	0.63	0.60
		14+16	18.60	7.39	2.34	0.90
	Green bean pods	1+ 3	2.76	1.49	28.04	9.38
		2+ 4	3.22	1.43	38.63	9.64
		5+ 7	120.82	7.49	16.90	6.99
		6+ 8	14.12	4.34	47.08	14.48
		9+11	8.51	4.65	20.04	3.12
		10+12	13.49	6.13	15.68	4.00
		13+15	191.39	18.71	8.99	4.46
		14+16	64.76	23.16	15.06	5.39

Table 7: Transfer factors for Cs-134/137 and Co-57/60 in different plant organs and related to dry matter.
Radioactivity and soil treatment in tables 1-4.

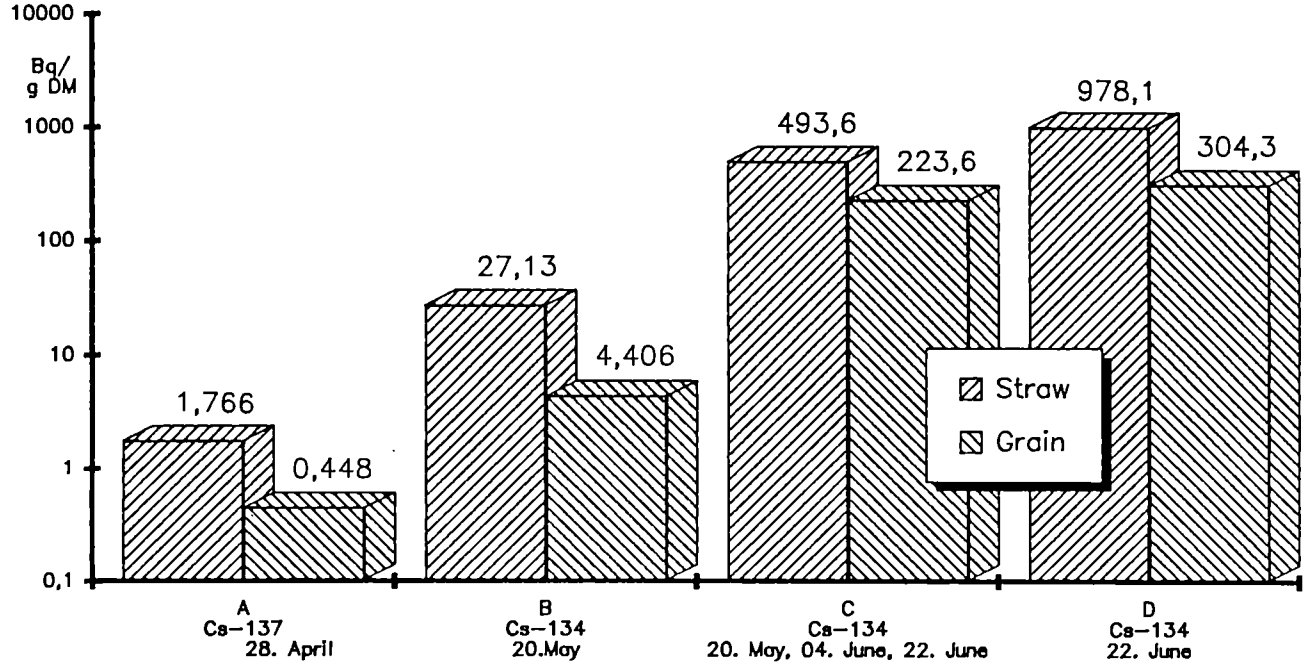
Year	Plant organ	Lysimeter No.	Mean transfer factors Cs-134	Cs.137	Co-57	Co-60
1985	Spring barley grains	1- 8		3.26 E-3		1.86 E-2
		9-16		1.90 E-2		1.24 E-3
	Lettuce	1- 8		5.90 E-2		1.77 E-1
		9-16		2.23 E-1		5.37 E-2
1986	Spring barley grains	1+ 3	1.52 E-2	2.62 E-3	3.12 E-2	2.11 E-2
		5+ 7	1.03 E-1	7.64 E-3	7.46 E-3	2.08 E-2
		9+11	1.76 E-2	1.51 E-2	4.38 E-2	4.48 E-3
		13+15	1.28 E-1	2.45 E-2	5.11 E-3	3.21 E-3
1987	Carrot roots	1+ 3		3.44 E-3		2.35 E-2
		2+ 4	1.06 E-2	3.82 E-3	3.93 E-2	2.08 E-2
		5+ 7		2.42 E-2		2.33 E-2
		6+ 8	8.19 E-2	1.75 E-2	4.27 E-2	1.48 E-2
		9+11		2.50 E-2		1.87 E-2
		10+12	5.35 E-2	2.85 E-2	1.55 E-2	8.54 E-3
		13+15		4.01 E-2		6.58 E-3
		14+16	2.14 E-1	5.17 E-2	7.64 E-3	6.27 E-3
1988	Spring wheat grains	1+ 3		1.41 E-3		4.95 E-3
		2+ 4	2.02 E-3	1.51 E-3	1.34 E-2	7.32 E-3
		5+ 7		4.73 E-3		5.16 E-3
		6+ 8	6.76 E-3	4.31 E-3	9.24 E-3	7.84 E-3
		9+11		5.52 E-3		9.59 E-4
		10+12	6.33 E-3	6.95 E-3	2.12 E-3	8.46 E-4
		13+15		1.08 E-2		1.34 E-3
		14+16	2.15 E-2	1.62 E-2	1.02 E-3	1.10 E-3
	Lettuce	1+ 3		1.76 E-2		1.15 E-1
		2+ 4	1.71 E-2	1.42 E-2	2.31 E-1	1.19 E-1
		5+ 7		3.98 E-2		1.27 E-1
		6+ 8	5.43 E-2	3.70 E-2	1.68 E-1	1.38 E-1
		9+11		2.26 E-1		4.30 E-2
		10+12	1.89 E-1	1.93 E-1	5.73 E-2	3.33 E-2
		13+15		1.92 E-1		2.98 E-2
		14+16	4.68 E-1	3.25 E-1	2.80 E-2	2.39 E-2

Table 8: Transferfactors for Cs-134/137 and Co-57/60 in different plant organs and ralted to dry matter.
Radioactivity and soil treatment in tables 1-4

Year	Plant organ	Lysimeter No.	Mean transfer factors Cs-134	Cs-137	Co-57	Co-60
1989	Potato tubers	1+ 3		6.83 E-3		1.90 E-2
		2+ 4	8.66 E-3	8.73 E-3	3.87 E-2	2.41 E-2
		5+ 7		1.59 E-2		2.07 E-2
		6+ 8	2.09 E-2	1.66 E-2	2.47 E-2	2.13 E-2
		9+11		4.00 E-2		1.05 E-2
		10+12	3.41 E-2	3.71 E-2	9.47 E-3	8.15 E-3
		13+15		3.74 E-2		6.58 E-3
		14+16	7.54 E-2	6.42 E-2	9.40 E-3	7.49 E-3
	Green bean pods	1+ 3		1.62 E-2		1.02 E-1
		2+ 4	1.37 E-2	1.22 E-2	1.36 E-1	8.12 E-2
		5+ 7		8.24 E-2		7.52 E-2
		6+ 8	5.50 E-2	3.64 E-2	1.57 E-1	1.20 E-1
		9+11		4.41 E-2		2.99 E-2
		10+12	5.49 E-2	5.37 E-2	5.18 E-2	5.31 E-2
		13+15		2.15 E-1		4.86 E-2
		14+16	2.56 E-1	2.01 E-1	5.94 E-2	4.48 E-2

Figure 1: Uptake and translocation of Cs−137 and Cs−134 after irrigation on soil and spring barley at different developmental stages, 1987

A: Application of Cs−137 18.5 MBq after sowing onto soil (n=4)
B: Application of Cs−134 1.85 MBq at develop. stage 21 (n=2)
C: Application of Cs−134 1.85 MBq 1/3 at develop. stage 21, 30, 39 each (n=1)
D: Application of Cs−134 1.85 MBq at develop. stage 39 (n=1)



RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-B-056-D

Gesellschaft für Strahlen-
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Title of the research contract:

Investigation of the behaviour of radioiodine in aquatic and terrestrial environments under the influence of biogeochemical processes.

List of projects:

1. Investigation of the behaviour of radioiodine in aquatic and terrestrial environments under the influence of biogeochemical processes.

Title of the project no.:

Investigation of the behaviour of radioiodine in aquatic and terrestrial environments under the influence of biogeochemical processes

Head(s) of project:

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Ingolstädter Landstr. 1
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Scientific staff:

H. Behrens, Dipl.-Ing.

I. Objectives of the project:

Investigation of radioiodine speciation in aquatic and terrestrial systems, especially its conversion into organic bond under the influence of biogeochemical processes.

Study of the role of enzymatically mediated reactions in the transformation of radioiodine into organic bond. Identification of relevant enzymatic activity in water and soil.

Study of sorption and desorption processes of radioiodine in soil/water systems by batch and column tests under the above given aspects.

Disposition of data for the discription of radioiodine migration in environments under the influence of biogeochemical processes.

II. Objectives for the reporting period:

1. Introduction

As a final report this presentation summarizes the obtained insights into the environmental chemistry of radioiodine and points out in which respects these insights may be of importance for the discussion of radioecological questions which arise in the context of radation protection. It seems selfevident that the obtained results are generally valid for the element iodione, however, this point should be emphasized as the behaviour of radioiodine strongly depends on the total chemical concentration of the element. Moreover, radioiodine freshly introduced into environmental compartments will undergo exchange reactions with the already resident stable iodine, which to a part determine the radioecological behaviour of the radionuclide.

The regarded system is the freshwater environment, as well surface waters (rivers and lakes) as ground water systems, the latter mainly the unsaturated zone. The most important aspect is how the chemical behaviour of iodine influences the transport of radioiodine in environmental systems, especially in soils into wich it is mainly introduced by atmospheric dry or wet precipitation. Atmospheric chemistry of iodine is not under consideration.

Generally, it has been confirmed and further detailed, that and in which way the environmental behaviour of iodine is governed by biogeochemical processes which result in the formation of organic iodine compounds from inorganic iodine. In detail the processes appear as enzymatic iodination of organics. Humic substances play the most important role as acceptors of the organically bound iodine. In the following, examples are given of the experimental work which characterize the processes, the resulting species of (radio)iodine and their environmental behaviour, and finally the radioecological consequences are discussed.

2. Behaviour of radioiodine in water from environmental sources

Iodide (I^-) could not be stated to be the main species of iodine in surface waters or soil water. If I^- is added to

such a water, it will be converted to a non-iodide species until an equilibrium is established in which only about 20 % of the iodine remain as I^- (Figure 1,a). If the I^- is removed from this solution, then the equilibrium is established again be forming some I^- from the converted Iodine (Figure 1,b).

By chromatographic separation it could be shown, that the converted iodine has become associated with the dissolved humic materials in the water (Figure 2). The reaction runs as well in untreated water as also in water which has been sterilized by filtration.

The process of I^--conversion is attenuated if the water has been heated moderately and is abolished completely if the water has been boiled.

Conversion of I^- runs at highest observed velocity if the concentration of iodine is below or on the order of 10^{-8} M which is also the concentration of naturally iodine in fresh water (0.5 - 2 ppb). If radioiodine is added at chemical concentrations one or several orders of magnitude higher, then the reaction is running more slowly but reaching the same equilibrium finally. It seems worth noting that at different chemical concentrations in contrast to the lower speeds of reaction the turnover rates are almost the same. The reaction does not proceed in water which has been sterilized by autoclaving and normally also not in water from deep aquifers. However, the reaction can be instigated in such waters by addition of microbially contaminated materials (small amounts of soil or even house dust) and proceeds with the growth of microorganisms in the water.

The conclusion of all the observations is that I^- is bound in surface and soil water in organics by enzymatically mediated iodination. Such iodinations could be simulated by adding iodinating enzyme systems (H_2O_2 or Glucose/Glucose oxidase as H_2O_2-generating system together with Horsereddish Peroxidase or Lactoperoxidase) to previously inactivated water, and were found to deliver the same iodination products as the natural system. The conversion of I^- is reversible by de-iodination reactions in the opposite direction. Iodate (IO_3^-) seems generally to be involved in the reactions via prior reduction to I^-. The iodination

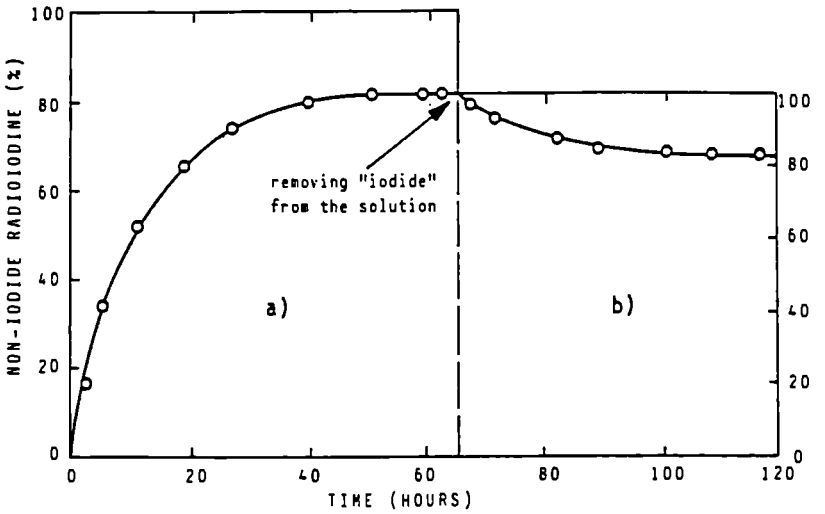

Figure 1: Formation of organically bound iodine from I^- in river water (a); re-establishment of equilibrium distribution after removal of I^- fom the solution (b). Added iodine concentration: 10^{-8} M

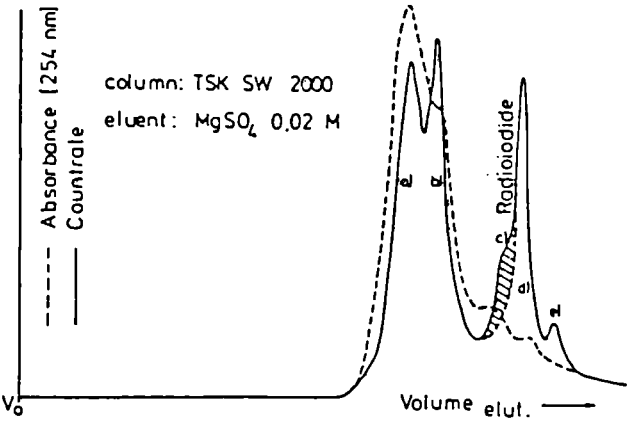

Figure 2: Size exclusion chromatographic separation of iodine compounds which have been formed from radioactive I^- in a soil water; the hatched area corresponds to I^- in equilibrium with the other iodine compounds. The elution profil of organics is represented by the broken line, the radioiodine profil by the solid line

process is only running under aerobic conditions with dissolved oxygen as part of the iodinating system. Under anaerobic conditions in contrast to iodination, the de-iodination process is continuing with final transformation of the iodine in the system to I^-.

In waters from different sources the reaction rates are different according to variations of microbial activity which is producer of the iodinating system. The dependence of the intensity of I-conversion in the aqueous phase could be demonstrated by variations in the state of microbial occupation (Figure 3). In similar ways, the strong decrease of iodine conversion in soil extracts with their sampling depth was demonstrated (Figure 4, left side). This effect can also be seen in the context of changes of density and quality of microbial population with the soil depth.

3. Behaviour of radioiodine in soil/water systems

In soil/water systems (batch shaking and column tests using soil samples from upper horizons) in the liquid phase the same processes as above occur. In addition a strong fixation of added iodine (added as I^-) on the soil matrice is noted. The process of this fixation is iodination as well, in this case of the solid humic materials of the soils. The process is expressed by strongly encreasing K_D-Values (distribution coefficient of added radioiodine tracer, see Figure 5). Cancellation of the iodination process by switching from aerob to anaerobic condition effects release of iodine from the solid phase as I^- by continuation of the de-iodination process. The process shows correlation with other redox-sensitive processes e.g. nitrification/de-nitrification (Figure 5). I^- generally does not sorb in soils and is found in the water phase only.

An example for the decrease of intensity of iodine conversions with sampling depth of the investigated soil/rock materials is shown in Figure 4, right side.

The desorption of iodine in anaerobic soil/water batch shows a remarkable detail: with increasing residence time of iodine in the solid phase it is desorbed more slowly. This can be interpreted with different strenght of binding of iodine at the various available sites. With increasing time

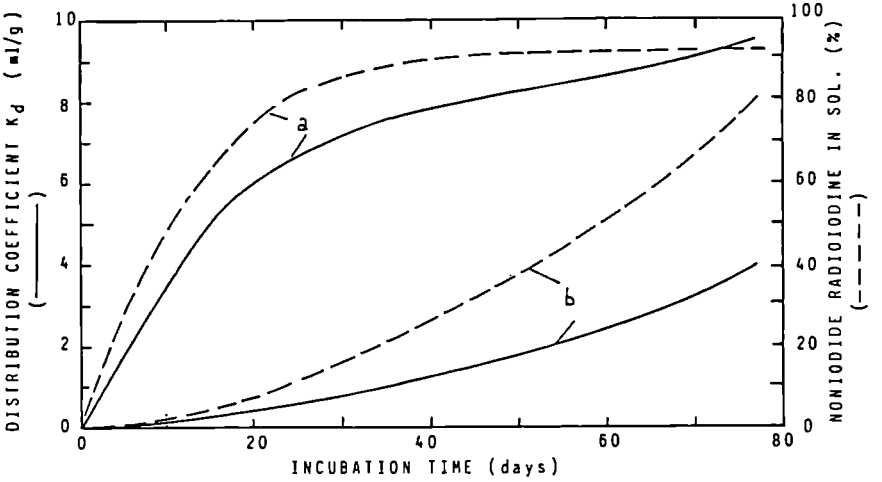

Figure 3: Variation of sorption of radioiodine (K_D ———) and of dissolved non-iodide radioiodine (organically bound radioiodine in solution, – – – –) over time in batch tests with microbially contaminated mineral sand.
Radioiodine added as I^- at 10^{-8} M.
a) Batch with microbially contaminated sand and ground water
b) same conditions, except autoclaving of the sand prior to the test

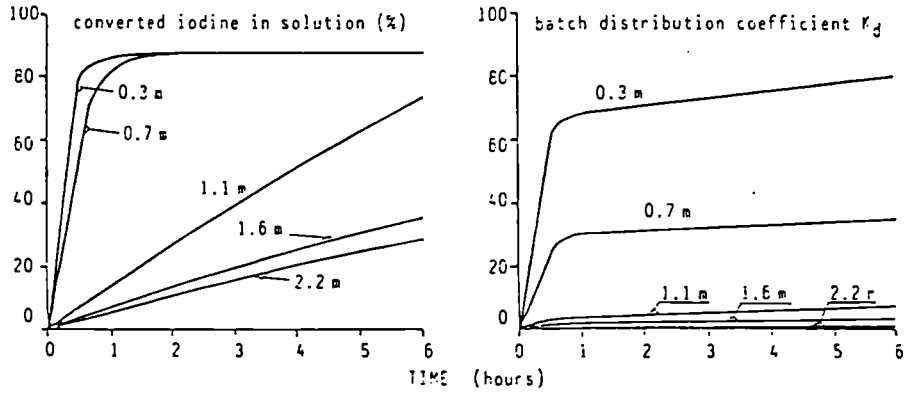

Figure 4: Conversion and sorption of radioiodine (added as I^-) in soil/water batch as a function of time, using sediment samples from different depths

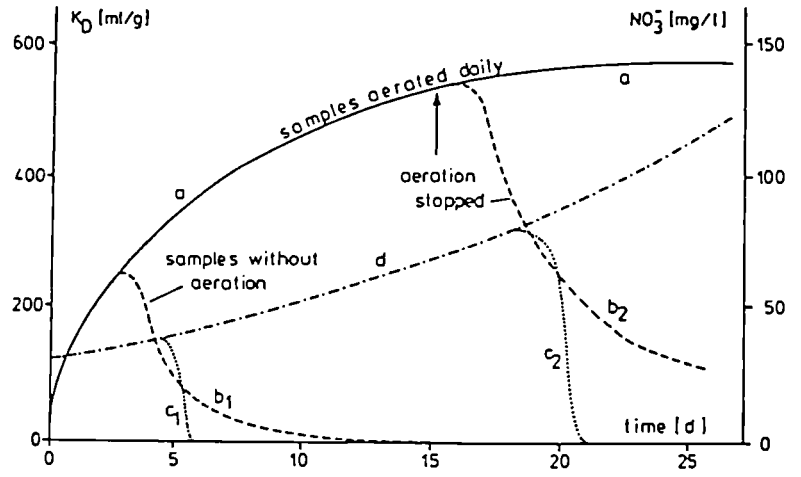

Figure 5: Iodine distribution coefficients (K_D) in soil/water batch. a) aerated samples; b_1) non-aerated samples; c) aeration stopped after 15 days
d, c_1, c_2) NO_3^--Concentrations in samples corresp. a, b_1, b_2

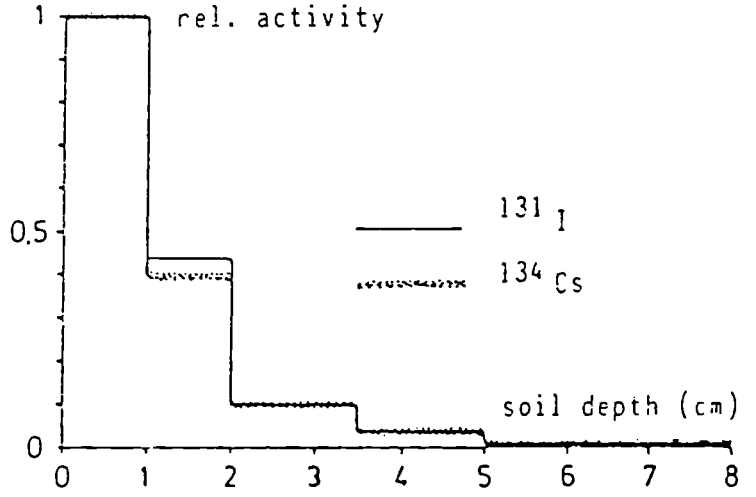

Figure 6: Distribution of ^{131}I and ^{134}Cs from Tchernobyl fallout in a soil profile; Munich, June 9th, 1986

of reaction the freshly introduced iodine will find more and more sites with stronger fixation. This can also explain the fact of decreasing bioavailability of environmental radioiodine with its residence time (SCHÜTTELKOPF 1978, SAAS 1981)

4. Transport behaviour of radioiodine in soils

In lab column experiments with highly homogeneous (sieved) soils, radioiodine injected with infiltrated water was found to be sorbed at the uppermost (entrance) layers. In contrast, in soils with natural structure, radioiodine applicated under simulated rain events was found with distribution into larger depth, typically down to about 15 cm soil depth. This is due to the fact that rain water infiltrates quickly through soil fractures and pores, in the course of which the reaction rate of sorption (iodination) of iodine is not sufficient to fix the iodine close to the soil surface. However, once sorbed in the deeper soil profile, iodine shows the same strong fixation and low mobility as in the homogeneous medium. This behaviour was also found at the occasion of monitoring Tchernobyl fallout. The depth of infiltration of radioiodine (^{131}I) showed strong correllation with that of ^{134}Cs, a radionuclide which also sorbs strongly, but based on quite different physico-chemical processes (Figure 6).

By the continuing iodination and de-iodination of dissolved/sorbed iodine a full reversibility of the sorption process is effected, which allowes application of the simple K_D-concept for the downward movement of radioiodine in soils with infiltrated precipitates according

$$R_f = \frac{1}{K_D * \gamma / w + 1}$$

R_f = retention factor
K_D = Sorption didtribution coefficient
γ = bulk density of dry soil
w = water content in a unit volumn of soil

With K_D's of some hundreds to about thousand (ml/g) residence times of radioiodine in soils layers of about 30 cm of several hundreds to more than thousand years can be expected.

5. Enzymatic iodinating systems

It seems to be established that enzyme systems based on peroxidases are mainly responsible for the observed iodination reactions. They are generally established in biochemistry, they are widely distributed in the animated environment and finally, in these investigations they could be detected in all systems in which iodination reactions were going on. Beyond this, whenever iodination occured also pronounced nitrification was observed. Until now it seems open wether or not nitrifiers can also effect iodination. However, some observations suggest this possibility: when Allylthiourea, an effective nitrification inhibitor was added to water or soil samples, the iodination in these media were strongly suppressed. In contrast, the more general bacteriostat sodium azide, influenced iodination to a much lower extent.

6. Modelling of processes of iodine conversion and distribution in soil/water systems

The reactions of iodine in solutions and the sorption in soils (iodination and de-iodination appear as of 1. order kinetics. On this basis a compartment model was developed for simulation of the above described processes (Figure 10). The fact of increasing sorption strength of iodine in sediments with time of incubation, was considered by installation of two boxes (F_1 and F_2) with different rates and strengths of iodine fixation. In Figure 7 the dynamics of iodine sorption in soil/water batch is shown, which simulates the results of a real experiment (Figure 8). Likewise simulations can be run with any other starting condition e.g.iodine fully sorbed or in any other distribution. Also the release of iodine off the organic bond by switching from aerobic to anaerobic condition can be simulated bei setting the rate constants of the iodination step to 0 (Figure 9).

7. Conclusions

The investigations have shown that environmental behaviour is strongly governed by biogeochemical processes.

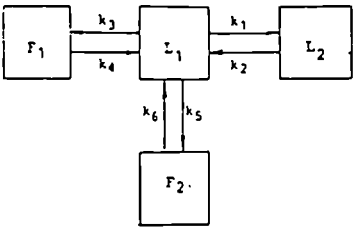

Fig. 7: Compartment Model of iodine conversion and sorption processes in soil/water systems.

L_1 represents the inorganic iodine in the system which is fully in solution as I^-; IO_3^- which will be reduced is not considered

L_2 represents the dissolved iodine in organic bond

F_1 and F_2 represent the iodine bound by solid soil organics

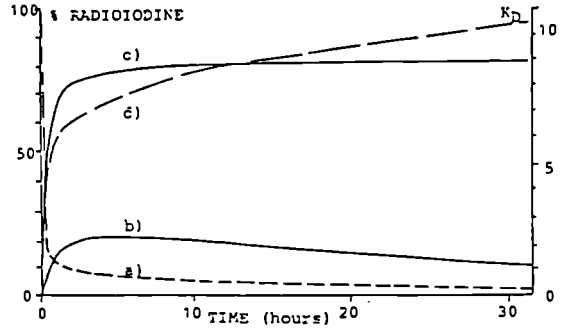

Fig. 8: Dynamics of radioiodine speciation in a soil/water batch experiment (loamy sand). Batch spiked at t=0 with ^{125}I (as I^-).

a) dissolved I^-
b) dissolved organic radioiodine
c) radioiodine bound by soil
d) distribution coefficient (K_D)

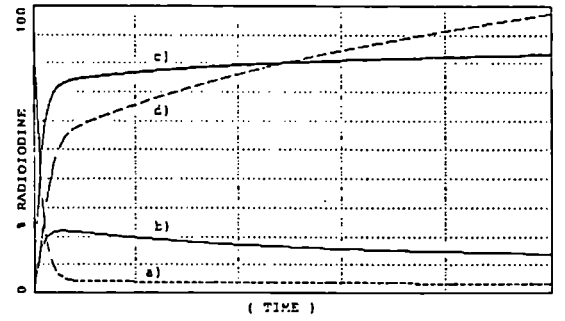

Fig. 9: Dynamics of radioiodine speciation in a soil/water batch as obtained by numerical simulation according model given in Fig. 1, starting with 100 % of radioiodine (as I^-) in solution.

k_1 =0.36 k_2 =0.09
k_3 =0.1 k_4 =0.006
k_5 =0.012 K_6 =0.0001

For a) - d), see Fig. 2

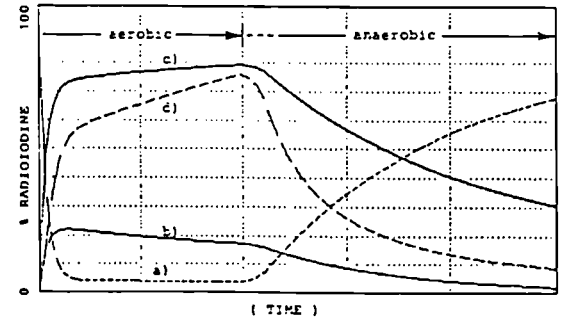

Fig. 10: Dynamics of radioiodine speciation in a soil/water batch by numerical simulation as in Fig. 3, but with change from aerobic to anaerobic conditions by tuning k_1, k_2 and k_3 to zero. This example represents a process which could occur when flooding a soil.

For a) - d) see Fig. 2

These processes effect transformation of (radio)iodine into bond to organic materials to a large extent.

The processes have been identified as enzymatic iodination of organics, mainly of humic materials. The necessary enzymatic systems seem to be produced by microorganisms which occur in surface aquatic and terrestrial environments. In materials from deeper aquifers normally no iodine converting activity is found.

The iodination processes are reversible, iodine can as well be released from the organic bond forming the inorganic I^- (de-iodination).

The iodination goes on only under aerob condition. In anaerobic media iodine is released from organic bond by continuation of the de-iodination process while iodination is abolished.

By the iodine sorption in soils, large retention times of radioiodine on its way from the atmosphere to grundwater can be expected which range in the order of several 100 years to about 1000 years. The iodine is lost however if the soil is braught in anaerobic conditions e.g. by water logging.

As the iodination processes are instigated by microbial activity, in laboratory experiments identity of the microbial state of materials and instrumentation has carefully be kept identical with field conditions if transfer of results to for consideration of processes in the field is intended (BEHRENS, 1986).

Aquifers are effectively protected against infiltration of airborne radioiodine by an intact organic soil cover.

Literature

F. BEHRENS: Zur Übertragbarkeit von Labor-Sorptionsdaten des Radioiods auf in-situ Verhältnisse. - Chemie und Migrationsverhalten der Aktinoide und Spaltprodukte in natürlichen aquatischen Systemen (Eds: J.I. Kim & E. Warnecke), 66. PTB Seminar, Braunschweig 1986

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Kernforschungsanlage Jülich
Postfach 1913
D-5170 Jülich

Contract no.: BI6-B-189-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. F. Führ
Institut für Radioagronomie
KFA Jülich GmbH
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D-5170 Jülich

Telephone number: 02461/616392

Title of the research contract:

Conversion of elementary tritium (HT) in agriculturally used soils, oxidation of HT to HTO and synthesis to organically bound tritium.

List of projects:

1. Conversion of elementary tritium (HT) in agriculturally used soils, oxidation of HT to HTO and synthesis to organically bound tritium.

III. Progress achieved:

FINAL REPORT:

1. Construction and test of experimental procedures including sampling in the field and storage of samples in the laboratory:

An ionisation chamber was included in a closed gas circuit to enable continuous recording of the tritium (HT) concentration in the air. The reaction product, tritiated water (HTO), was withdrawn from the head space by cooling traps. The interior of the ionisation chamber was gold-plated to minimize memory effects. Carrier-free HT is used, for the addition of hydrogen gas (H or D) dilutes the tritium and the specific activity resulting has to be taken into account. HT is stored in a glass vessel wherefrom it can be supplied to the experimental gas circuit by help of a syringe. HT concentrations varying over a large range do not have any effect on the deposition velocity measurements.

Soil samples were collected in stainless steel tubes, threaded at the lower end so that either a cutting edge (for penetration into the soil) or a gas-tight cap (for storage and measurement) could be attached.

Samples taken in this way can be stored in a refrigerator shich is preferable to storage deep freezing, which drastically lowers the deposition velocity or storage at room temperature, which often results in drying of the surface layer. If the soil surface can be prevented from drying the samples may be stored for about one year without changing their tritium deposition velocity.

To obtain a complete balance of tritium turnover systematic errors must be minimized. Thus in all samples tritium was oxidised to water (HT, organic material, soil), which is by cooling or passage through water. Finally all samples were

measured as tritiated water by liquid scintillation counting. The laboratory air was monitored by a air/gas-detector.

2. Formation of tritiated water as the first reaction product:

A tritium balance was made to ensure that tritiated water (HTO) is the first reaction product. The HTO formed from HT quickly distributes within the soil. In all the soil cores studied so far, catalytic conversion of HT to HTO was observed (soil profiles collected down to 16 cm). Nevertheless usually half of the HTO is found in the top 2 - 3 cm of the soil, except when the surface is dry. The HTO remains at the site of formation as diffusion downward is slow. HTO profiles of soil cores hardly change within the first week after exposure, but distinct shifts are visible after one month and more.

The HTO from soil may be obtained by distillation under vacuum (vaccum bridge with cooling finger at one end) or by the azeotropic procedure (toluene). Both methods give a maximum yield of about 95 %. The efficiency of both methods was tested with a double labelling technique, applying HTO and $H_2{}^{18}O$ simultaneously.

3. Study of the uptake of tritium from HTO into the soil biomass:

HTO can be taken up by man via two pathways: by an intake of organic material or by the uptake of HTO from vapour or liquid. Tritium of organic precursors can be incorporated very effectively. Therefore the uptake of tritium from HTO into the biomass of the soil was studied in incubation experiments. The double labelling technique was again used to ensure a complete absence of HTO and to distinguish between the exchangable and the non-exchangable organically bound tritium (OBT, the non-exchangable form is the OBT in a strict

sense). Taking into account the HTO losses by reemission and isotopic exchange between soil water and air humidity, only 1 $^{o}/_{oo}$ of the tritium present in the soil water will be taken up into the biomass by normal biosynthesis,- if one accepts a mechanical stirred soil as "normal". If microbiological synthesis is stimulated by the addition of glucose, the uptake of tritium increases. This was tested with 5 representative soils with different organic matter content, pH and land use. The increase of the biological activity was studied by URAS measurements (CO_2 release from the soil samples).

The results of the incubation experiments indicate that the formation of OBT in the soil can be neglected for dose calculations. The double labelling technique enabled a very low detection limit for OBT.

4. Laboratory studies of the deposition velocity and the effect of physical parameters and anthropogeneous influences as well as the role of microorganisms on tritium uptake:

The uptake and oxidation of HT in the soil should mainly occur by soil microbial activity, but other calatalytic activities cannot be completely excluded. Sterilised soil does not take up and convert any HT. Turnover activity irreversibly decreases and finally disappears at temperatures of 40 - 50°C. However, the final proof by isolation, characterisation and reinoculation of microorganisms is lacking. Quatitative study of the different steps of the radioecological pathway has been preferred to assess the effects of accidental and chronic release situations rather than elucidation of the (bio)chemical mechanisms exactly. But in future these studies will be necessary to understand the radioecological pathway more exactly.

The intensivity of dry deposition is characterized by the deposition velocity which by definition is not dependend on

the concentration of tritium in the headspace over the soil column. The deposition velocity is a term used in model calculations and experiments by air chemists and meteorologists. For arable land it may be a useful parameter if the air turbulence is sufficient. It is difficult to apply if one measures single leaves and soil samples to obtain an assessment of the deposition into whole plant communities and plant-covered soils (scale-up problem). The variation of the deposition velocity between representative soil types under different land use, its spatial and seasonal variation and the effect of parameters such as soil and air moisture were studies.

Most of the data were obtained from soil samples collected from a farmers field near Jülich (Merzenhausen), which is used for other studies at our institute. Samples of a pasture (meadow) were collected in the vicinity. Samples from forest soils were taken in a small stand of old beeches near our Research Centre. The upper layer of dry and loosely deposited leaves was generally removed mechanically.

The deposition velocities during summer season usually range between 1 to 10 * 10^{-4} ms^{-1}. At this time the ratio of deposition velocities field (arable land) and meadow/pasture and forest are about 1 : 2 : 10. While the deposition velocity into forest soil remains constant during the whole year, there is a significant winter minimum in pasture soil, which is more pronounced in arable soils. The porous space of the soil usually is closed by heavy and cold rainfalls, which usually start in October and continue to spring. Snow cover has a similar effect. Consequently the deposition velocities into field soil during winter are very low or even negligible. There is a significant correlation between soil moisture content and deposition velocity into meadow and into arable landcan be demonstrated. An increase in soil water content decreases the deposition velocity of elemental tritium. The temperature of the air and of the soil have no direct influence on the deposition velocity, but heat is

necessary to dry up the soil and thereby to expand the pore space.

It seems that the fraction of pore space available for diffusion is the most important factor. For example, biologically active soil of larger and homogeneous particle size such as that from sand dunes has a larger deposition velocity than soil formed of smaller particles, e.g. loess. At the other hand experiments with a standardizedcompression of the soil did not demonstrate a very pronounced effect. The hypothesis that the gas diffusion into the soil is the limiting step is supported by the observation that the deposition velocity hardly changes with temperature. From above 0 to 40 - 45° there is only a small increase. Usually the velocity of biochemical reactions is very sensitive to temperature. Above 40 - 45° enzymatic activity is irreversibly inhibited, and it stops completely at temperatures of about 50°.

Deposition of HT has been observed in all soils studied so far, including soil from a beach of high salinity or samples from higher altitudes in the Alps. Only soil close to or at water saturation does not show an uptake of HT. When reporting and using deposition velocities soil type, land use, water content and season must be taken into account.

In the vicinity of the Research Centre Jülich (radius 5 km around the centre), in France (up to 3 km distant from the release site) and in Canada (up to 1,5 km distant from release point) soil cores were collected over a short time to assess the spatial variation of the deposition velocity. The variation is surprisingly low and depends on the land use mainly (arable land > pasture/meadow > forest), except for the Canadian site (sandy soil). The type of crop on the arable land did not greatly change the deposition velocity.

5. Comparison between field and laboratory results of deposition velocities:

The most important result is the very close agreement between deposition velocity measurements made in the laboratory and deposition velocity calculated from field measurements during and following HT releases. There were two field experiments, one CEC funded release at Bruyeré-le-Châtel (B III of CEA/France) and one release at Chalk River/Canada. In both experiments a known amount of HT was released under defined weather and wind conditions. Both experiments are well documented and were reported to the scientific community at an international tritium meeting in Toronto.

At the end of the second release experiment the majority of the participants were of the opinion that the study of the radioecological pathway of tritium should be continued, but only after more intensive work in the laboratory. We did not agree becausen the release experiment was a success both as a test of instrumentation and methods as well as of international and interdisciplinary cooperation. Unfortunately this decision was the final action of the tritium group.

In France the deposition velocities in the three different types of land use and the spatial distribution of these values over the relevant area were measured before the tritium was released. Surprisingly the pattern there was very similat to that observed at an area around the Research Centre Jülich. Samples on three sites close to the release point were sampled in a time dependent series to assess the status on the date of release to compare with measurements elsewhere. The results were in close agreement with other samples we have collected at the German site.

On the day of release the atmospheric and soil conditions were suitable. The deposition velocities measured in the laboratory and those calculated from the concentration of HT

in the air plus the HTO concentration in the soil were in good agreement. This showed that the method to assess of deposition of HT by a laboratory procedure was successful. The way of sampling small soil cores decribed here should enable one to collect rapidly soil cores across a larger area and to get then a set of representative data. Before the release experiment there were severe doubts that there would be any detectable deposition of HT. The release experiment has clearly shown that the catalytic conversion of HT to HTO in the soil is important and that the laboratory method may be used in predictive models.

The delay before the French release enabled us to measure the background level of tritium in the vicinity of the centre. There was a clear relationship between background tritium activity in surface waters and the distance to the centre. Only water originating from ground water table (as assured by its ^{18}O content) invariably has a low tritium content.

In Canada a smaller amount of tritium was released, but the experimental conditions were better and the participants were more experienced. Again the distribution of the HT plume was close to the model predictions (Gaussian distribution pattern), as was the tritium activity of ground samples and air samplers. The soil cores were collected from the whole field and the adjacent forest. iThe deposition velocities measured later on at Jülich agreed well with the data obtained from field measurements obtained by others, such as Dr. Ogram from Ontario Hydro. The type of plant vegetation, including nitrogen fixing clover, had no influnce on the deposition velocity measured. Only samples taken from an area where the upper soil layer had been removed some years ago had lower deposition velocities. The deposition velocity in the forest soil was in the rsame ange as that of the area covered with grass and bushes. The reason may be that the soil mainly consists of sand and small-size gravel.

6. Radioecolocal pathway of HTO in the local ecosystem:

At the Canadian site the fate of the reaction product HTO was studied. In addition the water was labelled with ^{18}O. Four $1m^2$ plots, two including small bushes, were selected close to the release site (The release hight was about 1 m above ground level.) The water of two plots was labelled with $H_2{}^{18}O$ by spraying. Both labels (HTO as well as $H_2{}^{18}O$) reached the moss cover and the flat roots of the herbaceous plants very quickly. The label was seen in moss and plants directly after release and irrigation, but disappeared soon after the label moved downwards into deeper soil layers. The label reached the roots of the bushes hours later as shown by measurements of the sap of the stems. A rainfall next day favoured the downward movement of tritium and ^{18}O. The tritium and ^{18}O content of leaf water increased more early compared to their supply in the stem water pool. This is the result of an exchange between moisture at the soil surface and the leaf water (via air water vapour).

7. HTO re-emission after oxidation of HT at the soil surface:

In Canada a set of small soil samples (about 100 g weight), homogenized and sieved, was exposed to the plume. Their loss of HTO by re-emission under field conditions was measured (during the first hour about 5 % of the HTO formed). The reemission stopped at night, because the sample surface was covered by dew and the was little wind. The experiment has been repeated in Jülich several times. A set of 24 samples was exposed to HT in a gas-tight box. The initial amount was taken up within less than one hour. Then the box was opened and brought to the experimental area (lysimeter field). The results obtained in Chalk River were confirmed, but again it is not possible to distinguish between the effects of low air turbulence at night and the cover of the surface by dew.

Generally re-emission behaviour is more complicated behaviour than that of deposition. Re-emission depends on the air

movement across the soil surface and the activity of the plants and their roots. When the wind velocity was increased in a small laboratory system, using the same 8cm diameter soil cores used for the measurements of deposition velocity, the reemission also increased. HTO free air was introduced into the line. The HTO was produced by the reaction of HT with the soil. The re-emitted HTO was collected in three water traps, where the water was renewed each hour. A set of release curves was obtained, the amount of HTO re-emitted decreasing exponentially with time. Surprisingly no maximum of this loss was observed up to wind speeds up to 4 ms^{-1}. The relative humidity of the air flow had no significant influence on the re-emission (0 - 98 % relative humidity).

8. Modelling:

In order to assess the importance of the different steps of the radioecological pathway of tritium after a release of HT a numerical model was built using the official German regulations and formulas.

9. Characterisation of the deposition velocity into different soils:

The original aim of the study has been postponed because of the timing of the release experiments. In our R&D programme samples of typical soils now are tested . We try to moisten the soil samples in a standard way, but this is difficult. It is the aim of this study to report not only a single HT deposition velocity into a specific soil (which is valid only for its actual moisture content) but to determine the relation between deposition velocity and soil moisture content.

10. Publications:

All data discussed above have been published in proceeding volumes, progress reports or publications. Most of the basic

material is part of diploma theses submitted to the Fachhochschule Aachen - Abteilung Jülich. A list of the publications and the theses is attached. Internal progress reports, contributions to draft reports or to annual reports are not included.

11. Summary:

- A method has been developped determine the deposition velocity of elementary tritium HT into soil. Soil samples can quickly be collected from a larger area. Storage in a refrigerator under an aluminium cover up to one year does not alter the soil activity to convert HT to HTO.

- The decrease of the HT in the head space above the soil core is contineously recorded by an ionisation chamber. The tritiated water HTO which has been formed and is released into the gas space is removed by cooling traps. HT concentrations over a large range do not have any effect on the deposition velocity measured.

- Taking into account the volume of the gas space and the decrease constant of HT the deposition velocity of tritium is calculated. The deposition velocity can be used in model calculations and geochemical as well as environmental assessments.

- It is not completely proved that organisms are respronsible for the oxidation of HT to HTO, but sterilized and soil heated up to 50°C does not convert HT.

- HTO is the reaction product as shown by tritium balances. HTO may be obtained by vacuum or azeotropic distillation.

- The separation between HT and the non-exchangable organic form was carefully tested by double labelling technique using water enriched in ^{18}O. An uptake of tritium into the organic matter of the soil can be neglected.

- The deposition velocity depends on the type of the soil, especially the particle size. An artificial compression does not significantly alter the deposition velocity. The most sensitive parameter is the soil water content. Deposition velocity and soil water content are closely correlated (exception forest soil).

- The deposition velocity of arable and pasture soil show an annual cycle. From late autumn till early spring the water content of soil in Central Europe is high and the deposition velocity decreases or even is negligible. Forest soil has the highest deposition velocities and no minimum during winter is observed.

- Reporting deposition velocity data the type of soil, the kind of agricultural utilisation and the water content must be known. Collecting campaigns around the Research Centre Jülich and at two sites of the release experiments have shown that the method proposed here can be easily and quickly applied.

- The applicability of the method and the results of the studies at Jülich have been confirmed by two field release experiments, one in France and the other in Canada. The most important result was the close numerical agreement between the deposition velocities measured in the laboratory and those calculated from the measurements in the field. The data from the field experiment were supplied by other groups and independently determined.

- It was also shown that the spatial distribution of the deposition velocity is smaller than expected, if one directly compares data for arable land, pastures or forests.

- The oxidation product HTO is diluted in the local water cycle. The most important impact results from the re-emission of HTO from the soil. The re-emission depends on the wind velocity. Therefore a diurnal cycle is observed (low or no reemission during night).

- HTO is taken up into plant leaves from the soil by the transpiration stream and from the air water vapour by an isotopic exchange.

- Presently the relation between water content and deposition velocity under standardized conditions is studied.

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IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

see before

V. Publications:

V. 1. Publications in scientific journals, monographs,...:

Förstel, H. und Merches, G.
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RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-B-293-GR

GREEK ATOMIC ENERGY COMMISSION
N.R.C.P.S. "DEMOKRITOS"
GR - 153 10 Aghia Paraskevi, Attica

Head(s) of research team(s) [name(s) and address(es)]:

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Greek Atomic Energy Commission
GR - 153 10 Aghia Paraskevi, Attica

Telephone number: 00-30-1-6511212

Title of the research contract:

Behaviour of long-lived radionuclides in soil-plant systems of the mediterranean region

List of projects:

1. Behaviour of long-lived radionuclides in soil-plant systems of the mediterranean region

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List of projects:

1. Behaviour of long-lived radionuclides in soil-plant systems of the mediterranean region

Title of the project no.: B16-B-293-GR

Behaviour of long-lived radionuclides in soil-plant systems of the mediterranean region.

Head(s) of project:

Dr. C. Apostolakis/Dr. E. Papanicolaou

Scientific staff:

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V. Skarlou (Ms)	J. Bartzis	S. Synetos
G. Roubea (Ms)-Graduate student	P. Kritidis	

I. Objectives of the project:

The objectives of the project refer to the selection of regions in Greece with high degree of contamination and sampling of the main soil types - in various depths - and of the cultivated or indigenous plants grown on them; determination of the physicochemical parameters of the soil samples and the radionuclide concentration in the soil and plant samples; greenhouse experimentation with selected soil types and main agricultural crops to establish uptake rates, and laboratory studies to investigate translocation of radionuclides within undisturbed soil columns; correlation of analytical and experimental data and calculation of transfer factors from soil to plants and various products.

II. Objectives for the reporting period:

For the reporting period which covers the whole duration of the project of - as extended - 2 1/2 years (May 1st 1988 - Oct. 31st 1990), the following were completed:

III. Progress achieved:

A. Field soil and plant (sampling) studies

1. Methodology

Upon determining from previous investigations the regions in Greece (1,4) affected by fallout due to the Chernobyl accident we proceeded in selecting the sites - preferably cultivated fields - for soil and plant sampling.

During the whole operation of the project a total number of 103 soils were sampled in two depths (0-20 and 20-50 cm) and 31 soils in one depth using a metallic frame of specific dimensions (10x10x20 cm) for the sampling of the 0-20 cm layer and a standard soil auger for the 20-50 cm depth. On the same sites plant samples of the cultivated crops were taken on the spot or as close as possible in order to insure similar conditions. From all sites 82 wheat plants, 65 samples of alfalfa, 9 samples of sunflower, 7 samples of tobacco and 1 sample of a pepper plant were collected.

The samples were transported to the laboratory in plastic bags and were submitted to the usual preparation for analysis. The soil samples were air-dried, ground and passed through a 2 mm sieve. The plant samples were air-dried at 70°C and ground.

Generally accepted methods were used (3) for the determination of the physical and chemical characteristics of soils such as texture, calcium carbonate content, pH, cation exchange capacity and extractable cations.

For the determination of ^{137}Cs, ^{134}Cs and ^{40}K an HpGe detector was used with relative efficiency 20-22% with respect to NaI (Tl) 3x3 in crystal and energy resolution 1.9 keV at the 1332.5 peak of ^{60}Co and proper shielding and geometry of the container. An analyzer "Canberra model 90" with 21504 channels through which analysis of photopeaks is possible or a "Canberra model 35 plus" with 4096 channels with the analysis through an on line computer were used. The quantitative determinations were made at the photopeaks of 661.6 keV for ^{137}Cs, 604.7 and 795.8 keV for ^{134}Cs and 1460.7 keV for ^{40}K.

2. Results and Discussion

Soil data

The data for the physical and chemical characteristics of the soil sites indicate a range as to texture from sandy loam to clay loam to silty clay loam to clay, pH values from 4.8 to 7.8 with most values >6.5, low organic matter content (~1.8% on the average), exctractable (ammonium acetate) - K from 51 to 593 ppm with most values between 101 and 242 ppm, cation exchange capacity from 8.4 to 48.8 meq/100g of soil with most

values between 16.8 and 38.8 meq/100 g of soil.

The radio analyses of the soil samples from the 4 sampled regions i.e. Trikala, Karditsa, Grevena and Amyntaion indicate in the case of the surface soil samples a contamination with radiocesium which ranges from 3 to a little over 200 Bq/kg soil with most values between 7.8 and 137 Bq/kg soil. The contamination of the 20-50 cm soil layer is considerably lower than that of the surface layer, being from 1 to about 70 Bq/kg soil with the exception of the Trikala region where the difference in the upper values is much smaller.

The existing contamination of the examined soils ranges, with a small variability in approximately the same levels in all regions for the 0-20 cm soil layer. When the data is compared to results obtained in a previous survey (1986) a lower contamination is indicated with the exception of the Grevena region which indicates a wider variation in contamination during the 1988 survey, a fact which is partially due to a considerable increase in the number of sampling sites.

The obtained data on the ^{137}Cs content has been correlated to the determined soil properties examining their possible influence, under field conditions, on the translocation of Cs to a lower layer and or on the fixation of the element under certain soil conditions. In most cases there is a decrease in the Cs content of the 20-50 cm layer - in proportion to the surface content - with increasing pH values from 3.5 to 7.8 in the surface layer. In few cases the amount of cesium in the second layer exceeds that of the upper layer. Therefore these results are reported more as a trend although the "pattern" is substantiated as the number of sites and samples is extended.

The organic matter content of the soils of the examined regions is rather low (1-2.6%) in all sites and its effect is usually observed in soils with much higher content.

For most regions a definite trend of increasing cesium content with an increase in the amount of clay is established while the cation exchange capacity of soils which is influenced by factors such as amount and type of clay and organic matter content is expected to affect the cesium status in the soils. In the present study, data indicate that most of the values obtained present an increasing ^{137}Cs content with increasing cation exchange capacity of the soils, although at the lower values of CEC the correlation cannot be established.

Plant data

Corresponding plant samples indicate a contamination with ^{137}Cs which in comparison - for the same soil sites - is higher in alfalfa ranging from ~3.5 Bq/kg to 47.6 Bq/kg with most values from 8.0 Bq/kg to 13.0 Bq/kg) than in wheat (grain ~0.6 Bq/kg, straw ~2.5 Bq/kg with certain values being

higher). Few samples of tobacco leaves (3 month-old plants) indicated a contamination of about 4 Bq/kg while a pepper plant contained 4.6 Bq/kg and the sunflower content varied, being 3-4 times higher in the stems (6/14 Bq/kg) than in the seeds (~3 Bq/kg). An effort was made to calculate the transfer factor of cesium-137 from soil to plant and to try to correlate the transfer factors with certain soil properties.

The concentration ratio was much higher in alfalfa (0.116) than in any other of the collected plants which varied from 0.013 for wheat to 0.067 for sunflower, to 0.065 for tobacco and to 0.04 for the pepper plant, although their growth stage was not the same during the sampling.
The obtained data are of significant practical importance because they are based on measurements from soil and plant samples collected from fields under normal farming practices. Nevertheless for this reason, the range of the contamination of various crops and plant parts was wide and the variability of the calculated transfer factors high. Furthermore this high variability does not allow conclusions concerning the effect of the time on the availability of cesium-137 to the growing plants as well as to the calculated soil to plant transfer factors.

The contamination of grain or seed of the reported crops (wheat and sunflower) with cesium-137 is much lower than the contamination of plant material and this is of significant practical importance with respect to human nutrition.

The relation between concentration ratio (CR) and certain soil properties for wheat grain shows a trend for the concentration ratio to increase with decreasing pH, percent clay content and cation exchange capacity. Similar trend is observed for wheat straw and alfalfa hay.

The collaborating - as subcontractors - in the group scientists, M. Antonopoulos-Domis, A. Clouvas, A. Gagianas from the Nuclear Technology Laboratory of the University of Thessaloniki in cooperation with the Agronomy Department of the same University selected on the basis of their different but high levels of radiocesium deposition, three agricultural experimental farms in Northern Greece as natural radioecological laboratories. Agricultural products of annual and perennial plants were collected from these locations and properly prepared for the determination of their ^{137}Cs content using a high purity Ge detector connected with a multichannel analyser and a microcomputer.

The main experimental findings in the case of annual plants (cereals), may be summarized as follows:

1. Radiocesium contamination of grains is very low almost always less than 1 Bq/kg. The contamination of the vegetative parts (straw) is higher than 1 Bq/kg but always less than 10 Bq/kg.

2. For all crops the natural radioactivity due to K-40 is about two orders of magnitude greater than that due to radiocesium contamination.

3. For the first three years after the Chernobyl accident it is generally observed that the radiocesium contamination of wheat and barley appears to be time independent, the differences lying within experimental error.

4. Transfer factors relating radiocesium deposition to contamination of crops were found to be for wheat between 0.006 and 0.01 in good agreement with the mean value 0.01 obtained in other stages of the present project. However, the above TF values are one order of magnitude smaller than those deduced from field experiments in W. Germany and England.

In the case of perennial plants (fruit trees) the main experimental findings may be summarized as follows:

1. Contamination by root uptake is a small, if not negligible, fraction of total contamination of every year's new tree products i.e. fruits, leaves and annual shoots. Experimental evidence suggests that the principal source of new tree products contamination is the radiocesium inventory in the body of the plant.

2. A very small fraction of this inventory flows every year to new tree products.

3. Radiocesium concentration (Bq/kg) is almost the same in crops, leaves and annual shoots. For all three products, 1988 concentration is smaller than that of 1987 by a factor of three.

Further, by the collaborating group, a compartment model for long term contamination of perennial plant products is proposed. It predicts:

a) exponentially declining contamination levels of fruits and leaves,
b) a biological half-life of the contamination of the crops of the order of 0.7 y,
c) total accumulated rejection of radiocesium, over all years following deposition, to be one order of magnitude smaller than the inventory in the tree,
d) root uptake may be neglected not only for the first few years following deposition, but also for the long term contamination. Thus contamination levels may be described by a single exponential term.

It is important to note that the model predictions, are now experimentally verified as results of measurements show declining contamination levels of measured products with a biological half-life, of the order of 0.7 y, in very good agreement with the model predictions.

B. Greenhouse experiments

I. Sr-85 study

1. Methodology

For the planned greenhouse pot experiment to study soil to plant transfer factors of ^{85}Sr (1/2 life 64 days) for a number of important economic crops with varying rooting and growth habits, eight representative soil types that had great differences as to their physical and chemical properties (pH 4.1-7.5, cation exchange capacity 6.0-27.5 meq/100 g of soil; clay content 18.8-60.8%) were selected.

The procedure followed was the commonly used for greenhouse experiments i.e. large samples of the surface layer of the selected types were collected, brought into the laboratory, dried and passed through a 10-mm sieve. Pots were filled with 7 kg of air dry soil to which the appropriate amounts of fertilizers and radioactive material were added. The radioisotope selected for this work was ^{85}Sr, which was considered most suitable because of its half-life (64 days) and the easily counted gamma radiation (513.993 keV). For the mixing of strontium-85 with the soil, 0.02 mCi of ^{85}Sr (amount added per pot) were diluted to 400 ml of water. Fifty ml portions of this solution were added consecutively in the form of small drops on the top of soil layers about two cm thick having thus ^{85}Sr distributed in eight layers per pot.

The soils in the pots were watered to field capacity and left to stand for 1 month. At the end of this period wheat, lucerne, radish, string beans and cucumber were sown and lettuce seedlings were planted and irrigated. Each treatment was replicated five times bringing to 240 the total number of pots. The necessary steps were taken (irrigation, weed control, thinning, pest control) for the normal growth of the experimental plants.

The plants were harvested at maturity, separated when appropriate into edible and other parts, sliced into small pieces and counted for ^{85}Sr; representative samples were dried at 70°C for 48 hr, ground and used for the required chemical analyses.

Generally accepted methods were used for the determination of soil properties with proper modifications for the determination of exchangeable calcium and magnesium in calcareous soils.

For counting ^{85}Sr the already described system of an HpGe detector was used.

2. Results and Discussion

The calculated values of concentration ratio for all of the

used plants and their parts indicate a great variation depending on the soil type. More specifically the plant to soil concentration ratio in the wheat grain ranged between 0.03 and 1.39 for the tested soil types. For wheat straw the range was 1.02-21.27, for alfalfa hay 2.77-23.23 for lettuce 1.74-17.18, for radish roots 1.63-11.71, for radish leaves 4.27-16.27 for string bean pods and seeds 0.26-8.51, for string bean plant material 4.16-28.38, for cucumber fruit 0.42-2.95 and for cucumber plant material 7.62-36.49. For the calculation of these C.R. values both soil and plant samples were counted.

For crops where edible parts (grain, root, pod or fruit) and plant material (straw, leaves etc.) were separated it is important to indicate that C.R. values of the exible parts were much lower than those of the plant materials. The correlation, negative in all cases and significant or highly significant for most crops, between CR and soil properties was greatest for soil pH and decreased in the order pH > total clay plus silt ≈ cation exchange capacity > total clay.

Soil pH is known to be related to the degree of base saturation in which calcium plays a very important role. As calcium has been broadly used for the calculation of the soil to plant transfer of strontium (observed ratio), exchangeable calcium was first tested for its correlation with C.R. values of the harvested crops. In addition exchangeable Mg, exchangeable (Ca+Mg) and exchangeable bases, all expressed either in meq/100g or as percentages of their cation exchange capacity, were also tested.

The obtained values of the correlation coefficients show that exchangeable calcium, expressed in meq/100 g gave - in most cases - significant or highly significant correlations with C.R. of various crops or plant parts, while exchangeable calcium plus magnesium or total exchangeable bases (expressed also in meq/100 g) gave higher correlation coefficient. The situation is further improved (higher correlation coefficient and lower variability) if the above mentioned exchangeable cations are expressed as percentages of cation exchange capacity of the soil. It should be noted that only exchangeable (Ca+Mg) percent or total exchangeable bases percent gave significant or highly significant correlations with C.R. of all tested crops or plant parts. Nevertheless exchangeable magnesium alone did not give any significant correlation with C.R. of the tested crops or plant parts.

To calculate OR for the soil to plant transfer of ^{85}Sr, a specified Sr/exchangeable cations OR term (defined as ORex) was introduced in which exchangeable cations were used instead of soil (precursor) Ca. Compared to CRs the values of ORex were relatively constant and usually not related to soil properties. Between the fractions of ORex highly significant correlations were noticed for all tested crops or plant parts.

II. Ce-141 study

1. Methodology

In the overall approach to the project as well as in the final approved form, provision was made for additional studies to establish plant uptake rates of radionuclides from the soil. As radioisotopes of cerium (^{141}Ce, ^{144}Ce) were determined in samples of air and food products in Greece after the Chernobyl accident and little is known as to their behaviour in the food chain pathway a greenhouse experiment was planned in order to determine the transfer factor of Ce in the soil-plant system.

To this purpose five soil types were selected in central and southern Greece presenting considerable differences as to their physical and chemical characteristics (pH 4.5-7.8; cation exchange capacity 5.6-25.6 meq/100g soil; clay content 16.4-48.4%). Considerable quantity of the above soils was collected and transported to the laboratory. The previously described procedure was followed for the preparation for the establishment of the experiment. Pots were filled with 6 kg of air-dry soil to which the appropriate amounts of fertilizers and radioactive material were added. The radioisotope selected was ^{141}Ce with a half-life of 33 days and a γ-energy delay characteristic peak at 145.5 keV. The total activity of ^{141}Ce was 25 mCi in the chemical form of cerous chloride in 0.5M hydrochloric acid. The total amount of the radioisotope was diluted in water and it was distributed by adding 0.23 mCi uniformly and gradually in eight levels of soil in each pot.

The soils in the pots were watered to field capacity and, left to stand for 1 month. Four kinds of plants were used (soya, endives, onions and lucerne) and the treatments were replicated six times for a total of 120 pots. Two sampling times were set up at the end of 2 and 4 months. The collected plants were prepared, as described before, for the necessary determinations. Soil samples from each pot were taken for measuring the ^{141}Ce content at the 2-month sampling. Generally accepted methods (3) were used for soil and plant analyses and the HpGe detector - previously described - for the ^{141}Ce measurements.

2. Results and Discussion

A great variation exists in the calculated values of concentration ratio among the various plants and in the same plant grown on various soil types. It is understood that further studies can be initiated in some detailed form in order to clarify the effect of soil properties on the soil to plant transfer of radiocerium. From the present data it is indicated - using the values obtained from the determinations at the completion of a 4-month growth - that the CR value for soya ranges from 0.003 to 0.426 for the vegetative part and

from 0.001 to 0.049 for the bean. For endives the CR ranges from 0.002 to 0.195, for the onion leaves from 0.002 to 0.122 and for the bulb from 0.001 to 0.032 and for lucerne from 0.002 to 0.453. As it can be seen the obtained values indicate higher CR values for lucerne and soya followed by the endives and the onion plant, all compared grown on the same soil. In all but one of the cases the values of CR show for all plants the same differentiation in order of magnitude when compared to soil type.
The differences obtained among the various plant species used seem to be caused by the soil properties and particularly the soil pH as the CR decreases with increasing pH. The same tendency exists when the values of CR are compared to the soil cation exchange capacity.

C. Laboratory studies

1. Methodology

For the study of the migration of radionuclides in soil profiles five undisturbed field soil columns of characteristic soil types from two regions (Argolis and Ilia in Peloponnesus) of southern Greece were taken, according to a technique in use by our laboratory (2).

The selected areas were representative of two different climatic regions of the country, a humid and a semi-arid. The soil characteristics as determined in samples taken by auger on the sampling sites ranged from sandy loams to loams, silt loams, and to clays with varying pH (4.1 to 7.2), calcium carbonate (0 to 22%) and organic matter (1.3 to 3.1%) contents, and cation exchange capacity (7.4 to 30 meq/100g soil). The soil columns in the plexiglass were fit at the bottom with the necessary filtering system. Daily additions of small quantities of de-ionized water to the soil columns saturated the soil during a period of approximately one month.

Effecting a dilution procedure to the initial quantities of radioisotopes of ^{134}Cs and ^{141}Ce to be used as contaminants, an amount of 0.0025 mCi from each radioisotope was added to the top of the soil columns in a total volume of 100 ml of water to achieve a satisfactory surface distribution.

The daily additions of water continued and the amount of filtrate was collected daily and accumulated for weekly measurements of radioactivity.

After a two-month period of water addition the soil columns were left to drain for about a month, and the soil was pushed out of the plexiglass, sliced into 2 cm layers to a depth of 10 cm for detailed measurement of the radioisotope migration into the soil profile. The lower section was separated into three equal parts of approximately 18 cm thickness each and all the samples from the column were placed in special con-

tainers for measurement in the previously described system. The amount of ^{134}Cs was measured at 795.5 keV while that of ^{141}Ce was measured at 144.8 keV.

2. Results and Discussion

The weekly measurements of radioactivity in the filtrates from the columns considered either separately or in summation for the whole duration of the experiment, showed very small amounts of radioactivity passing through the columns which could be attributed to either of the added radioisotopes. In a general comparison there is an indication of lack of continuous diffusion of isotopes through the profile after the end of the fourth week of leaching, particularly in the soils with medium to high calcium carbonate content (14.6 to 24.6), while in the acid and near neutral soil a slight diffusion continued to the end of the sixth week. It is is noted that for this type of experimentation an approach for greater accumulation and concentration of the leachates should be applied. It is further indicated that in most profiles (4 out of 5) ^{141}Ce is more mobile than ^{134}Cs.

As to the amounts of radioisotopes withheld and determined within the soil profiles it is pointed out from the existing data that differences exist among the various soil types and between the isotopes used in this study. Thus ^{141}Ce seems to be more mobile than ^{134}Cs, as in all profiles the former is withheld at the proportion of 31.2% to 59% within the first 2 cm of the profiles as compared to 79.6% and 74.5% respectively for ^{134}Cs.

These differences tend to minimize as the 93% to 97% and in one case to 99.4% of the amount of isotopes added is withheld within the first 10 cm of the profiles. Nevertheless radioisotopes have been determined in varying amounts to the depth of more than 20 cm within the soil profiles and in some cases, small but traceable amounts have been determined at a depth of ~70 cm, that is at the bottom of the soil column.

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V. Publications:

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Under publication

Papanicolaou, E.P., Apostolakis, C.G., Skarlou, V., Nobeli, C., Kritidis, P.

1. Ratios of plant to soil concentrations of strontium-85 and their relations to the exchangeable cations for soils and crops of Greece.

2. Ratios of strontium-85 to cations for crops and soils of Greece.

Results to be published

1. Undisturbed column studies with ^{134}Cs and ^{141}Ce.
2. Greenhouse studies with ^{141}Ce.

SCOPE-RADPATH
REPORT ON PROJECT ACTIVITIES

1. Introduction and Overview

This report provides an account of SCOPE-RADPATH activities over the period from January to December 1990.

One of the main activities during this period has been to convene the First RADPATH Case-study Meeting (26th - 30th March, 1990) at University of Lancaster, U.K. Additional meetings which have involved the participation of RADPATH represenatives were held in: Zeleny Mys, U.S.S.R., 10th - 18th September, 1990; Luxembourg, 1st - 5th October, 1990; Stockholm, Sweden, (BIOspheric MOdel Validation Study (BIOMOVS) Meeting), 8th - 12th October, 1990 and Gomel, U.S.S.R., 15th - 19th October, 1990. Other project activities have been concerned with the organisation of future workshop activities, identification of topics requiring consideration, production of draft manuscripts for the final RADPATH report (to form a volume in the SCOPE series of publications), preparation and submission of proposals to secure further financial support for the project, and maintaining information dissemination activities. Further details about these activities are provided below.

The grant received from the Commission of the European Communities (CEC) has continued to provide invaluable assistance to the RADPATH project, by enabling support of investigations into the biogeochemical pathways of artificial radionuclides through technical workshops to examine results from experimental and modelling research studies.

2. Workshops and Meetings

2.1. Revisions to 1990 Work Plan

The first of the two meetings planned during 1990, the First RADPATH Case Study Meeting was, as outlined in the tentative workplan originally submitted (see RADPATH's Report to CEC in 1989), held at Lancaster (near Windscale/Sellafield), U.K. over the period 26th - 30th March, 1990. Provisionally a second case-study meeting was planned in November 1990 in Tashkent, U.S.S.R. The venue and dates of this meeting were subsequently revised, with an All Union Conference on Geochemical Pathways of Artificial Radionuclides Migration in Biosphere convened from 15th - 19th October, 1990, in Gomel, U.S.S.R.

Additional meetings not included in the 1990 Work Plan which have subsequently involved the participation of RADPATH representatives include: "Biological Aspects of the Consequences of the Chernobyl Atomic Power Station Accident", Zeleny Mys, 10th - 18th September 1990; "Comparative Assessment of the Environmental Impact of Radionuclides Released during Three Major Nuclear Accidents - Kyshtym, Windscale, Chernobyl", Luxembourg, 1st - 5th October, 1990 and the BIOMOVS Meeting, Stockholm, Sweden, 8th - 12th October, 1990.

It has not proved possible to secure the additional funding required to permit RADPATH's Final Review and Synthesis Workshop during 1991 to be held in Marin County, U.S.A. as previously planned. This matter was, therefore, discussed at an SAC Meeting during the Lancaster Case-study Meeting where SAC members agreed, in view of the expected large European input, that the Final Meeting should instead be held at University of Essex, U.K.

The need to convene a RADPATH meeting following the production of the synthesis volume has been noted. The possibility of holding an additional workshop in London during the final year of the project, to review and discuss issues relating to the conclusions reached in key areas of the RADPATH programme, has therefore been considered. Unfortunately, however, The Royal Society, London, in conjunction with which the planned meeting was to have been arranged, has been unable to provide the necessary funding to permit such a meeting to be held.

2.2. First Case Study Meeting, March 1990

The First RADPATH Case-study Meeting, held from 26th - 30th March, 1990, at the University of Lancaster, U.K., was organized by the Essex SCOPE Unit. In total, forty-three participants from some twelve nations, were present at the meeting. A review of the current status of the RADPATH programme was provided by Sir Frederick Warner, Chairman of the SCOPE-RADPATH Scientific Advisory Committee (SAC), who chaired the technical sessions. In the course of the meeting an overview of the status of aquatic RADPATH-related research programmes was gained, and detailed elucidation of the pathways of artificial radionuclides was provided by examining specific case-studies, namely Sellafield discharges and the Chernobyl accident release. Reports on information relating to the Soviet 1957 accident in the Southern Urals were also presented. Further discussions, within aquatic, atmospheric and terrestrial sub-groups, provided the opportunity to finalize detailed plans concerning the preparation of material for RADPATH's synthesis volume. Issues relating to future RADPATH activities, and the allocation of specific tasks in connection with the production of the RADPATH synthesis volume, were considered during the final plenary session discussions. Plans relating to future workshops, the dissemination of information derived as a result of the RADPATH programme and the publication of scientific reports were discussed.

A report of this meeting, including summaries of the technical presentations and SAC Meeting Minutes, is provided in Appendix II.

2.3. RADPATH Involvement in Zeleny Mys Meeting, September 1990

The 1st International Conference on the "Biological Aspects of the Consequences of the Chernobyl Atomic Power Station (APS) Accident" was held from 10th - 18th September 1990 in Zeleny Mys, U.S.S.R. Participants (including several RADPATH representatives) at the meeting considered the effects and genetic consequences of radioactive pollution on flora and fauna, radionuclide migration in ecosystems, and issues relating to dosimetry and management of livestock and game under contaminated conditions. A number of field-trips in the Chernobyl region were also undertaken. Further details about the meeting are given in Appendix III.

2.4. RADPATH Involvement in Luxembourg Meeting, October 1990

The Luxembourg meeting (1st - 5th October, 1990) on "Comparative Assessment of the Environmental Impact of Radionuclides Released during Three Major Nuclear Accidents - Kyshtym, Windscale, Chernobyl" was organized by the International Union of Radioecologists/Commission of the European Communities, with co-operation from SCOPE-RADPATH, who funded the attendance of several of the forty-six Soviet delegates present. Specific issues examined are discussed in detail in Appendix IV: These included

consideration of; source terms, dispersion, deposition and transfer of radioactivity, radiological implications and the effectiveness of countermeasures.

2.5. RADPATH Involvement in BIOMOVS Meeting, October 1990

In the course of the BIOMOVS Symposium, held in Stockholm (8th - 12th October, 1990), Prof. Charles Shapiro (RADPATH SAC member and Co-ordinator of the Atmospheric Pathways Chapter of RADPATH's SCOPE volume) presented an overview of the RADPATH project. A total of some sixty-five delegates from twenty-five countries (including five RADPATH participants) were involved in the Symposium, which marked the formal ending of the BIOMOVS study. Appendix V provides more detailed information about this Symposium.

2.6. Gomel Meeting, October 1990

The Gomel meeting, convened from 15th - 19th October 1990 in U.S.S.R., is reported to have involved a total of three hundred Soviet and twenty-five to thirty non-Soviet scientists, including a number of RADPATH represenatives. Poster and oral presentations were given on various topics including, radionuclide forms, distribution, migration, and monitoring following Atomic Power Station accidents. The fourth day of the meeting comprised an excursion to a contaminated region caused by the Chernobyl accident. A meeting summary is contained in Appendix VI.

3. Information Dissemination Activities

The SCOPE-RADPATH Unit has continued to produce, at intervals throughout the year, a Newsletter reporting on RADPATH-related topics. During 1990 five further issues of the Newsletter have been prepared for distribution to some 500 individuals, including members of both the scientific community and the interested public.

Considering lectures given by RADPATH scientists, during a Commonwealth Science Council visit to India in January 1990, Sir Frederick Warner presented a lecture entitled "After Chernobyl" at the Inaugural Session of the Nuclear Power - Advance Fuel Cycles Conference. The conference, which was arranged by the Indian Nuclear Society, was held at the Tata Institute of Fundamental Research, Bombay, India.

A 24-hour television marathon publicising the Chernobyl accident was organized on 26th April 1990 (the fourth anniversary of the disaster) by The Chernobyl Union, U.S.S.R. (in the formation of which Dr. Lev Khitrov, a RADPATH SAC member, has played a major role), in order to attract world attention to the lessons from Chernobyl and to raise funds to promote the activities of the Union. The programme was broadcast over the main channel of Soviet Central Television, and was seen within the Soviet Union and by many other countries in the area of Soviet satellite-beamed progammes.

Further activities have included, for example, a poster presentation about the RADPATH programme, which was exhibited in a Open Day display at the University of Essex, U.K., April 1990.

As previously noted, in Section 2.2 above, in order to publicise the findings of the RADPATH programme a volume in the SCOPE series of publications is being prepared. This volume, reviewing the present state of knowledge on the environmental pathways of artificial radionuclides,

comprises some eight sections: Introduction (definition of terminology, isotopes of interest, the nuclear fuel cycle, case-study data and maps), Atmospheric Pathways, Terrestrial Pathways, Aquatic Pathways, Urban Environment, Dosimetry and Assessment of Environmental Effects, Synthesis and Recommendations, Appendix (containing a glossary of terms, table of units, methods of analysis, sampling strategies and modelling). Completion of the volume is expected next year. Further details of chapter topics and authors are provided in Appendix VII.

4. Future Workshops

The Final Review and Synthesis Meeting is being organized by the RADPATH Unit from 13th - 19th April, 1991. Arrangements have been made with the Wivenhoe Park Conference Centre at the University of Essex for accommodation and meeting facilities for forty delegates. The technical agenda for this meeting, which will include a visit to the Sizewell Nuclear Power Plant, Suffolk, is presently under discussion. Planned activities include the review and revision of draft manuscripts in preparation for the ensuing SCOPE report. In addition the authors and members of the RADPATH Scientific Advisory Committee may hold a general session for the presentation of the proposed SCOPE volume, as developed to date, for the media and interested public. An outline agenda and a list of possible participants are provided in Appendix VIII.

5. Project Funding

Besides the financial support being provided by the CEC, funding has been obtained from The Leverhulme Trust (£ 54,900) to support a postdoctoral research assistant and part-time secretarial assistance in connection with the Essex Unit. In addition, the University of Essex is providing full use of its facilities and office space free of charge, and no charge is being made for the time which Sir Frederick Warner and Dr. Harrison devote to the project. To assist with general project activities, grants of £ 1,000 and £ 10,000 have been made by The Fellowship of Engineering and The Royal Society (London) respectively. The latter organization has provided supplementary funding, totalling just over £ 1,000, to cover per diem and additional accommodation expenses associated with the presence of five Soviet delegates in the U.K. prior to and following the First Case-study Meeting earlier this year. A further grant of £ 17,800 was obtained from The Wolfson Foundation towards the organization of a case study meeting to examine the pathways of radionuclides, particularly in connection with Sellafield discharges and the Chernobyl accident. The U.S.S.R. Academy of Sciences has also provided financial support covering, for example, expenses within the Soviet Union associated with the Gomel 1990 Meeting. Another source of support has been through the International Union of Radioecologists (I.U.R.) which has funded the attendance of several representatives to RADPATH meetings.

With regard to applications pending, support for the Final RADPATH Review and Synthesis Meeting Meeting (13th - 19th April, 1991) is presently being sought from UNESCO's (United Nations Educational, Scientific and Cultural Organization) MAB (Man and Biosphere) programme via Ms. Veronique Plocq (SCOPE Secretariat, Paris).

6. RADPATH Continuation Programme: 1992-1994

The need for a continuation programme following the conclusion of the

initial phase of the RADPATH programme in December 1991, after the publication of the synthesis volume, has been recognised and is presently under consideration.

A draft proposal for a continuation project was prepared by the Essex Unit in November 1990 following a meeting (in October 1990 at The Royal Society, London) with the International Union of Pure and Applied Chemistry (IUPAC), which expressed great interest in ensuring the continuation of RADPATH's work on an international basis. A number of possible areas for collaboration between RADPATH and IUPAC, arising from within existing programmes and from newly formulated projects, have been identified. Further details are provided in Appendix X.

Contract: Bi6-345 **Sector: A23**

Title: Transfer and conversion mechanisms of H-3 and C-14 compounds in the local environment.

1 Bunnenberg Niedersächs.Inst.Radioökologie

I. Summary of Project and Global Objectives

Fusion test plants and tritium handling facilities will contain large amounts of tritium, mainly in the highly mobile HT form. Safety analyses and licensing of these facilities require the availability of models to predict the environmental impact and dose consequences of potential tritium releases to the atmosphere. The evaluation of recent experimental field releases in France and Canada showed that reemission of tritium from soil after plume passage and deposition of HT is a key process in tritium behaviour, i.e., the reemission process dominates the inhalation and skin absorption dose after an HT release. However, no model exists as yet, to predict reemission rates on the basis of general or site-specific parameters.

Research in this project focusses on the dependence of the reemission rate on relevant soil physical and meteorological parameters (e.g., soil texture, moisture, temperature). Single parameter studies in a wind-tunnel/soil-column arrangement under controlled conditions are performed for this purpose. When principal relationships are established, the project proceeds towards more natural conditions with respect to dynamic meteorological conditions and to the effects of HTO soil profiles that result from deposition processes.

Head of Project 1: Dr. Bunnenberg

II Objectives for the reporting period

Completion of the experimental wind-tunnel/soil-column arrangement to simulate tritium reemission from soil under controlled environmental conditions.
Experiments with different temperatures.
Evaluation of collected air samples during and tritium profiles in soil columns after a reemission phase.

III Objectives for next period

Parameterization of the relation between reemission rates and air/soil temperatures.
Effects of soil physical conditions (soil texture, moisture content) on reemission rates.
In all investigations special attention will be paid to the question, to what extent information on evaporation may be used to predict HTO reemission for dose estimation purposes.

IV Progress achieved including publications

1. Methodology

The applicability of theoretical or empirical formulae describing transport and evaporation of water on HTO reemission from soils may be limited, as in tritium contaminated soils HTO is a solute in soil water and may move within and independent from the solvent because of individual concentration and vapour pressure gradients. In order to examine similarities or dissimilarities of H_2O and HTO reemission processes and to establish relationships to easily accessible environmental parameters, a wind-tunnel/soil-column device was built, which allows single-parameter studies under controlled soil physical and meteorological conditions. The device contains a soil column, which can be exposed to defined air streams. During the simulated reemission phase air samples are collected, and after each run the soil columns are disassembled in horizontal layers. The air and soil samples yield time-resolved HTO reemission rates and vertical soil profiles of moisture and HTO contents.

2. Results

The results presented here refer to reemission experiments with loess soil homogeneously labeled with tritium. Initial moisture contents (12 wt.%), humidity of tritium-free air (60%), and wind speed (1.6 m/s) were the same in all runs.

The air/soil temperatures (isothermal) varied between 10 and 30°C for different runs but were kept constant during each experiment.

The principal time course of the HTO reemission rate, defined as the fraction of the initial activity lost per unit time, is characterized by high values in the beginning and slow decreases during the starting phase in the order of one hour. In the following phase reemission rates decrease considerably faster with time. Depending on temperature the rates decrease to about 1/3 to 1/4 of the starting values within 12 to 20 hours.

The time behaviour of the reemission rate is generally the same for all temperatures tested. However, the activity loss within 20 h is about 2.5 times higher at 30°C than at 10°C. The corresponding water loss by evaporation increases by a factor of 4 within this temperature range. This temperature dependency is shown in Fig. 1, which gives the rates of relative losses (reemission rates) averaged over a 20-h reemission phase for tritium activity and soil moisture.

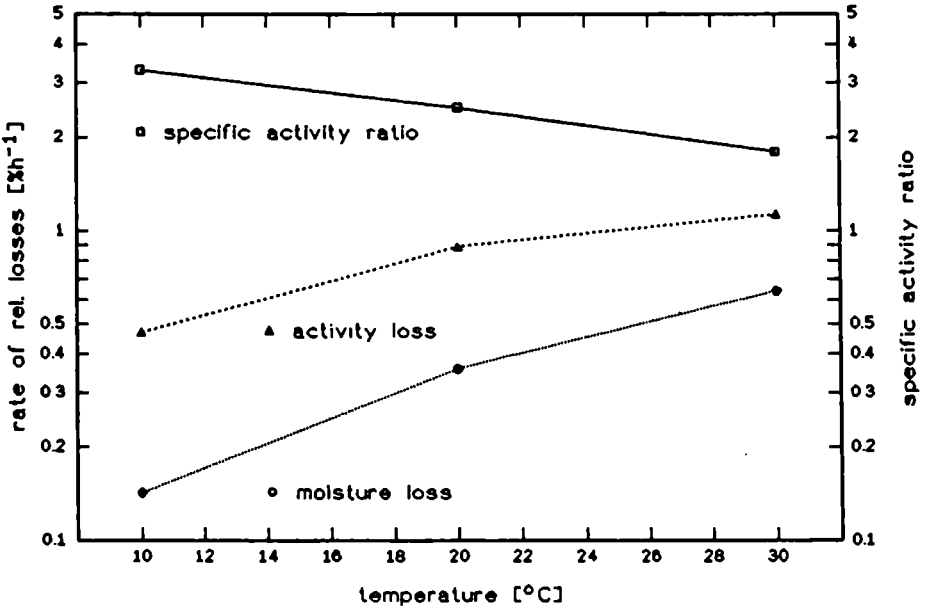


Fig. 1: Rate of relative losses (reemission rates) of tritium activity and soil moisture averaged over 20-h reemission phases and ratios of the specific activities of the evaporated water and the soil water for different temperatures.

The figure also clearly demonstrates that the reemission rate [%/h] of HTO as defined above is higher than that of H_2O for all temperatures. This finding has three important consequences:

- the specific activity of the evaporating water is higher than that of the soil water, i.e., the ratio of those two values (specific activity ratio) is greater than unity,
- the specific activity of the soil water decreases during a reemission phase and
- the resulting profiles in soil for HTO and H_2O contents differ distinctly.

As can also be seen from the figure, the specific activity ratio is the higher the lower the temperature and exceeds a value of 3 in case of 10^0C under the experimental conditions.

3. Discussion

The two-phase time behaviour of the HTO reemission rate under constant environmental conditions indicates that as long as the top soil layers are moist, HTO resupply occurs predominantly by liquid transport in soil. With proceeding reemission of HTO and H_2O the top soil dries out, and the much slower resupply by diffusion prevails, which reduces the reemission rate more significantly.

Specific activity ratios greater than unity were also found with HTO deposition processes, and they are in agreement with the understanding of molecular transports. It must be noted that this is not an isotopic effect but the result of the individual driving forces for HTO and H_2O movement. The finding proves that the prediction of HTO reemission rates on the basis of evaporation data via specific tritium activity of soil water would considerably underestimate dose consequences.

4. Publications

Schubert, K., Täschner, M., Bunnenberg, C.:
Wind-Tunnel Experiments on Tritium Reemission from Soil.
NIR-Jahresbericht 1990 (annual report), in press.

Täschner, M., Bunnenberg, C., Gulden, W.:
Maximum Permissible Amounts of Accidentally Released Tritium Derived from an Environmental Experiment to Meet Dose Limits for Public Exposure.
Fusion Technology (1991), envisaged publication Aug. 1991.

Contract: Bi7-011 **Sector:** A23

Title: The bio-availability of long-lived radionuclides in relation to their physico-chemical form in soils

1	Lembrechts	RIVM
2	Wilkins	NRPB
3	Sandalls	AEA Technology Harwell Laboratory
4	Cremers	Univ. Leuven (KUL)

I. Summary of Project and Global Objectives

Two of the major aspects affecting the behaviour of radionuclides in soil/plant systems, are studied : 1) availability in the soil liquid in relation to major characteristics of the soil and 2) the efficiency of the uptake process. The nuclide of main interest is Cs-137, although some experiments on transuranics are planned as well.

The speciation study concerns both naturally (well-characterised sites in Cumbria) and artificially labelled soils (lysimeters), and accentuates the quantification of chemical forms in the soil and soil solution. Amongst others ultrafiltration techniques are used to investigate the association of radionuclides with different molecular size fractions in solution and to monitor time dependent changes in speciation. By conducting batch equilibrium experiments and by studying soil solution and its associated radiolabelled soil the effects of various treatments on sorption are determined under laboratory conditions. The soil chemical parameters which regulate the specific interception potential of Cs, are quantitatively characterised in a broad range of soils, with particular attention being given to reversibility aspects and aging effects. As a result, it will be possible to estimate the effect of amendments on radiocaesium availability in problem soils.

The first part is complemented with experiments on bioaccumulation. Transfer along the soil / soil solution / plant pathway is studied under controlled conditions in phytotrons, and validated with references to field observations on upland soils from Cumbria. The time trend in uptake, im- and remobilization of radiocontaminants is studied throughout the growing period in order to explain changes in transfer and to extend the information derived from the detailed soil studies. Uptake from soils is compared with uptake from nutrient solutions in order to better distinguish between soil-specific and plant-specific phenomena.

The final goal is a generalized description of the relation between the concentration and species of a radionuclide present upon the solid phase of the soil, in the liquid phase and in biota.

Head of Project 1: Dr. Lembrechts

II Objectives for the reporting period

* Study the transfer of cesium along the soil/soil solution/plant pathway in phytotrons. In order to better distinguish between soil- and plant-specific phenomena uptake from soil is compared with uptake from nutrient solutions.
* Execute introductory experiments with nutrient solutions to describe the variability of the TF as a function of crop growth and composition of the nutrient solution (= NS).
* Begin experiments on transfer from soil to plant to (1) describe boundary conditions, (2) quantify yield and (3) allow for a first comparison with experiments on NS.

III Objectives for next period

* Compare the TF of Cs from nutrient solution to plant and from soil to plant for those physico-chemical parameters which most pronouncedly affect transfer.
* Monitor effects of changes in availability in the soil liquid on Cs accumulation by earthworms.
* Execute some illustrative experiments with plants and earthworms in order to test the relation between soil, soil liquid and organism for another radioelement, i.e. neptunium.

IV Progress achieved including publications

1. Uptake of Cs from nutrient solutions.

1.1. Materials and methods.

Fifteen lettuce seedlings, germinated in moist perlite, for five days in the dark and one day in the light, are grown on 11 L of nutrient solution contaminated with about 5 Bq ^{134}Cs/, for 3 weeks at most. They are grown in a controlled environment chamber. Various dilutions of the Steiner nutrient solution are used, on occassion enriched with one or more specific nutrients. The concentration ranges used are comparable to those observed in agricultural soils (Table 1). K, Ca and Cs are analysed in solution and plant, as well as conductivity and pH of the solution, water consumption, fresh and dry weight.

Table 1 : Ranges of a number of variables measured in the soil solution of the ITAL-lysimeters (with clay, loam or sand), in the soil solution of the preliminary pot experiments and in the various nutrient solutions (NS).

	Conduct. (mS/cm)	pH	K (mM)	Ca (mM)
Clay	2.4-0.41	7.9-7.0	2.8-0.18	21.4-1.30
Loam	1.8-0.34	7.9-4.9	2.6-0.20	20.1-0.80
Sand	3.6-0.29	7.4-4.5	5.5-0.36	13.6-0.45
Pot exp.	7.3-0.40	7.3-4.4	8.5-0.54	11.4-1.30
NS	4.3-0.30	7.9-4.1	16.7-0.06	11.4-0.12

1.2. Observations.

* In most cases the Ca-, K- and Cs-concentration of the NS, its pH and conductivity do not change during the experiment.
* Plant growth is exponential throughout the experiment. The average relative growth rate is 0.30.
* Within the concentration-ranges studied the Ca- and K-concentration of the plant do not depend on that of the NS.
* The TF solution - plant varies between 2.2 and 19.
* The observed ratio (OR = [Cs/K]plant / [Cs/K] substrate) varies between 0.003 and 0.287. K has a minor effect on Cs-uptake within the K-range studied (Figure 1), or, the OR increases directly proportional with the K-concentration of the substrate.
* The TF decreases with increasing Ca-concentration of the NS (Figure 1). If the concentration of all other nutrients is increased at the same time, no supplementary reduction in TF is realized.
* Changes in pH between 4.5 and 7.5 do not affect the TF.

2. Transfer soil - plant.

2.1. Materials and methods.

The moisture content of two sieved, field-moist, sandy soils is adjusted to 75% of the field capacity. After mixing ^{134}Cs (100 Bq/g) and fertilizer (Sporumix PG, 0.1-0.75 g/kg) thoroughly through the soil, it is incubated at 18 °C for 2 months. On each container with about 1 kg of soil one lettuce seedling is grown for about 3 weeks. Water loss due to evapotranspiration is replaced every day with deionized water. The soil solution is collected at harvest, by means of an immiscible displacement method, using chloroform as diplacent. The parameters analysed are given in paragraph 1.1.

2.2. Observations.

* The Kd of Cs varies between 250 and 550.
* The TF from soil solution to plant ranges from 1.1 to 34.8.
* The OR plant/soil solution ranges from 0.062 to 0.49.
* As the composition of the soil liquid sometimes pronouncedly changed in the course of the experiment, the transfer data on soils should be corrected for these changes. These corrections have not been made yet.
* The TF on the two soils is pronouncedly different. Changes in TF on each of the soils seem to be explained by changes in Ca-concentration of the liquid phase. The Ca-effect on soils is of the same order of magnitude as the effect observed on nutrient solutions. The difference between the observations on the two soils can, however, not be explained up to now (Figure 2).

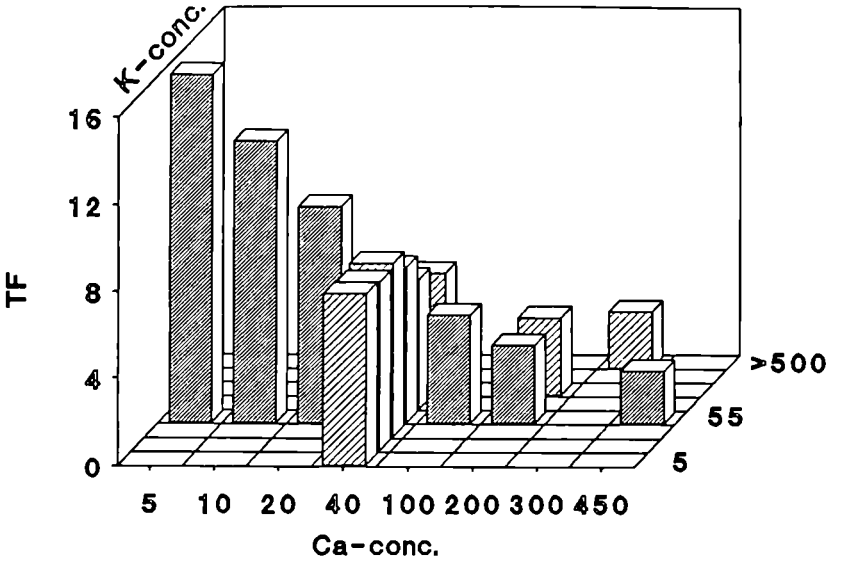


Figure 1 : TF of Cs from nutrient solution to lettuce plants as a function of the Ca- and K-concentration (in mg/L) of the nutrient solution.

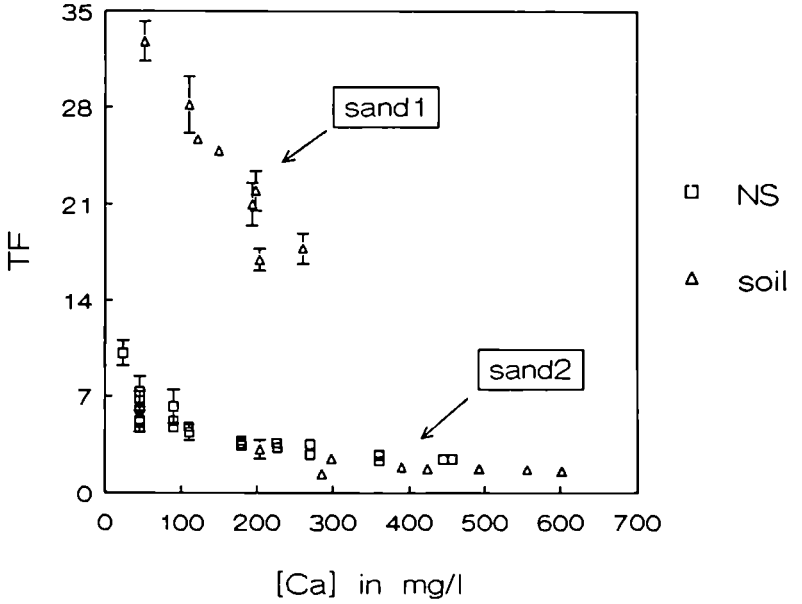


Figure 2 : Transfer of Cs (mean ± std. err.) from nutrient solution and from two sandy soils to lettuce as a function of the Ca-concentration of the substrate.

HEAD OF PROJECT : Dr B Wilkins/ Dr A Nisbet

II Objectives for the reporting period

1 To set up soil water sampling equipment in four plots in Cumbria. To relate time dependent changes in the concentration of radionuclide and stable elements in soil solution to their concentration in associated soils and pastures.

2 To conduct more detailed speciation studies on soil solutions derived for soils artificially contaminated with ^{137}Cs, ^{90}Sr, ^{239}Pu and ^{241}Am in 1983, using hollow fibre ultrafiltration.

3 To initiate batch equilibrium experiments under conditions representative of those found in situ to determine the effects of common agricultural treatments on radionuclide ad/desorption.

III Objectives for next period

1 To continue sampling and radiochemical analyses of soil, vegetation and soil solution in Cumbria over at least one year to characterise the soil-soil solution-pasture system for ^{137}Cs, ^{90}Sr, ^{239}Pu and ^{241}Am, as fully as possible.

2 To conduct more detailed batch equilibrium experiments on soil-soil solution equilibria to quantify the competition between nutrient ions and radionuclides for a range of soil types and treatments.

IV Progress achieved, including publications

1 Field Studies In Cumbria

Four of AEA's field plots in Cumbria were selected for more detailed investigation of radionuclide transfer along the soil-soil solution-plant pathway. The soil types were a deep peat (DP) and an acid brown earth (ABE) located in adjacent fields, and a peat ranker (PR) and an improved peat ranker (IPR) located close by. These upland sites are all grazed by sheep. In May 1990, six porous ceramic cup samplers were installed at each site adjacent to vegetation and soil sampling plots. Routine collection of soil solution started on a monthly basis in July 1990. Samples have been analysed for ^{137}Cs, ^{90}Sr, ^{239}Pu, ^{241}Am, K, Ca, NH_4, pH and conductivity. As it is recommended to allow several months for the cup samplers to equilibriate with the surrounding soil, the initial set of data presented here, should be interpreted cautiously.

SUMMARY SOIL SOLUTION DATA
(ranges for the period July-December 1990)

	pH	cond ($mScm^{-1}$)	K (mgl^{-1})	Ca (mgl^{-1})
ABE	4.2 - 4.8	0.04 - 0.09	1.0 - 2.1	0.4 - 0.9
DP	4.3 - 5.1	0.04 - 0.08	0.1 - 0.8	1.6 - 4.4
PR	3.8 - 4.2	0.10 - 0.23	0.2 - 0.4	0.4 - 0.9
IPR	6.0 - 6.4	0.07 - 0.15	0.4 - 0.8	4.3 -10.0

	^{137}Cs ($mBql^{-1}$)	^{90}Sr ($mBql^{-1}$)	^{239}Pu ($mBql^{-1}$)	^{241}Am ($mBql^{-1}$)
ABE	<10	15 - 48	0.1 - 0.5	0.1 - 0.5
DP	<10 - 20	27 - 45	0.2 - 1.7	0.2 - 0.4
PR	14 - 20	31 - 46	0.1 - 1.0	0.2 - 0.3
IPR	<10	26 - 32	0.1 - 0.4	0.2

The main features to note from these data are:
1 Very low pH, conductivities, K and Ca concentrations in the soil solution from unimproved soils. This is consistent with many published data for acid upland ecosystems, and a very marked contrast to agricultural ecosystems where pH >5.5, K > 10 mgl^{-1}, and Ca > 100 mgl^{-1}.
2 Activity concentrations of the nuclides in solution are also low and vary within a small range.

The results so far obtained suggest that most of the activity in these organic soils is held on the solid phase and is released into solution by desorption from the extensive cation exchange complex. Data from Cremers' infinite bath scenario tends to support this theory with complete and rapid desorption occurring within 10 hours in the same peaty soils. These soils maintain low concentrations in solution but have high replenishment factors.

To date, samples of vegetation have been analysed for ^{137}Cs, ^{90}Sr, ^{239}Pu and ^{241}Am. Their associated soils are currently undergoing the same determinations. Discussion of the relationship between the radionuclide content of soils, soil solutions and vegetation in the Cumbrian uplands will be deferred until the data set is complete.

2 Ultrafiltration studies on soil solution

A feasibility study begun in 1989 was extended to evaluate the potential use of hollow fibre ultrafiltration in determining the association of radionuclides in soil solution with different molecular size fractions. In January 1991 soil solution was collected from NRPB lysimeter soils that

had been artificially contaminated with ^{137}Cs, ^{90}Sr, ^{239}Pu and ^{241}Am in 1983. Ultrafiltration was performed on soil solution from loam, peat and sand soils, using hollow fibres with nominal molecular weight cut-off levels of 10^4 and $3x10^3$ daltons. The unfiltered and ultrafiltrate samples were analysed for ^{137}Cs, ^{90}Sr, ^{239}Pu and ^{241}Am and results compared to those from July 1989. Data so far available from the current, more detailed study, are consistent with those previously obtained and support the reproducibility of this technique. Up to 86% of the ^{239}Pu and ^{241}Am in soil solutions would seem to be associated with high molecular weight material (i.e. > $3x10^3$ daltons) which is probably not in a bioavailable form. Interesting differences in the molecular size distribution of ^{239}Pu and ^{241}Am before and after freeze/thaw events suggest changes in chemical form are brought about by these processes. The work may therefore be extended in the summer of 1991 to investigate changes induced by wetting and drying cycles. A paper summarising the study to date is in preparation.

3 Batch equilibrium experiments

Preliminary experiments involving the shaking of NRPB lysimeter soils and their associated soil solutions with a range of common agricultural treatments have been completed, and the distribution of radionuclides between solid and liquid phases determined after 24 hours. To date, experiments have been restricted to peat and loam soils using K, NH_4, Ca and NO_3 based fertilisers, applied in quantities equivalent to standard agricultural rates (i.e. 100 kg K ha^{-1}, 100 kg N ha^{-1}, and 3000 kg lime ha^{-1}). Potassium and ammonium were found to increase the activity concentration of ^{137}Cs in the liquid phase by factors of about 2 and 4 respectively, compared to controls. Calcium had little effect on the distribution of any nuclide, due to the already high calcium status of these particular soils (see table below). An increase in the desorption of ^{90}Sr following application of nitrate (as sodium nitrate) was thought to reflect the interaction between Na and ^{90}Sr (similar ionic radii) rather than NO_3. Initial results have been encouraging, so it is planned to extend this work to cover a wider range of soil types and treatments. A more comprehensive set of cations will be determined so that changes in soil-solution equilibria, induced by soil-based treatments, can be fully quantified.

Exchangeable K and Ca in experimental soils
(meq/100g dry soil)

	K	Ca
LOAM	47	770
PEAT	10	2410
TYPICAL ENGLISH LOAM	35-55	3-10

Head of Project 3: Dr. F.J. Sandalls

OBJECTIVES

(a) July 1990 - May 1991

The objectives for the period were:-

(i) to determine ^{134}Cs and ^{137}Cs in vegetation on the eight study sites as a continuation of a series of measurements begun in April 1987.

(ii) to provide samples of soil and vegetation to other members of the project team as and when required.

(iii) to determine, in soil water, the concentrations of those cations likely to influence soil-to-plant transfer of radiocaesium and

(iv) to investigate the degree to which plants differentiate between potassium and caesium at concentrations found in soil solutions.

(b) June 1991 - May 1992

The objectives for this period are:-

(i) to monitor the eight plots twice for ^{134}Cs and ^{137}Cs and to provide samples for other members of the project team.

(ii) to determine those stable elements in soil water which influence soil-to-plant transfer of radiocaesium.

(iii) to build and use a hydroponic system for studying relative root uptakes of potassium and caesium.

(iv) to determine, through laboratory measurements, the degree of association of caesium, strontium and possibly plutonium with humics.

PROGRESS

July 1990 - May 1991

Samples of vegetation were collected from the eight sites in upland Cumbria on May 30, September 12 1990 and May 15 1991. The ^{134}Cs and ^{137}Cs found in the 1990 samples are shown in Table 1. The 1991 samples are yet to be analysed.

Table 1

^{134}Cs and ^{137}Cs in Vegetation in May and September 1990

Site Ref	May 1990		September 1990	
	^{134}Cs (Bq g^{-1} dw)	^{137}Cs (Bq g^{-1} dw)	^{134}Cs (Bq g^{-1} dw)	^{137}Cs (Bq g^{-1} dw)
DP	0.60	1.24	0.90	1.79
PR1	0.34	0.83	0.44	1.04
PR2	0.27	0.65	0.33	0.76
IPR	0.43	0.95	0.44	0.98
PG	0.25	0.59	0.14	0.34
PB	0.07	0.16	0.025	0.06
IBE	0.03	0.11	0.14	0.34
BE	0.03	0.06	0.01	0.03

It is of interest to see how the ratio $^{137}Cs/^{134}Cs$ in vegetation has changed from 1987 to 1990. The ratios for each year at all sites are shown in Table 2. All values are quoted as of May 1986.

Table 2

Ratio $^{137}Cs/^{134}Cs$ in Vegetation, 1987-1990

Year	Ratios $^{137}Cs/^{134}Cs$							
	DP	PR1	PE2	IPR	PG	PB	IBE	BE
1987	1.9	1.8	1.9	1.9	1.5	1.5	1.7	2.1
1988	2.0	2.2	2.2	2.2	2.0	2.1	2.1	2.2
1989	2.1	2.4	2.3	2.3	2.3	2.4	2.3	2.1
1990	2.0	2.4	2.4	2.2	2.2	2.6	2.7	2.0
In soil 1987	2.0	2.2	2.1	2.3	2.4	2.9	2.6	2.3
In Air 1986	1.7 ± 0.1							

Correlation of TF_{134}'s and K in soil water at field capacity

In 1988, we produced plots showing a correlation between TF_{134} and potassium in soil water at field capacity. We then perceived the determination of potassium in soil water at field capacity to be a simple and rapid means of assessing the sensitivity of any given soil to potential radiocaesium contamination. In 1990, the potassium in soil water was again determined at each of the eight sites bu the correlation with TF_{134} was no longer evident. The levels of potassium in the soil water at field capacity in May 1990 compared with the levels in July 1988 are shown in Table 3.

Table 3

Potassium in Soil Water (Field Capacity) in July 1988 and May 1990

Soil Ref	1987 K^+ ($\mu g\ ml^{-1}$)	1990 ($\mu g\ ml^{-1}$)
DP	10	58
PR1	17	19
PR2	6	21
IPR	7	19
PG	14	32
PB	21	27
IBE	7	38
BE	35	23

There are several possible reasons why this relationship was not apparent in 1990. They are (i) inaccurate determinations of K^+ (ii) with the passage of time, the relationship no longer holds since much of the ^{134}Cs has undergone a "fixation" process and is not behaving in the same way as the potassium (ie selective adsorption): the degree of fixation will have been different on each of the different soils, probably depending largely on the amount of mineral matter in each soil and (iii) the levels of ^{134}Cs are now difficult to determine accurately.

NH_4 +, Na and Cs in soil water at field capacity ($\mu g\ ml^{-}$)

Sufficient water was added to the soil samples (topmost 5cm layer) to bring them to field capacity. The soil water was then expressed, filtered and analysed for ammonium, sodium, potassium and caesium. The results of these determinations are shown in Table 4.

Table 4

NH_4^+, Na and Cs in soil water at field capacity ($\mu g\ ml^{-}$)

Site Ref	Na	Cs	NH_4
DP	24.5	0.4	17
PR1	13	5.3	31
PR2	8.25	5.1	42
IPR	11	0.6	0.3
PB	9.5	4.8	25
IBE	9	0.7	39
BE	5.5	0.1	43
PG		4.7	

Note: The ICP-MS technique may prove to be unreliable for the very low concentrations of Cs: further investigation is called for.

DO PLANTS DIFFERENTIATE BETWEEN POTASSIUM AND CAESIUM IN ROOT UPTAKE?

The reported soil-to-plant transfer factors vary enormously from species to species but this may simply be a reflection of (i) the alkali metal demand of the species and (ii) the ratio K/Cs in the soil where the plant derives its nutrients rather than deflecting the ability of the plant to differentiate between K and Cs. We have therefore used a hydroponic system (NFTl) to grow tomatoes and determine the amounts (more specifically the relative amounts of K and Cs in the NFT solution, and in the shoots, fruits and calyces. The NFT solution was also analysed for other ions and these results are shown in Table 5. Samples were collected for analysis in June and August 1990.

Results

Table 5

Potassium and Caesium in Tomato Plants

June 1990

Sample	K (mg/gdw)	Cs *(mg/gdw)	Ratio K/Cs
Fruit	43 ±6	5.0×10^{-5}	8.6×10^{5}
Shoots	44 ±3	1.2×10^{-4}	3.7×10^{5}
Calyces	24 ±3	1.0×10^{-4}	2.4×10^{5}
	K (mg/ml)	**Cs (mg/ml)**	
NFT Before	325 ±8	3.1×10^{-5}	1.05×10^{7}
NFT After	309 ±6	3.05×10^{-5}	1.01×10^{7}

August 1990

	K (mg/gdw)	Cs *(mg/gdw)	Ratio K/Cs
Fruit	38 ±2.4	$0.14 \pm 0.02 \times 10^{-3}$	2.7×10^{5}
Shoot	45 ±2.4	$0.17 \pm 0.04 \times 10^{-3}$	2.6×10^{5}
Calyces	29 ±1.4	$0.012 \pm 0.0007 \times 10^{-3}$	2.4×10^{6}
	K(mg/ml)	**Cs(mg/ml)**	
NFT Before	319 ±20	0.000875 ± 0.0778	3.6×10^{5}
NFT After	307 ±36	0.00145 ±0.212	2.1×10^{5}

For K, the means and SDs are from AAS and ICPMS.

Cs in the redistilled HNO_3 used for oxidation of samples was not determined: this could be a source of error.

Conclusion

It is intended to repeat the measurements using either an NFT system containing radiocaesium or to determine the Cs in the present samples using neutron activation analysis.

Head of project 4: Prof. A.Cremers

II. Objectives for the reporting period

Research efforts were focussed on the following objectives: (a) characterization of soils in terms of specific interception properties for radiocaesium and identification of problem soils; (b) the identification of soil chemical parameters which regulate the availability of radiocaesium; (c) the reversibility of radiocaesium sorption and the possible effects of aging on reversibility.

III. Objectives for the next period

The objective for the next period are: (a) optimization and upscaling of the methodology for measuring Cs-availability in soils: (b) extension of the methodology to soils contaminated by Cs and Sr, using γ and LS-counting; (c) radiocaesium availability measurements in aged soils for which soil-to-plant transfer studies have been carried out in various lysimeter studies in the Community; (d) soil-plant transfer studies of radiocaesium.

IV. Progress achieved

1. Specific sorption potential of radiocaesium in soils

A series of podzol soils (forest, pasture, agricultural, A & B horizons) from the Belgian Campine region, three lysimeter soils (sand, loam, peat) studied by NRPB, and three Belgian soils of varying texture (loamy sand, loam, clay) were characterized in terms of specific radiocaesium interception potentials using the AgTU masking technique described in earlier reports. These parameters are defined as the product of the number of micaceous frayed edge sites and the trace Cs-to-K and Cs-to-NH_4 selectivity coefficient. These quantities are represented by the symbols $[K_D.m_K]$ and $[K_D.m_N]$ respectively. The results are shown in Table 1.

Table 1 Specific radiocaesium interception potentials of soils

Soil	C.E.C. (meq/100g)	$[K_D.m_K]$ meq/g	$[K_D.m_N]$ meq/g	$K_c(N/K)$
Clay	36.4	6.64	1.04	6.4
loam	10.5	3.30	0.52	6.3
loamy sand	11.2	1.64	0.29	5.7
sand	10.8	2.75	0.46	6.0
loam	16.1	4.20	0.68	6.2
peat	133.0	0.14	0.034	4.1
podzol 1	18.3	0.15	0.045	3.4
2	3.6	0.42	0.060	7.0
3	4.5	0.26	0.049	5.2
4	10.1	0.13	0.034	3.8
5	10.6	0.28	0.066	4.2
6	4.5	0.11	0.019	5.8

It is seen that $[K_D.m_K]$ values cover a wide range (6.6 to 0.1 meq/g). The lower end of the scale 0.1-0.3 meq/g), as obtained for podzols coincides with the results obtained on Cumbrian peat soils. Since these values are a direct measure of S/L distribution behaviour (along with soil solution levels of K and NH_4), these two groups of soils clearly fall in the category of problem soils. The NH_4 to K selectivity coefficient in the specific sites, as obtained from the ratio of $[K_D.m_K]/[K_D.m_N]$ covers the narrow range of 4-7 (as obtained for reference micaceous clays); this would indicate that the nature of the specific sites in these various soils is identical.

2. Desorption potential of radiocaesium

In order to assess the desorption potential of radiocaesium a comparative study was carried out using a variety of displacing agents. Two groups were considered: (a) bulky ions or complexes showing high selectivity for the non-specific sites;(b) common salts. In group (a) the following chemicals were used (concentrations and symbols shown in parenthesis): silver-thiourea (0.015M AgTU, silvermethyl thiourea (0.015M AgMTU), silverethylenethiourea (0.015M AgENTU), bistrimethylammoniumethane (0.01M BTM-2), bis-trimethylammoniumhexane (0.01M BTM-6), Copper-ethylenediammine (0.01M CuENDA) and trimethylammonium (0.01M TMA). The second group includes 1M solutions of KCl, NH_4Ac and $CaCl_2$. Soils were labelled with Cs-137, equilibrated for 24 hrs, centrifuged and submitted to three consecutive desorption treatments ((S/L ratio = 1/50). In all treatments, except $CaCl_2$, the major displacement yield is obtained in the first treatment. In the case of $CaCl_2$ however, desorption yields in the three treatments were comparable although slightly decreasing. Results are shown in Table 2.

Table 2. Desorption yields (%) of radiocaesium from soils as obtained by these consecutive extraction treatments using various displacement agents (see text)

	Clay	Loam	loamy sand	Podzol-1
BTM-6	0.3	0.5	1.9	1.4
BTM-2	0.3	0.3	0.6	4.1
TMA	0.4	0.4	1.4	10.6
AgENTU	0.3	0.5	1.3	10.8
AgMTU	0.5	1.0	2.0	21.8
AgTU	1.1	2.5	6.8	40.9
CuENDA	2.8	7.4	11.8	33.9
$CaCl_2$	0.7	1.8	3.8	56.4
NH_4Ac	12.4	35.9	56.3	62.5
KCl	13.4	48.5	88.7	75.5

These data require various comments. It is seen that for the first group of ions, desorption yields in the clay, loam and sandy loam are quite low. Such result could be expected on the basis of the bulky nature of these ions. However, the podzol data show a quite different behaviour (1 to 40% desorption yield). The fact that BTM-6 fails to displace any significant amount of caesium in the podzol indicates that caesium is in fact localized in the specific sites. The finding that the other bulky ions exhibit much larger desorption yields would indicate that the nature

of these sites in podzol soils is quite different, contrary to what one might expect on the basis of sorption studies (section 1). The pronounced diversity in the desorption behaviour is further confirmed in the data obtained by conventional treatments (K, NH_4, Ca). The main conclusion of this study is that the desorption of radiocaesium from specific sites (which, on the basis of sorption characterization, were considered to be quite similar in the various substrates) may be drastically different in various soils.

3. Intrinsic availability of radiocaesium: a new method

We have developed a new procedure in which the limitations, inherent to some currently used methods, are eliminated. The method is based on the use of an "infinite bath" scenario in which radiocaesium desorption can proceed into a liquid phase of essentially zero activity. Such boundary conditions can be generated by a dialytic equilibration between the soil sample and a sulphonic acid resin (either in the K- or the NH_4-form) in a dilute solution of KCl or NH_4Cl (10^{-3}M). The total amount of resin is such that the radiocaesium level in the liquid phase is lowered by at least one order of magnitude, as compared to its level in equilibrium with the soil. The process can be monitored by regular radioassay of the activity collected on the resin. In practice, the experiment is carried out in liquid scintillation vials containing 1-2g resin and 100-200 mg soil in a dialysis membrane. At regular time intervals, the soil is transferred to a new resin bed and the activity collected is assayed by LS-counting.

For all podzol soils studied radiocaesium desorption is completed within a day in spite of very low K concentrations in the liquid phase (figure 1). For peat soils from Cumbria, complete desorption - or very nearly so - is obtained within 10 hours (figure 2). In contrast, desorption yields in the brown earth soil drops to about 50%. The sequence coincides well with the $[K_D.m_K]$ value of these soils. This finding is somewhat surprising in that desorption yield can hardly be expected to be related to the number of specific sites. This finding is all the more surprising since desorption studies carried out on illite clay demonstrated that near 100% desorption yields could be obtained. Consequently, it must again be concluded that specific sites, which may otherwise seem quite similar in regard to caesium sorption selectivity, may exhibit extremely diverging behaviour in desorption properties.

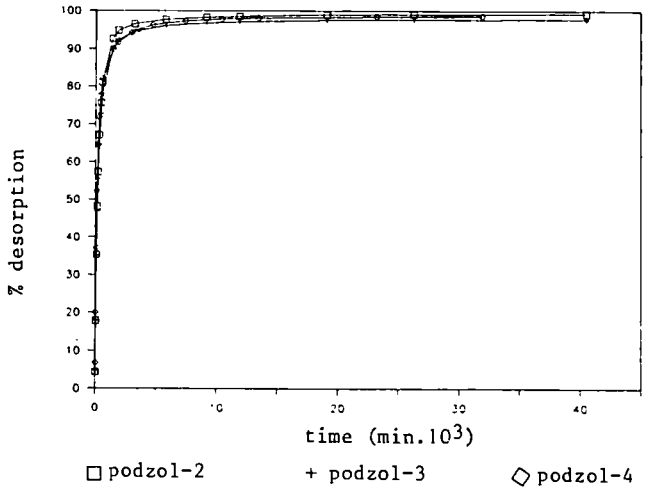


Figure 1. Radiocaesium desorption from podzol soils using sulphonic acid resins. Aging time: 1 day (10^{-3}M KCl)

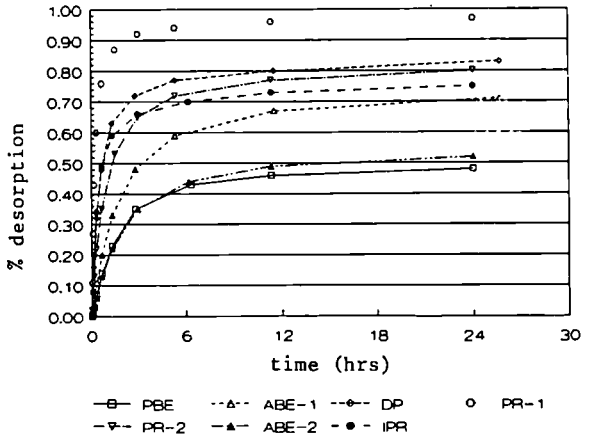


Figure 2. Radiocaesium desorption from Cumbrian Soils using sulphonic acid resins. Aging time: 1 day.(10^{-3}M NH_4Cl)

Contract: Bi7-018 Sector: A24
Title: Factors affecting radiocaesium transfer to ruminants.

1	Howard	ITE
2	Vandecasteele	CEN - SCK
3	Mayes	McAulay Land Use Research.Inst.
4	Belli	ENEA
5	Stakelum	Agric. and Food Develop. Authority
6	Colgan	NEB
7	Assimakopoulos	Univ. Ioannina
8	Unsworth	Univ. Nottingham
9	Jones	Univ. Uppsala Agricult.Sciences

I. Summary of Project and Global Objectives

There are a number of animal and dietary factors which have an important effect on the transfer of radiocaesium to ruminants, but which have not been fully investigated. Similarly, few attempts have been made to understand results in terms of processes and mechanisms. This programme is aimed at identifying and understanding the most important factors which affect the transfer of radiocaesium to animal tissues.

The first phase of a 2 year programme is described. The programme involves 9 laboratories in 6 countries: Belgium, Ireland, Greece, Italy , Sweden and the United Kingdom; scientists from Norway (K. Hove) have also contributed on an informal basis. The research studies are structured to compliment each other and experimental and sampling protocols have been agreed so that results are directly comparable.

A number of experiments have used the true absorption coefficient to measure uptake in the gut. The technique has the advantage that it does not need equilibrium between tissues and feed. It is also a more direct method of establishing differences in the gut transfer of radiocaesium under a variety of different dietary and animal conditions. The technique involves the dual administration of two isotopes of radiocaesium. One isotope is administered orally, the other is administered as a intravenous infusion to estimate endogenous excretion from body tissues back into the gut. Transfer of dietary radiocaesium across the gut wall can then be calculated taking endogenous excretion into account.

Other integrated aspects of the study include measurements of the soil adhesion to pasture grass and fodder. Allied to this study the availability of radiocaesium in different types of soil for absorption in the gut is being measured.

The programme splits into three topic areas. The two experimental topics of (i) animal and (ii) dietary factors affecting the transfer of radiocaesium provide information to the third topic area, modelling.

Head of Project 1: Dr. Howard

II Objectives for the reporting period

i) Co-ordination of programme;
ii) Commence metabolism cage studies to investigate the effect of age on the transfer of radiocaesium to lambs (a collaborative study with MLURI);
iii) Collection of samples from two sites to determine the extent of soil adherence to vegetation;
iv) To conduct joint experiment with MLURI on the dynamics of radiocaesium in sheep tissues to provide data for a model of radiocaesium behaviour in ruminants developed by Nottingham University;
v) To compare the availability of radiocaesium for transfer to sheep from different environmental sources in a collaborative study with MLURI.

III Objectives for next period

i) Co-ordinate completion of programme and preparation of collaborative publications and final report;
ii) To finish the metabolism cage experiments investigating the effect of age on the transfer of radiocaesium to lambs; data analysis and preparation of publications;
iii) Completion of gamma-analyses of samples for 'soil adhesion' study. Determination of soil adhesion by chemical analyses (Ti^{+} and loss on ignition) including analyses of samples from CEN-SCK, ENEA-DISP and NEB.
iv) Gamma-analyses of samples from cattle studies being conducted at TEAGASC

IV Progress achieved including publications

1. Effect of age on the transfer of radiocaesium to lambs

The true absorption coefficient of radiocaesium has been measured in five groups of male Scottish Blackface lambs, aged approximately: 11, 16, 20, 30 and 39 weeks. A final study using lambs aged approximately 14 months will be conducted in late June 1991.

The animals were housed in metabolism cages and fitted with faecal collection bags to allow the collection of faeces and urine separately. The 11 week old lambs were bottle-fed ewe milk and were not offered solid food. Caesium-137 was added to the milk at each feed. The group of animals aged 16 weeks old received a mixed diet of both ewes milk and fresh grass. Half of the animals within this group were administered Cs-137 in milk; the remaining animals received uncontaminated ewes milk and were administered gelatin capsules containing ionic Cs-137 absorbed onto filter paper. All the subsequent age groups were offered a diet composed solely of fresh grass and Cs-137 was administered in gelatin capsules.

At the start of each experiment the lambs were administered Cs-134 as CsCl intraveinously. Faecal and urine collections were made daily.

The true absorption coefficient (A_t) for each group of lambs is shown in table 1. For comparison the value determined for lactating ewes using a similar experimental protocol is also shown. The results suggest that

there is no change in true absorption with age. However significant differences were found between animals receiving Cs-137 in milk compared with those administered Cs-137 in gelatin capsules with fresh grass. This may simply be due to a greater availability of radiocaesium in milk or may be connected to the fact that radiocaesium present in milk passes directly to the abomasum, whereas Cs-137 in solid food and capsules would enter the rumen. The A_t value of approximately 1.0 obtained for the two groups of lambs receiving contaminated milk is the highest value possible and indicates that complete absorption has taken place.

Table 1. The true absorption coefficient of radiocaesium determined in lambs of different ages.

Approximate age	n	Diet	Cs-137 source	A_t (mean±SE)
11 weeks	4	Ewes milk	Ewes milk	1.00±0.001
16 weeks	5	Ewes milk & fresh grass	Ewes milk	1.02±0.007
16 weeks	5	Ewes milk & fresh grass	Gelatin capsule	0.85±0.022
20 weeks	5	Fresh grass	Gelatin capsule	0.80±0.016
30 weeks	5	Fresh grass	Gelatin capsule	0.82±0.015
39 weeks	5	Fresh grass	Gelatin capsule	0.84±0.022
Lactating ewes	3	Lucerne pellets	Gelatin capsule	0.84±0.130

2. Soil adhesion study

Routine sampling of soil, vegetation and faeces from two site in Cumbria was completed in April 1991. Radioanalyses to determine Cs-134 and Cs-137 activity concentrations in the samples is ongoing.

3. Radiocaesium dynamics

Fifteen 1-2 year old female sheep, housed in metabolism cages, were administered Cs-134 directly into the rumen. The sheep were fitted with both bladder and blood catheters. Three sheep were killed 0.5, 1, 4, 12 and 20 days after dosing and a range of tissues taken for gamma-analyses. Samples of blood, urine and faeces were taken at 1, 2, 4, 8, 12, 20, 30, 47, 72 and 96 hours after radiocaesium administration.

Gamma-analyses of the samples are largely completed and the data has been supplied to Nottingham University to incorporate into the model.

4. The bioavailability of radiocaesium from different environmental sources

The true absorption coefficient of radiocaesium in non-lactating ewes has been determined for three environmental sources: Calluna vulgaris and upland grassy vegetation both collected from upland areas of west Cumbria (UK) which was contaminated by fallout from the Chernobyl accident; and silt from the Ravenglass estuary which is contaminated by marine discharges from the Sellafield Reprocessing Plant. The results are presented in table 2.

Table 2. True absorption coefficients for Cs-137 in ewes from three different environmental sources.

Cs-137 Source	n	A_t (mean±SE)
Grassy vegetation	4	0.88±0.093
C. vulgaris	2	0.67±0.034
Ravenglass silt	3	0.12±0.018

The availability for transfer of Cs-137 from both herbage types was significantly higher than from Ravenglass silt ($p<0.01$). Differences in At between C. vulgaris and grassy vegetation were only significant at the 10% level. The availability from grassy vegetation was similar to that of ionic radiocaesium presented in table 1.

Publications:

Beresford, N.A. & Howard, B.J. (in press)
The importance of soil adhered to vegetation as a source of radionuclides ingested by grazing animals. Sci. Tot. Environ.

Crout, N.M.J., Galer, A., Howard, B.J. & Beresford, N.A. (in press)
A comparative assessment of the impact of the Windscale and Chernobyl accidents on 137Cs levels in upland lamb in west Cumbria using the RUINS model. In: Seminar on Comparative Assessment of the Environmental Impact of Radionuclides Released During Three Major Nuclear Accidents: Kyshtym, Windscale, Chernobyl. CEC/IUR:Luxembourg.

Galer, A.M., Crout, N.M.J., Beresford, N.A. & Howard, B.J. (1990)
Modelling radio-caesium transfer in upland ecosystems. In: 1st Int. Conf. on the Atomic Power Plant Accident at Chernobyl. p 264. Moscow.

Howard, B.J., Beresford, N.A. & Mayes, R.W. (1990)
Factors affecting radiocaesium transfer to ruminants. In: Proc. SCOPE RADPATH meeting. RL.13.90. Lancaster 26-30 March 1990.

Head of Project 2: Dr. Vandecasteele

II Objectives for the reporting period

Participation in the study on the extent of soil adhesion in different pasture systems and on various fodders, including monthly sampling of grass and sheep faeces at two different locations in Belgium with different pedological characteristics; winter fodder has also been sampled and soil samples have been taken from all sites when possible. Commencement of a study on the effect of feed characteristics on radiocaesium transfer to sheep: highly contaminated vegetation has been produced in a greenhouse for feeding to sheep and *in vitro* extraction techniques have been investigated.

III Objectives for next period

1) Soil adhesion study: finish monthly sampling, complete sample analysis for radiocaesium, Ti, or mineral content (by LOI);
2) Forage type feeding trials: two types of contaminated forage produced under controlled conditions will be fed to sheep housed in metabolic cages; availability of bioincorporated radiocaesium will be determined by measuring radiocaesium activity concentrations in urine, faeces and blood. The effect of forage treatment will also be assessed by feeding forage fresh and after drying, ensilaging and freezing;
3) Breed study: a study to looking at radiocaesium uptake in different sheep breeds, comparing the two main Belgian breeds (Suffolk and Texel).

IV Progress achieved including publications

1) Soil adhesion study

The sampling protocol agreed within the CEC group has been followed. Two permanent pastures on two sites with contrasting soil types have been selected in Belgium : at the experimental farm of the SCK/CEN (sandy soil) and at the University of Louvain-La-Neuve farm (loamy soil). Grass and faeces samples were taken monthly, starting in May 1990 ; sampling will continue until May 1991. Soils at the two sites were sampled in June and November 90 and another sampling will be taken in May 1991.

The following winter fodder samples were collected :

Mol:	LLN:
hay	hay
grass silage	grass silage
maize silage	ground barley
beet roots	

Most of the Belgian sheep breeders buy in their winter fodders, so it is not always possible to obtain a sample of the appropriate corresponding soil. However we have managed to locate a dairy farmer who was willing to cooperate and identified the soil corresponding to his winter fodders. Unfortunately it was to late to sample at the time of cutting. Therefore, sampling of the feed will only take place at the beginning and the end of the feeding period.

2) Forage type feeding trials

A practical method for producing contaminated plant material was tested successfully. Rye-grass was sown in PVC trays (100 x 84 x 7 cm) filled with a synthetic, water-retaining polymer (Na polyacrylamide resins, Aquastock R°, Sodetra, France). This substrate has a pure water retention capacity up to 150 times its dry weight, has a low exchange capacity and supplies the plants with a suitable foothold. After harvest the resin can be dried to its original volume thereby limiting the volume of radioactive waste. The vegetation was contamination by adding Cs-134 as CsCl to the nutrient solution. Three successive cuts were possible, the first one contained more than 16% of the radioactivity initially introduced into the system.

Different forage species will be highly contaminated using this method and subsequently mixed with similar uncontaminated material and fed to sheep to determine the relative availability of radiocaesium incorporated into different forage plant species. Cs-137 will simultaneously be infused into the jugular vein to determine the true absorption coefficient. The contaminated plant material will be fed in different forms (fresh, silage, dried and frozen) to assess the influence of processing on the availability of radiocaesium for uptake.

In vitro estimation of radiocaesium availability

In parallel to the intended *in vivo* measurements of radiocaesium uptake an *in vitro* digestion method was used to investigate whether such a technique could be used to obtain a rapid estimation of the bioavailability of radiocaesium associated with plant material. Three types of herbage (Italian rye-grass, red clover and maize) were internally contaminated by adding Cs-134Cl to nutrient solution. Extractions were performed on these three sources presented in 3 different states; either fresh, dried and frozen. Additionally maize was also contaminated by injection of Cs-134Cl into the stem at flowering. The dried, pulverized leaves from this maize were used in both *in vivo* and *in vitro* studies.

Vegetation samples were incubated at 39°C in test tubes with cow rumen liquor and an artificial saliva buffer for 48 h. HCl and pepsin were then added and the tubes were incubated for another 48 h period before being neutralised with NaOH and incubated for one more day.

At each step, the contents of three tubes were filtered through a nylon cloth with calibrated pores of 50 μm The activity recovered in the filtrate was compared with the initial activity within the plant material. For each series of tubes, vegetation standards contaminated with a known amount of Cs-134 as CsCl were also incubated to assess the digestive potential of the rumen juice used in each test, by measuring the repeatability of the digestibility and Cs extractability of each series of tests. A fixed amount of the same batch of each plant material was contaminated with Cs-134Cl, cut into roughly 1 cm pieces prior to extraction.

The digestibility of grass and clover was estimated at each step and found to be similar; drying or freezing the plant material prior to the test did not significantly modify the digestibility compared with that of fresh material, except in the case of maize when the digestibility of the frozen material was higher than that of oven-dried plants; that of fresh material was the lowest. The extractability was high (almost 100%) for all species and all treatments.

This extraction procedure was also carried out on maize which had been fed to sheep in an earlier *in vivo* experiment. Maize was contaminated at flowering by an injection of Cs-134 into the stem and harvested at maturity. A small amount of finely (2 mm) ground leaves was fed to four young male sheep, whilst four others received ionic Cs-134 (CsCl). In the *in vivo* study the availability of radiocaesium incorporated into maize was 25% lower than that of ionic radiocaesium. However the Cs-extractability measured *in vitro* was comparable with that of ionic radiocaesium added to uncontaminated dry maize.

The discrepancies between these two sets of results may be tentatively explained in two ways. Firstly, part of the radiocaesium activity passing the 50 μm nylon frame could still be associated with small plant fragments and would not be able to cross the gastro-intestinal barrier. Repeating the extractions using ultracentrifugation to remove <50 μm plant fragments or using a 20 μm filter would avoid this problem. Secondly the finely ground labelled plant material may pass comparatively quickly through the animal stomach, thereby reducing the potential for microbial attack in the rumen and the contact time in the absorptive parts of the GIT. Studies feeding the same forage species ground to different sizes would help to see whether this effect is important. For forage type feeding trials, it is probably better to take easily digestible plant species and to compare them with plant species with a high fibre content (e.g. grass versus straw, young grass versus old grass).

The *in vitro* digestion method described here may be useful for rapidly estimating the availability of radionuclides associated with forage plants, but the technique needs further development and its reliability needs to be verified.

Publications and presentation of studies:

Pollaris, K. (1990)
Relative bio-availability of caesium incorporated into plant material to sheep", communication at the IUR Workshop on "Plant-animal transfer IUR-WG", Neuherberg, April 23-25, 1990.

Vandenhout, S. (1990)
Test de digestibilite in vitro applique a des aliments contamines au Cs-134, Memoire de Fin d'Etude ISIP Bruxelles, CEN/SCK Mol, Sep. 1990.

Vandecasteele C.M., Fagniart E., Van Hees M., Hurtgen C., Burton O. & Kirchmann R. (1990)
Comparative study of the behaviour of radiocaesium and radiostrontium from different source terms in pasture systems, Communication at the All Union Conf. on the "Geochemical pathways of artificial radionuclides migration in the biosphere", Gomel (USSR), October 13-21.

Head of Project 3: Dr. Mayes

II Objectives for the reporting period

1. To evaluate methods of estimating the true absorption coefficient of radiocaesium in sheep eating different vegetation types, animal experimentation, sample preparation and analysis, data preparation;
2. To conduct studies to quantify the relative rates of absorption and recycling of radiocaesium in different parts of the digestive tract of the sheep, as affected by the radiocaesium source and type of diet: commencement of animal experimentation;
3. Examination of the effect of age on radiocaesium absorption in sheep (jointly with ITE, Merlewood), animal experimentation; samples preparation.

III Objectives for next period

1. To evaluate methods of estimating the true absorption coefficient of radiocaesium in sheep eating different vegetation types, preparation of papers for publication;
2. To conduct studies to quantify the relative rates of absorption and recycling of radiocaesium in different parts of the digestive tract of the sheep, completion of animal experimentation; sample preparation and analysis; data preparation; preparation of paper for publication;
3. Examination of the effect of age on radiocaesium absorption in sheep (with ITE):completion of animal experimentation; sample preparation.

IV Progress achieved including publications

1. Variation in true absorption of different sources of radiocaesium

The purpose of this study was to estimate the availability to adult sheep, in terms of the true absorption coefficient, of radiocaesium present in two vegetation types, heather (C.vulgaris) and hill grass. The use of a second isotope of radiocaesium, administered as a continuous intravenous infusion was chosen as the means of obtaining an estimate of the transfer of dietary radiocaesium across the gut wall. There are, however, a number of methods of estimating such transfer. Thus a second objective of this work was to compare two such methods, so that recommendations could be made for establishing a procedure for future routine use.

The contaminated heather and hill grass were harvested from separate sites in west Cumbria, England in summer, 1990. The vegetation was stored at -20^{o}C until use. Both vegetation types contained mainly Cs-137 which had originated from the reactor accident at Chernobyl; it is probable that the radiocaesium was contained within the plant tissues.

Eight mature Scottish Blackface ewes were used. They were housed in metabolism cages after previously grazing a pasture containing predominantly perennial ryegrass. In order that the animals would become accustomed to the experimental diets, four animals received freeze-stored uncontaminated heather and four animals were given freeze-stored hill grass which was also uncontaminated. After 10 days, catheters were inserted into the jugular vein and into the bladder. An infusion of Cs-134 into the jugular vein was begun, concurrent with changing the heather and grass diets to the respective contaminated vegetation types (1 kg dry weight per day in two feeds). Total collections of faeces and urine were made; the bladder catheters allowed the collected urine to be free of faecal contamination. The Cs-134 infusion, feeding of

contaminated vegetation and excreta collections continued for 7 days. Two sheep were removed from the heather treatment due to loss of appetite in one animal, and breakage of the infusion catheter in the other animal.

Samples of vegetation and faeces were dried (80°C) and ground. The daily urine and dried faeces samples were bulked over the first three and final four days prior to analysis for gamma-emitting nuclides. Samples of vegetation, faeces, urine and Cs-134 infusion solution were subjected to gamma analysis using a Ge detector.

It was proposed to compare two methods of estimating the true absorption coefficient. In Method (1) the turnover rate of dietary Cs-137 through the blood plasma pool (the irreversible loss rate) is estimated from a knowledge of the rate of infusion of Cs-134 into the blood, and the ratio of Cs-137:Cs-134 activity concentrations in the blood plasma; as the radiocaesium in urine is derived only from that in the plasma, the Cs-137:Cs-134 ratio in urine is used. The true absorption coefficient is estimated as the quotient of the plasma Cs-137 turnover rate and the dietary Cs-137 intake rate. In Method (2) the true absorption coefficient of Cs-137 is estimated from measurements of the intake, total faecal excretion rate and the faecal excretion of endogenous Cs-137; faecal endogenous Cs-137 is the rate of transfer of the nuclide from the blood to the faeces and can be estimated from a knowledge of the urinary excretion rate of Cs-137 and the ratio of the faeces: urine excretion rates of Cs-134.

In both methods it is assumed that, after absorption, both nuclides are in the same form. As long as the animals were not contaminated prior to radiocaesium administration, and that the infusion and feeding of both radionuclides began at the same time, tissue deposition and depletion would have no effect upon the true absorption estimate. Corrections can be made for any Cs-134 in the diet, as long as it is in the same form as the dietary Cs-137.

Because gamma analyses have not been completed results are only available for the true absorption coefficient estimated by Method (1). The true absorption coefficient for the heather was 0.67 ± 0.034 (SD), and 0.88 ± 0.093 for the hill grass. These results suggest that heather is less available. It is interesting to note that the true absorption coefficient for hill grass from a different site in Cumbria, collected in 1987, with measurements being made in 1988, was 0.87 whereas the value for ionic Cs-137 was 0.84. These results suggest that, from a year after the Chernobyl accident (or possibly earlier), the radiocaesium from that source in grass was behaving as ionic caesium.

2. Radiocaesium absorption in the ruminant gut

Little is known of the behaviour of radiocaesium in the digestive tract of the ruminant, and how it is affected by differences in the physiological conditions within the gut, brought about by dietary differences. With information of such behaviour, and a better understanding, better prediction models describing radiocaesium transfer to ruminants could be developed. Furthermore, an improved understanding of the modes of action of various countermeasures would result.

Rates of true absorption of radiocaesium and its return to the gut in secretions at various sites along the gut can be measured using similar techniques to those used for determining the true absorption coefficient, with extra sophistication through the use of sheep which have been surgically prepared with cannulas for sampling and marker administration. The purpose of the work which has recently begun, is to examine the behaviour of radiocaesium in the gut of the sheep using two dietary sources of Cs-134, ionic and that present in an organic soil 2 years after injection. Two diets will be used, a grass (perennial ryegrass) and a cereal-based concentrate diet; the former represents a high moisture-content roughage diet whereas the latter is a dry diet of high starch content. These diets can be expected to generate large differences in conditions within the digestive tract which might influence the behaviour of radiocaesium.

Due to difficulties in obtaining large numbers of surgically-prepared sheep it was decided to make measurements in two experimental periods, allowing 6-8 weeks between each period for decontamination of the animals. At present, only the first experimental period has been completed.

Eight mature Scottish Blackface ewes, each fitted with a ruminal fistual and 'T'-shaped cannulae at each end of the small intestine (duodenum and terminal ileum) were housed in metabolism cages and offered freeze-stored perennial ryegrass 800 g DM per day). After about 10 days, continuous intraruminal infusions of Cr-51-EDTA and Ru-103-phenanthroline digesta-flow markers began. At the same time the sheep were fitted with jugular blood catheters and bladder catheters and a continous intravenous infusion of Cs-137 started. Four of the animals received Cs-134 infused into the rumen together with the flow markers; the remaining sheep received Cs-134 in contaminated soil (10 g per day), administered orally twice daily in gelatin capsules. In order to quantify water exchanges betwen the blood and gut simultaneous respective infusions of deuterium oxideand tritiated water were administered into the jugular vein and into the rumen. Total collections of faeces and urine were made for 4 days; the bladder catheters were then removed. In order to estimate the microbial growth rate in the forestomachs, daily oral dosing with S-35 was carred out. Over a 48-hour period, and 10 days after beginning infusions, samples of duodenal and ileal digesta were removed alternately at 12-hour intervals. The samples were centrifuged in order to obtain liqid and solid fractions and to extract microbial material from the duodenal digesta; the fractions were freeze-dried. Blood samples were taken for deuterium and H-3 analyses. Immediately after completion of digesta sampling, all infusions were terminated. Total faecal colections were made at frequent intervals, so that the rates of decline in faecal concentrations of the digesta-flow markers could be used to estimate the rates of passage of material along the gut.

As has been already mentioned, the experimental work with the animals has not been completed and no results are yet available.

3. Effect of age on radiocaesium absorption

The examination of the effects of age on radiocaesium absorption in sheep has been reported by ITE (contractor 1).

Publications:

Mayes, R.W., Eayres, H., Beresford, N.A. & Howard, B.J.
Changes with age in the absorption of radiocaesium in sheep. Paper to be presented at the workshop: "Age-Dependent Factors in the Biokinetics and Dosimetry of Radionuclides", Schloss Elmau, Germany, November 1991.

Howard, B.J., Beresford, N.A. & Mayes, R.W. (1990)
Factors affecting radiocaesium transfer to ruminants. In: Proc. SCOPE RADPATH meeting. RL.13.90. Lancaster 26-30 March 1990.

Head of Project 4: Dr. Belli

II Objectives for the reporting period

The main objectives within the period has been to agree experimental and sampling protocols and to begin the two studies outlined below:
- to study the availability of radiocaesium associated with two different soil types for absorption in the sheep gut and its transfer to sheep milk;
- to study the extent of soil adhesion on fodders and grazed pastures
  1 - Selection of representative sampling sites
  2 - Samplings of soils and cattle fodders.

The activities are carried out in a north-eastern region of Italy (Friuli-Venezia Giulia Region) and include both laboratory and field studies.

III Objectives for next period

The objectives for next period are:
- completion of all outstanding analyses on the samples collected from the two studies carried out in the first year;
- to conduct an experiment of the effect of vegetation species in the diet on radiocaesium uptake by sheep, or to proceed further with the soil ingestion studies;
- preparation of the final report and papers for publication in the open literature.

IV Progress achieved including publications

1. Soil adhesion study

Methodology
In Spring 1990 suitable sites were identified on the basis of soil and vegetation characteristics. The following sampling sites were selected:

Lowland	Upland
alfalfa grassland (A)	polyplyte meadow (3 sites)(D)
alfalfa-fescue grassland (B)	Dactylis glomerata grassland (E)
maize (C)	grazed pasture (F)

At sites A-E vegetation and the superficial soil layer (0-2cm depth) have been sampled at each cut during the growing season from may to october 1990. One soil sample of 0-30cm depth was taken in may. During winter 1991 fodders from sites A-E were sampled directly from the relevant farms. At site F vegetation and the superficial soil layer (0-1cm depth) have been sampled weekly during the cattle grazing season from july to september. One soil sample of 0-30cm depth was taken in july.

At all sites, the vegetation and soil characteristics have been determined and the meteorological conditions (rainfall and temperature) continuously monitored. Radiocaesium content of all samples will be carried out.

The extent of soil contamination of the vegetation will be evaluated by Ti analysis of vegetation and soil samples. These will be carried out by the Institute of Terrestrial Ecology (ITE).

2. Availability of ingested soil

The availability of radiocaesium associated with ingested soil for transfer into body tissues of grazing animals was assessed by indoor feeding trials, using 2 types of contaminated soil and sheep housed in metabolism cages.

A clayey and a loamy soil, artificially contaminated in lysimeters by RIVM several times from 1981 to 1983 was administered to lactating ewes. Caesium-137 activity concentrations in the soils were 17000±170 Bq/kg and 18000±180 Bq/kg dry weight respectively.

Each soil type was fed to six ewes, housed in individual metabolism cages. The sheep used were an Italian meat breed, called Bergamasca, and are typical of the Alpine region. The ewes that were allocated to each experimental group were selected on the basis of age, live weight and milk production in the previous lactation periods.

During the experiment the ewes were fed as follows:
- lucerne pellets administered "ad libitum";
- 100 g of barley straw;
- 20 g of a vitamin supplement.

The ewes were given an inital adaptation period in the metabolism cages of 20 to 40 days. The contaminated soil was administered orally each day for 33 days. Each ewe received 100 g of contaminated soil, suspended in 200 ml of water, using a oesophageal catheter, connected with a syphon to a 500 ml bottle, closed with a non-returnable valve. After day 33 a decontamination phase of 14 days occurred.

Milk was collected from each ewe with the following frequencies:
- daily from day 1 to day 27 after the commencement of soil administration;
- once every 3 days from day 28 until day 33, ie at the end of soil administration;
- daily from day 34 to day 47 when the experiment ended.

Blood was also sampled weekly. Cs-137 activity concentrations were determined in all samples by gamma-spectrometry, using HPGe detectors.

Initial Results

The daily intake of Cs-137 for each ewe was 1691±33 Bq/d for the ewes administered clayey soil and 1770±35 Bq/d for the ewes fed with loamy soil.

Caesium-137 activity concentrations measured in the milk from day 1 to day 20 of soil administration are presented for each soil type (Table 1).

Table 1. Cs-137 (Bq/l) in milk from ewes administered clayey and loamy soil.

Days of soil administration	Soil Type	
	Clayey soil	Loamy soil
	Cs-137 (Bq/l mean±SD)	
0	<0.3	<0.3
2	0.5 ± 0.1	1.0 ± 0.1
6	0.6 ± 0.1	1.1 ± 0.2
9	0.7 ± 0.1	1.2 ± 0.3
13	0.6 ± 0.1	1.1 ± 0.2
16	0.6 ± 0.1	1.1 ± 0.2
20	0.7 ± 0.1	1.1 ± 0.1

For each soil treatment Cs -137 activity concentrations in milk reached an equilibrium after only a few days.

Both of the two soil types used, a clayey and loamy soil, had a clay content exceeding 10%. Initial transfer cofficient values have been obtained of 3.5×10^{-4} d/kg for the clayey soil and 6.2×10^{-4} d/kg for the loamy soil. These comparatively low values suggest that radiocaesium was not removed from the binding sites on the clay minerals in the sheep gut.

Head of Project 5: Mr. Stakelum

II Objectives for the reporting period

The objective of the programme at TEAGASC is to determine the true absorption coefficient of radiocaesium in dairy cows fed fresh herbage. A further objective of the experiments is to determine the principal sites of digestion and resecretion of ionic radiocaesium in the digestive tract of the dairy cow. In the reporting period a suitable experimental protocol has been agreed with MLURI and the experiments have just started. This study will serve as a comparative study for lactating sheep fed a similar herbage and receiving radiocaesium via the rumen in an identical manner.

III Objectives for next period

To complete the experimental feeding trials and analyze samples for radiocaesium content, digestibility, nitrogen, sulphur, Ru-103 and Cr-51 content.

To interpret results, compare with the equivalent sheep experiment concurently conducted by MLURI and to produce final report and papers for publication.

IV Progress achieved including publications

Experimental protocols have been designed and the experiments have just begun. The radiocaesium is being fed to the cows in two sources;- as ionic or soil bound radiocaesium. The cows will be fed fresh grass close to their appetite limit.

The experiments are being carried out with a grass only or grass plus concentrate diet to examine whether different rumen fermentation conditions will alter the transfer coefficients. Blood, urine, and milk will be analyzed to compare the different methods of estimating endogenous excretion of blood infused radiocaesium. Endogenous excretion must be estimated in order to calculate true absorption.

To avoid repetition further details of the experimental procedure are presented in the report by MLURI. The two studies at TEAGASC and MLURI are currently in progress at the time of writing. Therefore there are no publications as yet.

Head of Project 6: Dr. Colgan

II Objectives for the reporting period

Carry out soil adhesion sampling programme in Ireland as specified under agreed sampling protocol

- monthly sampling of vegetation from a range of permanent pasture sites commencing May 1990 for 1 year. Also collect soil samples from these sites;
- sampling of stored winter fodders and appropriate soil samples;
- Prepare all samples for analysis.

III Objectives for next period

Compile and statistically analyze all environmental data and sample analysis produced during the previous twelve months field work for the preparation of a final report. The influence of parameters such as soil characteristics, plant morphology and biomass and rainfall on the soil contamination of vegetation will be considered. Comparisons will be made between the findings of the work in Ireland and that of the other participating countries.

IV Progress achieved including publications

Soil adhesion study

Eleven months of sampling have been successfully carried out according to the originally agreed project protocol.

Samples of permanent pasture, and three winter fodders - hay, silage and fodder beet were taken from both mineral and organic soils. Permanent pasture mineral soil sites were compared in an upland and a lowland situation. Pasture vegetation and sheep faeces samples were taken monthly from each field site. Winter fodders were sampled at the time of harvesting and also three times from storage during the period of feeding to animals. Rainfall was recorded for each site prior to each sampling event.

Vegetation was sampled on an area basis to give information on biomass production. Twelve replicate areas were taken at each sampling and these were bulked into three replicates for analysis. Faeces was sampled randomly from the fields and divided into three replicates for analysis.

Soils were sampled from the pasture fields twice throughout the sampling year to date - in June 1990, November 1990 and will be sampled again in April 1991. Soil samples from the winter fodder sites were sampled at the time of harvest. They were sampled on an area basis and twelve replicates representing each field were bulked into three replicates for analysis.

To describe the soil type at each site physical and chemical characteristics were determined for samples taken from a profile down to 30cm deep.

All samples to date have been prepared and analysed for the radionuclides - Cs-137, Cs-134 and K-40 and most of the physical and chemical characteristics of the soils required by the protocol have been determined to date - pH, bulk density, moisture content, % clay, silt and sand, organic content and exchangeable potassium.

All samples will be analysed for titanium or mineral content (LOI) to determine the level in each soil type and the level of titanium (or mineral content) in the corresponding vegetation due to contamination by soil.

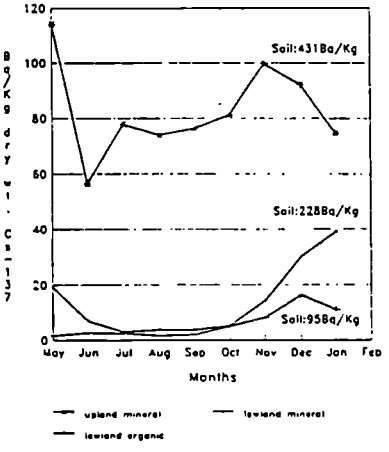
Initial Results

The levels of radionuclides in the vegetation and faecal samples have reflected the levels in the associated soil. They have shown seasonal trends similar to those previously recorded in the literature which follow the patterns of growth and senescence of the vegetation (see Figure). Uptake of Cs-137 and Cs-134 by vegetation was lower in the well managed, mineral soils which are treated with potassium fertiliser than on the low quality, upland mineral sites and the organic soils.

Initial comparisons of radionuclide levels in corresponding soil and pasture samples suggests that significant soil adhesion is occurring. Samples of permanent pasture from the site with the lowest soil nutrient status and resultant poor production of very thin grass, appear to have significantly enhanced radioactivity, probably due to soil adhesion to this low growing vegetation. Rainfall may prove to be an important factor in such sites. A comparison of radionuclide levels in pasture vegetation and faeces indicates that soil contamination of fodder also seems to be occurring, probably by direct consumption of soil during grazing.

Specific farming practices are suggested as having a role in the extent of soil contamination of winter fodder crops. Tossing of hay during the drying process may incorporate soil into the fodder. Fodder beet in particular may be subject to significant soil adhesion because these root crops are not washed prior to feeding and in some cases the animals graze the beet and their leafy tops directly from the ground. Such contamination pathways may be limited by modifying harvesting and storage procedures for winter fodders.

Changes with time in Cs-137 activity concentrations in grass at 3 of the pasture sites in Ireland (Bq/kg dw)



Head of Project 7: Prof. Assimakopoulos

II Objectives for the reporting period

The first year was devoted to agreeing experimental protocols, and to proceeding with the following parts of the study:-

- To study possible variations in the transfer coefficient for sheep milk during an entire lactation period;
- To study seasonal variations in soil adhesion to grass in pastures grazed by sheep;
- To begin a study on the availability for absorption of radiocaesium in soil taken from around the Chernobyl plant;
- To develop more realistic animal models for trace element transport.

III Objectives for next period

Completion of the studies begun in the previous period and conducting a further study:-

- Analyse all remaining samples from soil adhesion and lactation study;
- Finish experiment and analyse remaining samples from study measuring the transfer coefficient for radiocaesium to ewes' milk from ingested soil;
- Preparation of the final report and papers for publications in the open literature.

IV Progress achieved including publications

1. Effect of stage of lactation on radiocaesium uptake

Experimental Protocol

A group of five lactating ewes, which are a pre-selected number of weeks into the lactation period, are segregated from the flock for three weeks. During the first "acclimitization phase", the animals are fed uncontaminated wheat for 1 week and both their daily intake and milk production are monitored. During the second week, the "contamination phase", the animals are fed wheat harvested in northern Greece during the summer of 1986, contaminated with radiocaesium at a level of 1200 Bq/kg dw. Daily intake, milk production and radiocaesium activity concentration in the milk are monitored during this phase. The same parameters are also monitored during the third week, the "decontamination phase", during which the animals are returned to an uncontaminated diet. Thus, each stage of the total lactation period is characterised by three weeks of data. The sheep are milked, and the samples bulked, twice daily. Hence the contamination and decontamination phases yield 28 data points giving radiocaesium activity concentration

Initial Results

Five groups of animals were studied during the spring of 1990, mainly as a test of the protocol and the model. Systematic measurements began in November 1990. Two hundred ewes, which gave birth between 30 October and 2 November 1990 were selected for the experiment. Data has been collected from 21 groups of animals, covering the entire lactation period this year. About 75% of the samples obtained have analyzed for radiocaesium concentration.

Sample collection for the experiment is now complete. Data has also been collected which will enable us to investigate the influence of several other factors affecting the transfer coefficient. For instance, milk from the sheep used in week no. 15 has not been bulked, but has been collected individually for each animal in the group. These data will be used to determine the variability (and hence estimate the experimental error) associated with the measurement of transfer coefficient in these experiments.

2. Soil adhesion study

Two pastures, of approximately 1.2 hectares each, were selected for this investigation. The soil of Pasture A was predominantly clay and that of Pasture B, near lake Pamvotis, was predominantly a sandy soil. A detailed soil analysis for the pastures was conducted in April 1990. Both pastures are within 5 km and are owned by the Ioannina Agricultural Research Station. During the spring and summer months they are systematically grazed by the Stations sheep. Daily precipitation data, recorded by the Ioannina Agricultural Research Station, will be used in the analysis of the experiment.

Samples of soil, grass and faeces were collected from both pastures, according to the protocol agreed for this experiment, every 15th of the month, starting on 15 May 1990. The samples were treated according to the experimental protocol and measured by XRF for Ti concentration. Although appreciable levels of titanium could be detected in the soil samples, Ti concentrations in the grass and faeces samples were found to be below the detection limits of conventional XRF (about 30 to 50 $\mu g/g$, depending on background elemental concentrations). All samples of grass and faeces were thus measured through a variation of the XRF method, developed in a different research project of the NPL, at the Nuclear Research Centre "Demokritos" in Athens. This method, usually referred to as XSQR (Karydas, A.G. and Paradellis T. Coal XRF analysis using a proton induced copper x-ray beam, J. of Coal Quality, 9:39-43; 1990), is able to detect concentrations of light elements of the order of 1 $\mu g/g$ or better. Using this method Ti concentration in the order of 20 $\mu g/g$ in grass samples and 70 $\mu g/g$ in faeces samples were measured. About half of the samples collected to date have been processed.

3. Soil ingestion study

Twenty kg of heavily contaminated surface soil were collected from a site near the Chernobyl reactor during a visit of members of the NPL to Zeleny Mys in November 1990. Due to the high activity concentration in the soil, the sample was delivered to the N.R.C. "Democritos" for processing. The soil sample was dried and sieved. The processed soil, with a Cs-137 activity concentration of 47 kBq/kg dw was encapsulated in gelatin capsules of 8 g capacity. Feeding of the capsules (two per day) to animals commenced on 21 March 1991. The study is being carried out according to a protocol also being followed by ENEA in a similar experiment.

Eight lactating ewes were placed in metabolic cages. The experiment involved a seven day contamination period, followed by a decontamination phase of the same duration. Samples of milk, faeces and urine were collected and will be measured for radiocaesium activity concentrations. Most of the milk samples have already been analyzed.

4. Modelling

A general multiple-compartment model for the transport of trace elements through animals has been devised and implemented in a computer programme. The model has been calibrated and validated with the help of data obtained in ad hoc experiments. A novel feature of this model is that it explicitly takes into account temporal variations in the system's volume compartments. It is thus particularly suited for the description of milk accumulation in the mammary gland. the model has been used to determine Fm values in the lactation and soil ingestion studies described above.

Publications:

Assimakopolous,P.A., Ioannides,K.G. & Pakou,A.A. (1991)
Milk: A programme for the analysis of milk contamination and decontamination data. Internal Distribution report. Nuclear Physics laboratory. Jan 1991. 21pp + discs. University of Ioannina: Ioannina.

Assimakopoulos,P.A., Ioannides,K.G. & Pakou,A.A. (in press)
A General Multiple-compartment Model for the Transport of Trace Elements through Animals. Health Physics

Assimakopoulos,P.A., Ioannides,K.G. & Pakou,A.A. (in press)
Validation of a General Multiple Compartment Model for the Transport of Trace Elements through Animals. In: Symposium and Workshop on The Validity of Environmental Transfer Models, Stockholm, 8-12 October, 1990.

Head of Project 8 Dr. Unsworth

II Objectives for the reporting period

1. Determine feasibility of in vitro caesium uptake by muscle cells;
2. Develop the RUINS model to consider adhesion of soil particles as a source of radiocaesium to grazing animals;
3. Investigate the use of the sheep model for simulating changes in transfer with animal age.

III Objectives for next period

1. Develop a physiologically based gut transport and caesium metabolism model;
2. Investigate the incorporation of countermeasures in the physiological model;
3. Investigate sensitivity of RUINS model predictions of animal contamination to ecological factors such as soil partitioning, grazing and season.

IV Progress achieved including publications

1. Modelling studies

Soil Adhesion Sensitivity

Previous work at Nottingham has led to the development of the RUINS package which is intended to simulate transfer of radiocaesium in the soil-pasture-grazing animal agro-ecosystems. Within this study this package has been developed to demonstrate its sensitivity to factors related to the adhesion of contaminated soil to vegetation surfaces. The factors studied are:

1. Availability of radiocaesium in soil relative to plant incorporated material, set by a parameter A_{EX} (which takes a value between 0 and 1).

2. Fraction of vegetation radiocaesium which is attributed to soil contamination;

3. Importance of soil type and the differing availability from various soil components.

These studies are at an early stage, nevertheless they indicate potentially useful areas of research.

Figure 1 shows simulated sheep muscle concentration of radiocaesium following the consumption of contaminated vegetation and soil using parameters appropriate to upland sites in Cumbria, UK, and with a number of assumed soil availability factors. The soil adhesion rate used in the model was such that the contamination due to soil adhesion was approximately 50% of the total vegetation activity (Figure 2).

Figure 3 shows equivalent curves for a lowland site, using the same soil adhesion rate. At the lowland site the soil fixation capacity is much higher and root uptake by plants correspondingly lower. Therefore, the contribution of soil to the radiocaesium contamination is much higher than in the upland case (Figure 4). Therefore, the sheep radiocaesium muscle concentration shown in Figure 5 demonstrates a much greater response to changes in the relative bioavailability of soil radiocaesium.

The fixation of radiocaesium in soils is a well known phenomena and crucial in restricting the supply of radiocaesium for plant uptake. It is therefore, a reasonable assumption that radiocaesium 'fixed' in the soil will behave differently in the gut of an animal than the labile or exchangeable radiocaesium. Simulations have been performed to test the importance of such assumptions. The results for the upland soil is shown in Figures 5, for comparison the curves for A_{EX}=0.0 and A_{EX}=1.0 are reproduced from Figure 1. The results for the lowland soil is shown in Figure 6, for comparison the curves for A_{EX}=0.0 and A_{EX}=0.1 are reproduced from Figure 3. The line denoted ^{EX}LABILE is calculated by making two assumptions.

a) Radiocaesium fixed in the soil is completely unavailable in the soil.
b) Radiocaesium held in a labile form is as available as plant incorporated material.

Because of the different kinetic properties of the two soils this results in a much lower overall adsorbtion from the lowland soil compared with the upland situation. Preliminary results from Belli (Italy) and Hove (Norway) demonstrate these types of differences. One consequence of these assumptions is that, in the model simulation, availability from the soil as a whole will change with time as the fixation process occurs.

Physiological Animal Model

A multicompartment model of lamb radiocaesium metabolism has been developed under previous research programmes, this continues to be developed and validated in collaboration with partners within this CEC project. In particular through collaboration with MLURI/ITE improvements are being made to the dynamics of absorption and secretion within the gut.

However, the main thrust of the Nottingham work is to extend the animal model so that its parameters are based upon the underlying animal physiology, rather than arbitrary 'fits'. This presents a considerable scientific challenge, but nonetheless has significant potential benefits.

i) A model based upon well founded physiological principles can be applied to animals other than sheep, greatly extending the relevance of the overall projects work. ii) It will provide a sound theoretical framework, within which counter measures could be developed and understood.

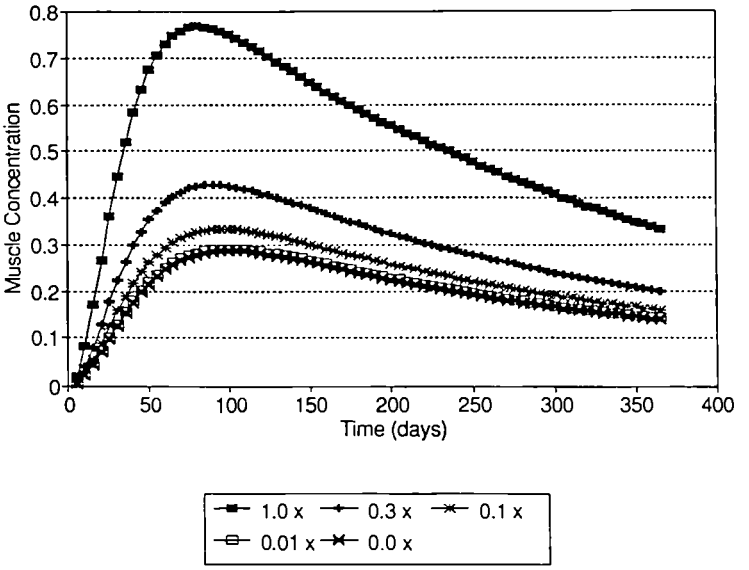


Figure 1 Effect of varying A_{EX} on upland sheep contamination.

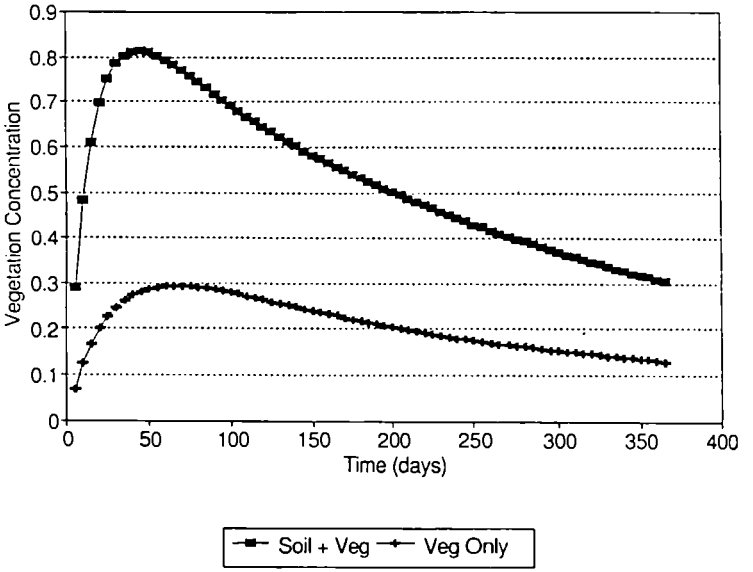


Figure 2 Contribution of soil to radiocaesium contamination of upland vegetation.

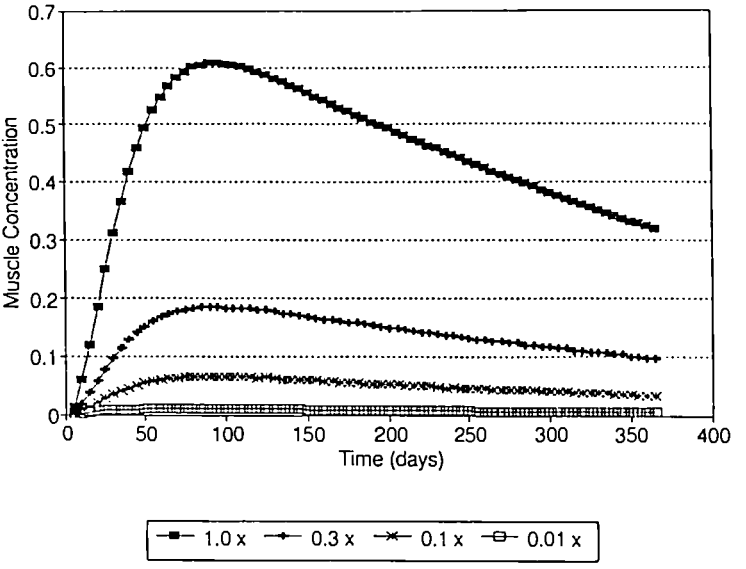


Figure 3 Effect of varying A_{EX} on lowland sheep contamination.

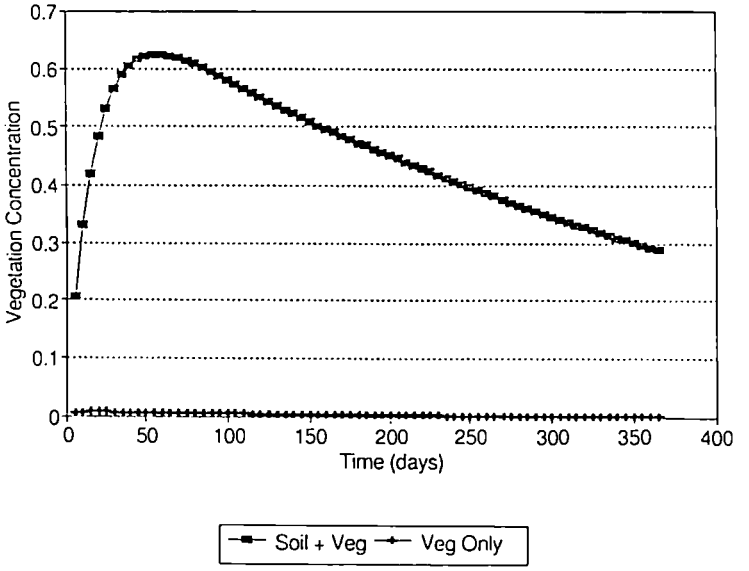


Figure 4 Contribution of soil to radiocaesium contamination of lowland vegetation.

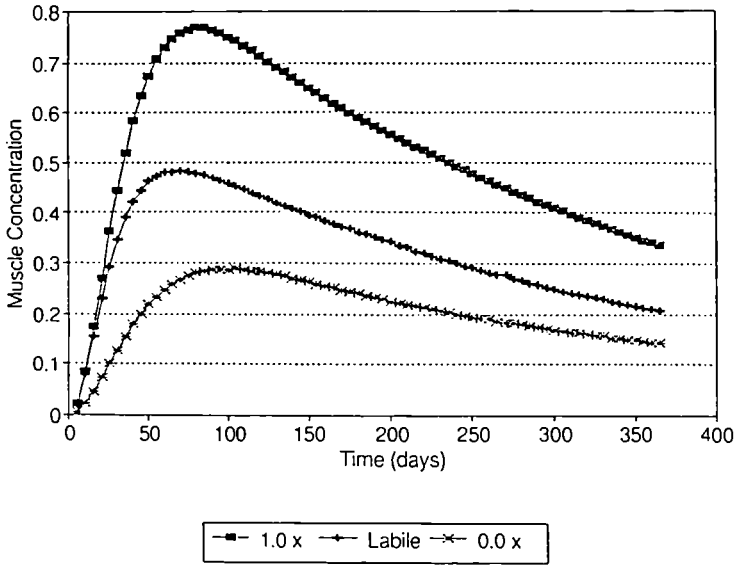


Figure 5 Comparison of upland sheep contamination assuming partitioned and uniform availability.

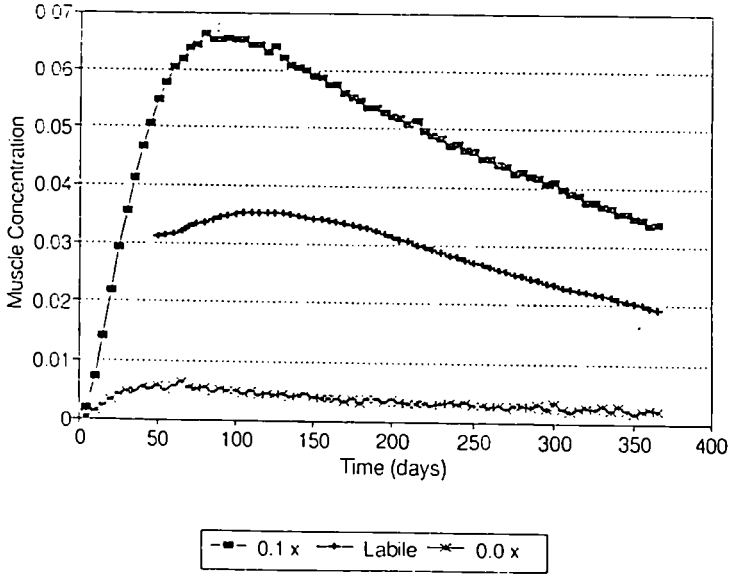


Figure 6 Comparison of lowland sheep contamination assuming partitioned and uniform availability.

2.Experimental studies

In parallel with the modelling programme a limited experimental programme is under way in collaboration with the Applied Biochemistry Department at Nottingham (P J Buttery). These experiments are at a very early stage and results will not be presented here. The objectives are to characterise the cellular uptake and efflux using standard in vivo culture techniques. The results are intended to relate directly to the modelling studies.

Publications:-

Crout, N.M.J., Galer, A., Howard, B.J. & Beresford, N.A. (in press)
A comparative assessment of the impact of the Windscale and Chernobyl accidents on 137Cs levels in upland lamb in west Cumbria using the RUINS model. In: Seminar on Comparative Assessment of the Environmental Impact of Radionuclides Released During Three Major Nuclear Accidents: Kyshtym, Windscale, Chernobyl. CEC/IUR:Luxembourg.

Galer, A.M., Crout, N.M.J., Beresford, N.A. & Howard, B.J. (1990) Modelling radio-caesium transfer in upland ecosystems. In: 1st Int. Conf. on the Atomic Power Plant Accident at Chernobyl. p 264. Moscow.

Head of Project 9 Dr. Jones

II Objectives for the reporting period

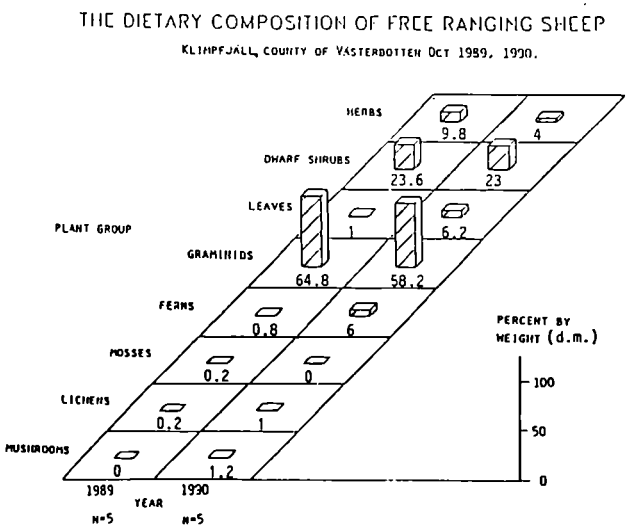
To study the uptake and transfer of radiocaesium from plants to sheep grazing freely in a natural ecosystem. The study area is situated in the Swedish mountains at Klimpfjäll in the county of Västerbotten (Latitude 65N: Longitude 15E)

III Objectives for next period

To continue the studies of free ranging sheep and also to study the uptake and transfer of radiocaesium to ewes milk during standardised feeding of the animals. By using this procedure it will be possible to obtain comparable results from different laboratories and breeds of sheep in Europe.
A study to measure radiocaesium transfer to ewes milk using standardised feeding conditions will be performed this year. This was delayed due to the late agreement between the Swedish government and CEC regarding participation in the radiation protection program.

IV Progress achieved including publications

A flock of free ranging sheep have been followed during the grazing period in a mountaneous area of Sweden. The botanical composition of ruminal samples taken at slaughter of the lambs is shown in the figure (mean of five animals). A comparison with the previous year shows that the intake of ferns and mushrooms was greater in 1990 than in 1989. This difference in feed intake may explain the higher radiocaesium levels found in mutton 1990 (mean 860 Cs-137 Bq/kg; range 677 to 1258) compared with 1989 (mean 1660 Cs-137 Bq/kg; range 1550 to 2084).



Progress Report

Contract: Bi7-009 **Sector: A25**
Title: Deposition of radionuclides on tree canopies and their subsequent fate.

1	Minski	ICSTM
2	Belot	CEA - FAR
3	Rauret	Univ. Barcelona
4	Ronneau	Univ. Cathol. Louvain-la-Neuve

I. Summary of Project and Global Objectives

It is known from studies of the deposition of conventional pollutants to forest canopies that trees are highly efficient in their interception and retention of airborne particulate materials. This has also proved to be the case for radioactive aerosols following the Chernobyl accident and as a consequence large areas of forest throughout Europe are still contaminated to some extent by ^{137}Cs. Following initial capture of radioactive fallout nuclides such as ^{137}Cs may then become incorporated into foodchains with the possibility of their ultimate transfer to man, especially *via* wild food products.

While much is known about the deposition processes involved in the initial contamination of grassland areas with radioactive fallout very little knowledge exists which relates directly to the mechanisms of deposition, accumulation, transfer and recycling of radionuclides within forest ecosystems. It seems likely that these processes will be considerably more complex within the latter ecosystems which comprise canopies of considerable architectural complexity and morphological variety, often underlain by herbaceous understories. The overall objective of this project is to provide an insight into the physical processes operative in such complex ecosystems which result in aerosol capture, recycling and loss. This is being approached at various levels within the project by each member group.

CEA - FAR is performing detailed studies of the effects of wind speed and particle size on the deposition of aerosols onto individual leaves and twigs of Pine, Spruce and Holm Oak. These experiments make use of a relatively narrow cross section wind tunnel into which dye-laden aerosols are released. The data so derived, and that from the other groups, will be used to assist in the validation of a multi-layered model currently being developed by this group.

The Univ. Cathol. Louvain-la-Neuve is carrying out similar wind tunnel studies of deposition onto isolated trees and twigs using thermo-generated UO_2-Cs aerosols, considered to be representative of the particulates which emerge from reactors undergoing catastrophic failure. The data resulting from these studies are being interpreted in the light of deposition data collected from a forest site contaminated by conventional chemical pollutants (Donon in the French Vosges).

Imperial College is studying the deposition processes of Cs-labelled silica aerosols in a wind tunnel of sufficient size to allow the construction of 'model' canopies of Spruce, Pine and Oak saplings above which a well characterised turbulent boundary layer can be established. This allows the effects of canopy architecture and morphology to be taken into account when deriving deposition parameters for the validation of the model being developed at CEA-FAR.

The Univ. Barcelona is deriving data on the deposition and recycling of aerosols within instrumented catchments situated in the Prades mountains of NE Spain, a site which provides conditions typical of southern European forest ecosystems. As well as characterising the properties of aerosols falling into these catchments this group is also investigating the physico-chemical behaviour of ^{134}Cs, ^{85}Sr and ^{110m}Ag within forest soils.

Head of Project 1: Miss Minski

II Objectives for the reporting period

To investigate and quantify dry deposition of labelled silica aerosols to a canopy of Norway Spruce (*Picea abies*) saplings in a wind tunnel.

To follow the time course of field loss of radioactivity from the tissues of contaminated Spruce saplings under field conditions.

III Objectives for next period

To further investigate and quantify dry deposition processes to a canopy of Spruce saplings and to extend these studies to Holm Oak (*Quercus ilex*).

To investigate and quantify resuspension of labelled silica aerosols from a Spruce canopy within a wind tunnel.

To extend field loss studies both under natural field conditions and under an artificial rainfall regime. This will involve use of a rain simulator which will also be used to determine the interception of contamination applied as a wet deposit.

IV Progress achieved including publications

The bulk of work in the first year has been devoted to the characterisation of processes involved in the dry deposition of labelled aerosols to a canopy of Spruce saplings arranged in a wind tunnel.

The dimensions of the working section of the wind tunnel are 6m x 0.8m which allowed the construction of a canopy of 120 saplings spaced four-abreast at regular intervals along the tunnel; this was considered to be the simplest and most homogeneous canopy configuration which should yield the most consistent deposition pattern. The average canopy height was 35cm.

A monodisperse silica aerosol with mean aerodynamic diameter of 1μm was labelled with $^{133}CsCl$ and liberated above the canopy at a steady wind speed of 4.9 ms^{-1} for a duration of 108 minutes. The mean air concentration of the aerosol during the experiment was determined using an array of isokinetic air samplers arranged within the boundary layer. Deposition onto the canopy itself was determined by destructively harvesting 40 trees taken from the last 2m of the tunnel, the region in which a fully developed turbulent boundary layer exists above the canopy. Randomly interspersed between these trees were 40 filter papers placed on the soil surface which were analysed to assess the extent to which the deposit was able to penetrate the canopy.

All analyses were by instrumental neutron activation analysis (INAA) which made use of the $^{133}Cs(n,\gamma)^{134}Cs$ reaction to allow the determination of deposited ^{133}Cs as ^{134}Cs. Checks on

uncontaminated saplings revealed that the 'background' concentration of ^{133}Cs within the Spruce tissues was substantial (in the order of 1 $\mu g\ g^{-1}$) and highly variable. In the regions of the canopy which received the least deposit of labelled aerosol this caused a serious interference with the absolute deposition values obtained and, accordingly, these regions were taken as a baseline of 'zero' deposition. Deposition to all other regions is expressed in relation to this baseline.

Sampling of the saplings for analysis was carried out such that 8 categories of tissue were defined as shown in Table 1.

Table 1 : Parameter values obtained for dry deposition to a canopy of Spruce saplings within a wind tunnel.

	Depositional Surface	V_g (cm s^{-1})	r
Upper canopy	1. New growth, needles	0	0
	2. New growth, stems	0.062	0.020
	3. Old growth, needles	0.412	0.065
	4. Old growth, stems	0.624	0.099
Lower canopy	5. New growth, needles	0.724	0.240
	6. New growth, stems	2.288	0.757
	7. Old growth, needles	1.574	0.297
	8. Old growth, stems	3.715	0.701
Soil Surface	1. Under trees	0.014	-
	2. Between trees	0.006	-
			r_{total} = 0.998

Contamination of each tissue category was determined on a surface area basis and a mean aerosol flux rate to each surface calculated for the duration of the experiment. From this value and the mean aerosol concentration above the canopy deposition velocities (V_g) were calculated for each tissue category, defined as

$$V_g = \text{Flux to plant surface / air concentration}$$

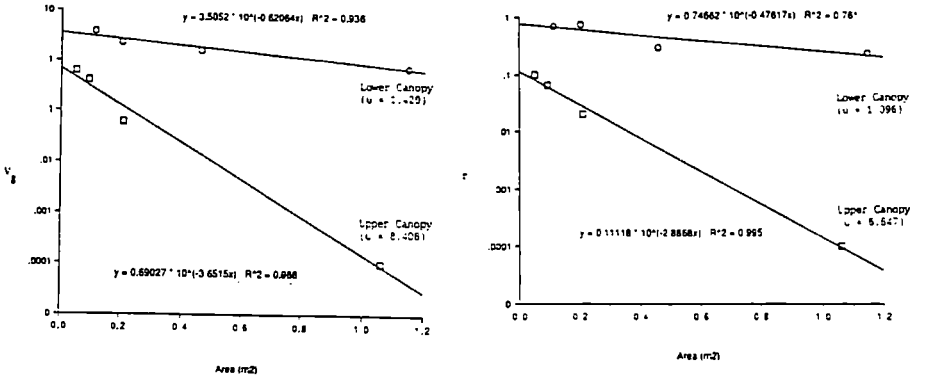
These are shown in Table 1. This table also shows the values of V_g determined for deposition to the soil surface at positions either directly under trees or between trees. These values are significantly different ($p<0.001$) and indicate that deposition was greatest **underneath** trees.

As a measure of the efficiency of capture of the aerosol by the canopy a series of interception fractions (r) were also calculated. These relate the deposition to the soil surface under a canopy to the deposition onto the canopy itself and can be calculated empirically from

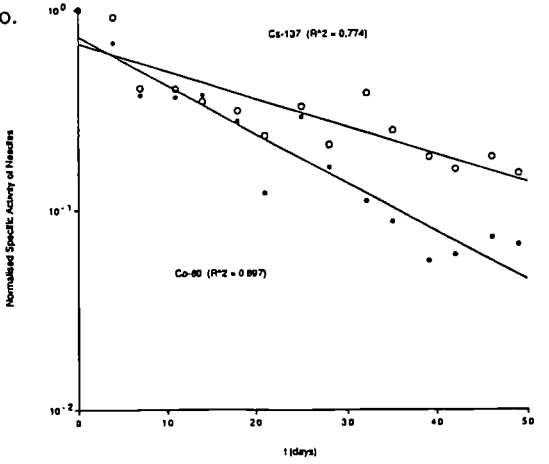
$$r = V_{gplant} / (V_{gplant} + V_{gsoil})$$

The values of r shown in Table 1 were calculated assuming that vertical penetration of the aerosol downwards through the canopy would occur either in the outer canopy (new growth) or in the inner canopy (old growth) regions. The total interception fraction, calculated for the canopy as a whole, is also given and indicates that 99.8% of the depositing aerosol was intercepted by the canopy, with only 0.2% of the deposit reaching the soil surface.

Figure 1 shows that the derived values of V_g and r correlated well with the density of tissues (measured as a surface area) within upper and lower regions of the canopy, although the deposition of aerosol to each of these regions seemed to be distinct from the other. The correlations obtained were also negative which is the inverse of the relationship between aerosol capture and herbage density commonly seen in herbaceous canopies.



An experiment to determine the loss of radioactive contamination from the leaf surfaces of spruce trees under field conditions (field loss) has also been carried out. This involved immersion of Spruce tree crowns in an aqueous solution containing ^{137}Cs and ^{60}Co in order to achieve an initially high and reproducible level of contamination of external leaf and stem surfaces (the soil in which these saplings were growing was protected from contamination at all times so that the root uptake pathway was excluded). The time course of contamination was followed over a period of approximately 50 days and is shown in Figure 2. This figure indicates that loss of both ^{137}Cs and ^{60}Co followed an approximately exponential pattern with time; biological half lives were calculated from these exponential relationships and were 21.8 days for ^{137}Cs and 12.4 days for ^{60}Co. Analysis of variance suggests that the rate of loss of these two radionuclides is significantly different ($p<0.001$) with ^{137}Cs being lost more slowly than ^{60}Co.



Head of Project 2 : Y. Belot

II Objectives of the reporting period

The general objective of the study was to determine in a wind tunnel the deposition rates of dye-laden particles on twigs of spruce trees as a function of particle size (0.1-1 μm) and wind speed (2-8 $m.s^{-1}$), these data being necessary to determine by means of a model the deposition rates of these particles on forest stands of given architecture.
The work described in the present report is a continuation of the work initiated during the precedent period. The deposition rates on spruce twigs obtained during the first period were surprisingly high compared to those obtained on other obstacles using particles of the same sizes. This excess of deposition could be tentatively explained by the morphology of the spruce twigs, but it was felt that this could be due, more likely, to the influence of an excess of electrical charges on particles or on the twig itself. Therefore, we decided to revise our experimental procedure and to focus our attention on the conditions of our experiments which might induce electrical charges differing from those encountered in the field.

III Objectives for the next period

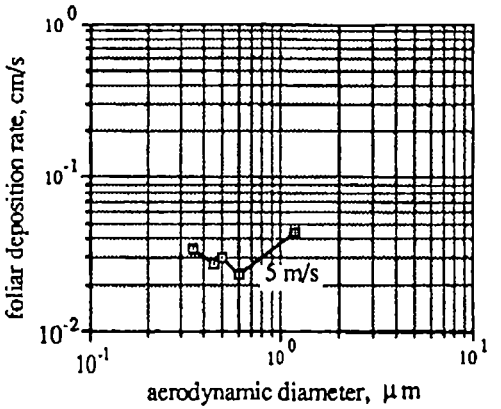
To furher investigate and quantify dry deposition processes on canopy components of spruce trees (*Picea abies*) and extend these studies to components of pine trees (*Pinus sylvestris*) and Holm Oak (*Quercus ilex*).
To further develop and utilize a multi-layered model of aerosol deposition to a forest canopy taking account of micrometeorological conditions, canopy architecture and deposition rates on individual twigs.

IV Progress achieved including publications

New procedure
The spinning-top aerosol generator formerly used was replaced by an ultrasonic generator such as used in inhalation therapy. This type of generator was somewhat less satisfactory as concerns the monodispersity of particles, but more adapted to the generation of high concentrations of small particles and much more easy to control.
The standard conditions used in most of exposure experiments were the following. The wind tunnel was filled with prefiltered ambient air taken from the outside of the laboratory. The aerosol generator was operated during 5 minutes, and the dry particles obtained from evaporation of droplets were made to circulate in the tunnel during 15 minutes in order that the particles equilibrate with the small ions naturally formed in air by the ionizing radiations of radon. The test twig, previously dipped into water and shaken to eliminate suspended drops, was then placed across the working section of the tunnel and exposed to the generated aerosol. During the exposure time (generally 5 minutes) the particles present in the tunnel were sampled through a cascade impactor and a single filter to determine size characteristics and total concentration. After exposure, the fluorescence of twig, impactor plates and filters were determined by conventional measurement.
The size characteristics of the aerosol used in each experiment were determined from the cascade impactor data by using a calculation procedure which derives a log-normal distribution from the impactor data, taking account of the detailed response functions of the impactor.

Experimental results
The spruce twigs used in our experiments were bearing 850-1400 needles of 0.6-2 cm lenght and 0.07-0.20 cm width. The total developed surface area of needles on each of the twigs was 200-300 cm^2, and the surface of defoliated twigs was of 20-50 cm^2. The deposition rates (Vd) given hereafter refer to the developed surface area of leaves.
The emphasis in the experiments was to determine effects of tracer particle diameter on dry

deposition rates. This was achieved by exposing spruce twigs to aerosols of different aerodynamic mass median diameter (MMD), respectively 0.35, 0.45, 0.50, 0.60 and 1.20 µm, and corresponding geometric standard deviations (GSD) of 1.30, 1.35, 1.35, 1.50 and 1.60. The Vd's obtained under standard conditions for a wind speed of 5 $m.s^{-1}$ are presented in the following graph.



In the size subrange of interest, the foliar deposition rate does not change very much with the mass median diameter of the aerosol, its average value is about 3 x 10^{-2} $cm.s^{-1}$. The value value obtained is 4 times lower than that formerly obtained without taking care of electrical charges. Other experiments have shown that, in this size subrange, the foliar deposition rate was nearly proportional to the square root of the wind speed.

Model for deposition to stands of trees

A resistance model for deposition of particles to a forest canopy of given architecture is under development. The turbulent transfer of particles to the canopy from the surrounding air is modeled by direct analogy to Ohm's law for electrical circuits, in which a current (or flux) is calculated as the ratio of a potential gradient and a resistance. In this case, the flux is that of particles to the canopy surfaces, the potential gradient is the difference in particle concentrations between the air and the receptor surfaces. The resistances, either represent resistance turbulent diffusion of particles betweeen layers in the canopy, or the boundary-layer resistance to transfer of particles in a given stratum to the canopy components. Theses last resistances are directly related to the foliar deposition rates measured in the experimental part of the work.

Head of Project 3: Dra. Rauret.
II Objectives for the reporting period.

1st. Selection of the study area and installation of the field instrumentation.
2nd. Establishment and exchange of methodology for sampling, for laboratory treatments and to determine chemical composition or activity.
3rd. Start of the experimental work in order to establish a) the properties of aerosols, b) the distribution of radionuclides in different forestal ecosystems and c) the migration of ^{134}Cs, ^{85}Sr and ^{110m}Ag in soils.

III Objectives for next period.

1st. Study of the properties of the aerosols collected in the experimental forest and their interactions with leaves.
2nd. Continuation of the study of the distribution of the above mentioned radionuclides and their relationships with soil properties.
3rd. Study of the migration in soil of ^{134}Cs, ^{85}Sr and ^{110m}Ag according to organic matter decomposition in the soil.

IV Progress achieved including publications.

1) Selection of study area.
1.1) Extensive studies.
An extensive sampling carried out in the northern forested areas of Catalonia showed that radiocaesium from Chernobyl was always the lower fraction of total radiocaesium activity.
12 plots have been selected from this sampling, with different combinations of species, lithologies and altitudes, to study the role played by different soil characteristics in the radionuclides behaviour.
1.2) Intensive studies.
Four plots have been selected in an homogeneous climatic area combining two different types of vegetation and lithology. In this area there is an experimental instrumented watershed with meteorological stations, which has been equipped with four static aerosol collectors and one high volum sampler. The study area characteristics are:
- Location: Poblet forest (Tarragona) in NE of Spain (lat. N 41° 13'-41° 24', Long. E 0° 55'-1° 12', Alt. 900 m).
- Typical mediterranean climate, mean annual temperature of 13°C , mean precipitation 570 mm.
- Schists and granites lithology.
- Vegetation: Holm oak - Quercus ilex - and Scots pine - Pinus Sylvestris - forests.
Progress:
The first plot (holm oak forest, schist lithology) was sampled during the last autumn.

2) Exchange and establishment of methodology.
2.1) A methodology for leaf aerosols was elaborated in a meeting hold in the " Université Catholique de Louvain" (Belgium) to contact, discuss and approve the following methodology to be used in the laboratory of Plant Physiology of Barcelona University. Material to be used: leaves of Quercus ilex L. Once lyophylized the samples are to be observed, without any kind of alteration,with a Scanning Electron Microscopy (SEM) including an Energy Dispersive X-Ray Analyzer in order to determine the morphology, distribution and composition of deposited particles on the leaves. Measurement of the size of particles: using and Interactive Binary Analizer System (IBAS Kontron).
2.2) This methodology has been elaborated from the discussion carried out in

two meetings with the researchers of the program "Transfer of radionuclides in forest: biological aspects" coordinated by Dr. Wirth.

A) Sampling plot is 25 x 25 m^2, with homogeneous lithology and vegetation.

B) Samples.

- Vegetation: A choice of 10 co-dominant trees must be done, sampling two upper third branches of opposited sides of the tree. For evergreen species it is necessary to sample during winter when the physiological activity of leaves is low.

- Forest floor layers: To avoid the spatial variability due to canopy heterogeneity it has been proposed to sample at 0.5 m from the tree trunk, taking separately the different organic layers: L (litter), F_1 (O_1), F_2 (O_f) and H (O_h) when they are present.

- Mineral soil: If it is possible to separate the different mineral soil horizons in the field, different samples will be taken for each horizon. If the separation is not possible, a sampling in different depths (0-2.5 cm, 2.5-5 cm, 5.0-15.0 cm) should be done.

C) Number of samples: The number of sampling points will be 16 and they will be grouped into 4 samples (each one made up of 4 single samples).

2.3) The extensive study showed the key role of litter decomposition and faunal activity in the radionuclides migration in the forest floor and mineral layers.

Field experimentation has been designed to describe these processes in an holm oak forest:

"In situ" incubation of contaminated green leaves in plastic cores. The contaminated green leaves replace the original litter layer and then the decomposition process is studied in field conditions. At the same time, another core with an exchange cationic resin bag between the contaminated green leaves and the F layer allow to discriminate the soluble and the particulate radionuclides migration.

Incubation of classical litter bags is used for the calibration of this new field method.

Periodical sampling of the remaining polluted leaves, the different underlying forest floor layers and the first 5 cm of mineral soil, will allow to model the radionuclides migration.

Seasonally, during 2 years, 4 points are sampled. Each point is composed of:

- One core with polluted green leaves.
- One core with polluted green leaves and exchange cationic resin.
- One core with non-polluted green leaves.
- One litter bag with green leaves.
- One litter bag with brown leaves.

2.4) An specific methodology has been established in this project to measure gamma activity (by high resolution gamma spectrometry with intrinsec Ge) of atmospheric aerosols, polluted leaves and rest of samples.

a) For atmospheric aerosols retained by filters, the influence in the efficiency of the counting geometry and of the acid treatment of the sample in the geometry to homogenize it has been studied. After these studies it has been established for the measurements a 50 cm^3 geometry without acid treatment.

b) Samples of entire leaves to be placed in field cores to study migration processes. Provided that it is not possible to work with standard density conditions (grinded sample) or to prepare standards with the sample conditions, we established, with samples of the same characteristics (entire leaves with artificial aerosols), correction factors for each radionuclide in order to correct the measured efficiency to the one of the standards used in the calibration of the spectrometer.

These measurements have been carried out to know the homogeneity of aerosol deposition on the leaves.

Quercus ilex leaves contamination was carried out in CEN Cadarache (France), with green leaves recently sampled in the study area.

c) The rest of samples were dried at room temperature and homogeneized. Measurement times were from 40000 to 80000 s.

3) Results

3.1) Study of aerosols on leaf surfaces.

The use of lyophylized samples without any other kind of pretreatment is a good methodology in order to preserve the samples of any surface alteration before their observation with Scanning Electron Microscopy. Our results show that deposited particles on the leaves are non spherical and mainly consisting of soil material: Si, Al, K, Fe. Size distribution data indicate that most of the deposited particles have a diameter larger than 10 µm. Particles are located in the adaxial surface of the leaf and their distribution is not regular if not they are retained in the trychomes of the basal part of the leaf near the mid vain.

3.2) Extensive study.

Tree canopies had no detectable radioactivity or radioactivity mostly coming from Chernobyl. The activity detected in leaves sprouting after the Chernobyl accident was attributed to retranslocation from older ones.

Total ^{137}Cs activity in the litter layer increased from L to H and was related to the degree of litter decomposition (fig.1).

It was assumed that a major ^{137}Cs fallout fraction in F and H was incorporated by mechanical soil mixing from faunal activity.

Radiocaesium activity in the upper 15 cm of mineral soil came mostly from weapon fallout deposition.

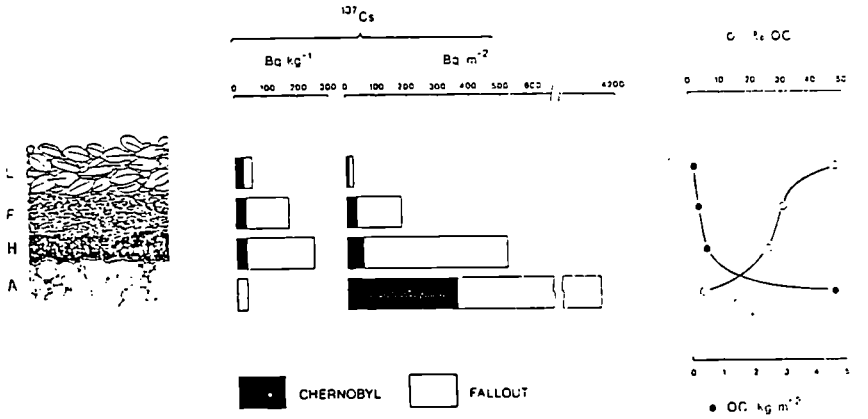


Fig.1. Comparative distribution of ^{137}Cs (Bq/kg) and % O.C. mean values in the forested ecosystems studied.

3.3) Intensive study.

Soil: Typic Xerochrepts; A horizon (0-25 cm) pH: 4.8, 18 % clay content and 1.74 % organic matter.

Organic layers: L 5.5±0.9 Mg.ha^{-1}
F 15.7±8.8 Mg.ha^{-1}
H 38.8±23.5 Mg.ha^{-1}

Table 1. Range of activities of some radionuclides (Bq/kg)

	Ac-228	Pb-212	Bi-214	Pb-214	Tl-208	Cs-137
F1	< 24	< 14	< 18	< 19	< 9	22-51
F2	< 17	<11-22	< 12	<13-18	<6-11	52-113
H	23-38	22-38	14-22	17-25	4-13	100-211
A1	33-43	7-33	11-35	26-36	12-15	33-123
A2	40-46	36-49	26-38	29-42	13-16	10-67

3.4) Migration studies.

The frequency diagram (fig.2) shows the activity of the polluted leaves placed in the cores for the migration studies. A gaussian distribution for the three radionuclides in the ninety samples measured, but also a relatively high dispersion in spite of homogenization process carried out in the distribution of the polluted leaves in the ninety different bags.

The relative quantity of the three radionuclides is constant for all samples.

Fig.3-4 show radionuclide percentages in the forest soil layers after 15 days and 45 days of polluted leaves field incubation. It can be noticed that there is an evident evolution with time and that radionuclide migration decreased according to the sequence: Sr ≈ Cs >> Ag. The measured rainfall during the first sampling was 36 mm and during the second sampling 30 mm.

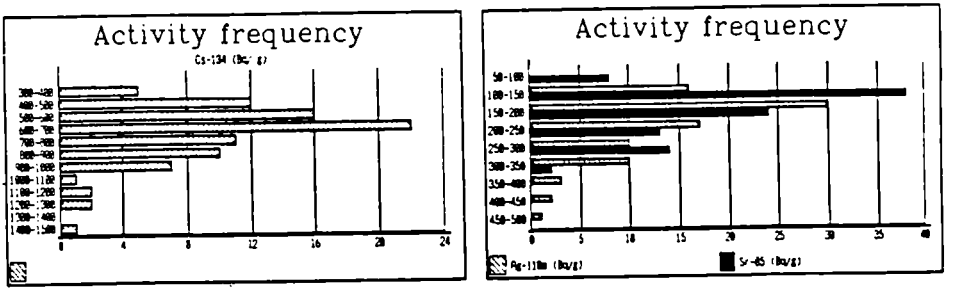


Fig.3. Leaves activity frequency.

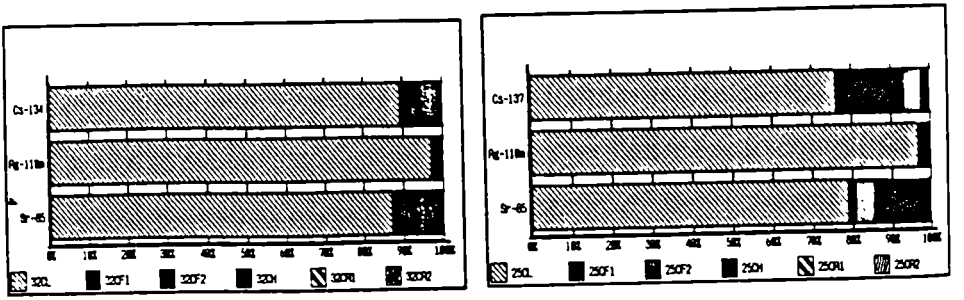


Fig.4. Radionuclides percentages in forest soil layers.

Head of Project 4: Prof. Ronneau

II Objectives for the reporting period

Defining the physico-chemical characteristics of thermo-generated UO_2-Cs aerosols by XPS and XRD. Building a wind-tunnel and starting to characterize its aerodynamic performances; first deposition experiments with non-radioactive aerosols. Continuation of forest samples collection and ^{137}Cs determinations (including samples from Chernobyl).

III Objectives for next period

Study of the physico-chemical characteristics of thermo-generated UO_2-Cs-Fe aerosols by means of XPS, XRD and Mössbauer spectroscopy. Study of deposition on trees in the wind-tunnel. The fate of ^{137}Cs in forests will be studied in the region of Chernobyl and compared with the observations made in Belgium and in france.

IV Progress achieved including publications

- Physico-chemical characteristics of thermo-generated Cs-UO_2 aerosols.

* Binding energy values of core and valence electrons have been determined in synthetic cesium uranates by XPS. This preliminary study was necessary before analysing the more complex spectra of aerosols uranates (see "X-Ray Photoelectron Spectroscopy of Cesium Uranates", by A.H. Al Rayyes and C. Ronneau: to be published in Radiochimica Acta).

* A thourough XPS and XRD study of thermo-generated aerosols allowed to determine the chemical nature of the aerosol surface which is very important as far as their environmental behaviour is concerned. It appeared that the core of the aerosols is constituted mainly by Cs uranates which are insoluble, while the surface of the particles is greatly enriched in soluble cesium (see: "X-Ray diffraction and photoelectron spectroscopy of cesium-urananium aerosols", by A.H. Al Rayyes and C. Ronneau: to be published in J. of Environmental Radioactivity).

- Behaviour of radiocesium in forest ecosystems.

* The migration ability of cesium in forest soil columns (undisturbed profile) following a surface contamination has been investigated. This study has put forward the risk to mislead the interpretation of activity measurements if expressed on a weight basis. Cesium speciation suggests that mineral components combined with the organic fraction in upper organic horizons could play a role in the high Cs retention in the surface layer of the soil (see: "Study on the bioavailability of radiocesium following contamination of the forest soil", by Y. Thiry and C. Myttenaere, presented in the "Seminar on comparative assessment of the environmental impact of the radionuclides released during three major nuclear accidents: Kyshtym, Windscale, Chernobyl; CEC (DG XI) - UIR Meeting, October 1 - 5, Luxembourg (1990)).

* A comparative synthesis of the distribution in the different compartments of a Belgian (Vielsalm) and a Soviet (Chernobyl) forest ecosystem has been released. Sequential extractions of forest soils from Chernobyl have shown their weak capacity to desorb cesium. Transfer from soil to plant seems related to root system development (see: "Behaviour of ^{137}Cs in forested polygones of the Chernobyl contaminated zone", by Y. Thiry, L. Sombre, C. Myttenaere, C. Ronneau, Y.A. Kutlahmedov and V.S.

Davydchuk, presented in the All-Union Conference: "Geochemical pathways of artificial radionuclides in the biosphere"; Gomel, USSR, October 15-19, 1990).

* The *in situ* determination of aerosol deposition mechanisms is still in progress. Rain water and aerosols are regularly collected above and under coniferous canopies (tower of DONON, Vosges, France). The numerous results obtained so far are still under examination and interpretation.

CYCLING OF RADIOCESIUM AND - STRONTIUM IN NATURAL ECOSYSTEMS

Aim of the Project

The Chernobyl accident led to an enormous amount of measurements of Cs 134 and 137 activities in wild berries and mushrooms. These data which are varying widely are valuable for decreasing actual radiation exposure to man but they are not of any help in understanding the behaviour of radionuclides in forest ecosystems. The project "Cycling of radiocesium and -strontium in natural ecosystems" investigates on the fate of radiocesium and strontium 90 in natural ecosystems in Belgium, Germany, Sweden and Italy in order to improve the knowledge of the cycling mechanisms and the understanding of their short and long term behaviour.

The experimental strategy is focusing on the fate of radiocesium and strontium 90 after their deposition in forest systems. For a better understanding of the migration particular attention is paid to differences in the adsorption and fixation of radionuclides in various horizons of undisturbed soil. Soil and plant parameters, such as organic matter content, exchange capacity, potassium concentrations in plant and soil, as well as mycelium and rooting depth will be analysed to describe the soil plant-transfer. The plant - herbivore interactions will be investigated by a special observation of the migration of mooses in selected areas in Sweden and their seasonal consumption habits. Further studies are concerned with the loss of radionuclides from the ecosystem by run off, the distribution of cesium and strontium within an ecosystem and the potential radiation exposure to man. Therefore the whole body activity of radiocesium is measured in groups who preferably consume mushrooms, wild berries and moose meat.

Based on the measurements transfer equations will be tested and discussed and better radioecological models for the long and short term cycling of cesium and strontium will be proposed.

The setup of the total project may be subdivided into the following topics:

1. Introduction - Aim of the investigations
2. Methods and materials, sampling strategy
3. Classification of the sampling sites
   (spectrum of the considered exosystems)
   Extendend discription of the jointly investigated sites:
   Vindeln (S), Passo Pura (I)
   Summary of the other sites

3.1. Soils

3.1.1. Classification of soils (FAO-system, National- and US-classification)

3.1.2. Soil parameters (organic matter, texture, concentrations of nutrients, density, pH, ...)

3.2. Description of the vegetation (plant species)
3.2.1. Forest stand (age, density, site quality, growth rate ...)
3.2.2. Understorey vegetation

4. Interception
4.1. General overview
4.2. Micropattern of deposition
4.3. Mapping of deposition

5. Direct uptake of cesium and strontium via the aerial parts of plants
5.1. General
5.2. Amount of direct absorption dependent on radionuclides and plant tissues
5.3 Time dependency of uptake
5.4. Equations for direct uptake

6. Behaviour of cesium and strontium in different soil horizons
6.1. Chemical physical behaviour (K_d)
6.2 Biological fixation
6.3 Transport of radionuclides by animals (earthworms, rodents)
6.4. Migration - Migration equations

7. Transfer of cesium and strontium from soil into plants
7.1. Uptake mechanisms
7.2. Plant availability of cesium and strontium
7.3. Rooting depths
7.4 Concentrations of nutrients
7.5 Mycorrhiza
7.6. Transfer equations

8. Translocation of cesium and strontium within plants
8.1. Distribution within plants
8.2. Time dependent changes of the distribution within plants
8.3. Comparisons of distribution dynamics beween cesium/potassium and strontium/calcium
8.4. Translocation equations

9. Transfer of cesium and strontium from feed to animals (mooses, rodents)
9.1. Metabolism of cesium and strontium in animals - in comparison with potassium and calcium metabolisms
9.2 Seasonal feed patterns
9.3 Seasonal migration of animals
9.4 Variation of cesium and strontium concentrations in feed depending on locality and time of the season
9.5. Population development
9.6 Transfer equations

10. Distribution of cesium and strontium within ecosystems
10.1. Distribution between soil - understorey vegetation - forest stand - animals
10.2. Seasonality of turn-over-rates
10.3. Time dependent loss from the systems
10.4. Model

11. Exposure
11.1. External exposure
11.2. Internal exposure
- Inhalation
- Ingestion
11.3. Whole body counter measurements

Head of Project 1: Dr. Wirth

II Objectives for the reporting period

During the first year the transfer of Cs 137 and Sr 90 from different soil horizons into mushrooms and plants has been investigated in different natural forest ecosystems. The influence of different parameters such as K and Ca on the transfer of the radionculides has been analysed.

III Objectives for next period

Continuation of the studies focusing on the aspect of time-dependency of the migration and transfer of the investigated radionuclides. Furthermore the quantitative distribution of Cs and Sr within different ecosystems will be measured. Transfer and migration equations will be developed as well as models describing the cycling of the radionuclides and their potential exposure to man.

IV Progress achieved including publications

Distribution of Cs 137 and Sr 90 in the undisturbed soils

On the selected 3 coniferous ecosystems activities between 5,000 and 25,000 Bq/m² have been measured. Between 85% and 98% of the activity was found in the organic O- and L-horizons (Fig. 1, site Hochstadt).

The Sr 90-activities range between 280 - 630 Bq/m², implying that a great part of the total activity is due to fall-out. Still 40 - 85% of the total activity was found in the upper O- and L-horizons.

A summary of the measured site properties for the site Hochstadt is given in Fig. 2. The organic content, the N, P, K and Ca concentrations as well as the pH are characteristics for the different soil horizons in a coniferous system in Southern Germany.

The K_d-measurements indicate that Cs is weakly bound in organic horizons. In mineral horizons the K_d-values increase by several orders of magnitude and are similar to values found on farmland.

The mobility of Sr is about the same in organic and mineral horizons. (Fig. 3).

Cs 137- and Sr 90-Activities in mushrooms and autotropic plants

The Cs 137 activities in different mushrooms are mainly due to their mode of living. Significantly higher Cs activities have been measured in symbiontic species (range 50 - 20,000 Bq/kg freshweight) whereby saprophytes (< 5 - 3,000 Bq/kg freshweight) and

parasites (<5 - 1,300) show generally lower values. A frequency distribution of the Cs 137 activities in different types of funghis are shown in Fig. 4. The Sr 90 activities in mushrooms were below 1 Bq/kg freshweight.

Autotrophic plants which are mainly rooting in the organic soil horizons have Cs 137 activities which are not signicantly different from the activities in most of the mushrooms. The values ranged between 120 Bq/kg freshweight for raspberries and 1,340 Bq/kg freshweight for blueberries. Within one plant the Cs activity is distributed similar to the K-concentration.

The behaviour of Sr 90 was similar to Cs in green plants. The measured activities ranged between 7 Bq/kg freshweight in pine needles and 37 Bq/kg in blueberries.

Based on these results different transfer equations for Cs 137 are tested presently.

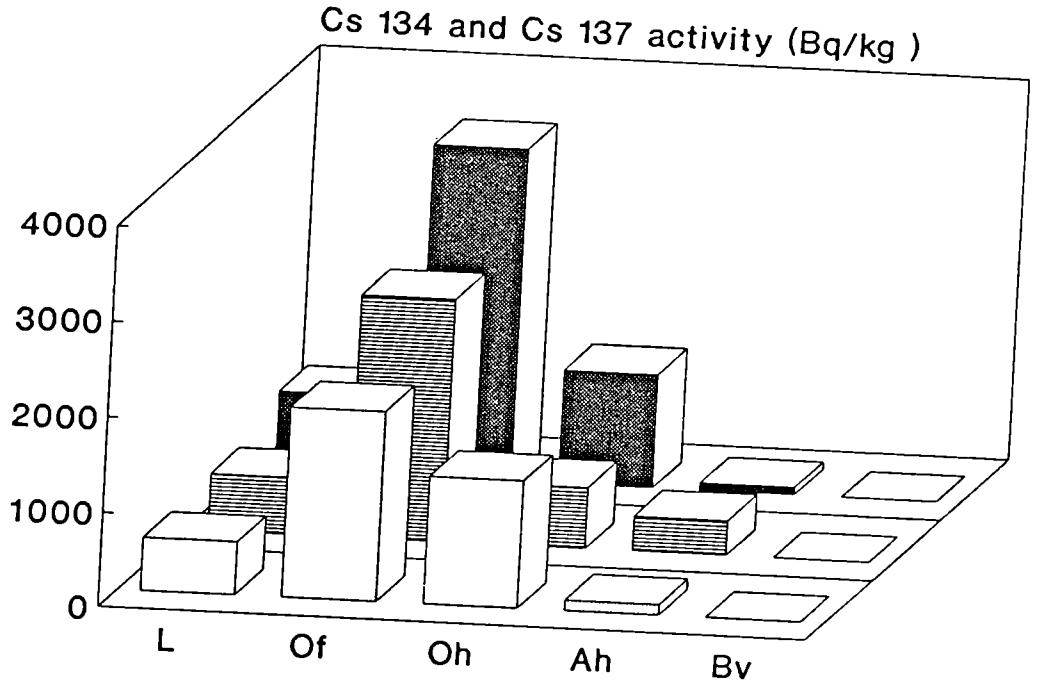


Fig. 1: Distribution of Cs 134+137 activity in different soil horizons of single samples collected at the site Hochstadt in 1990

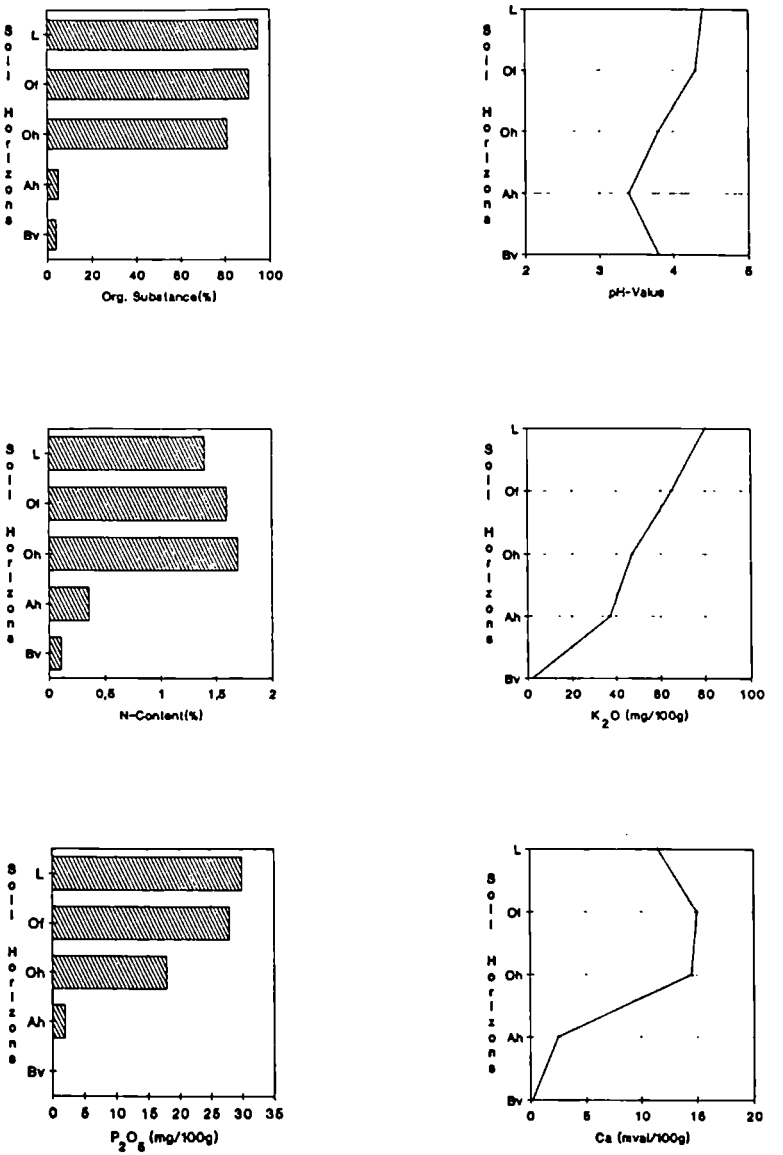
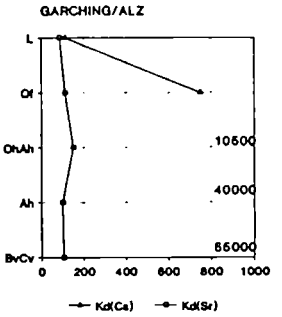
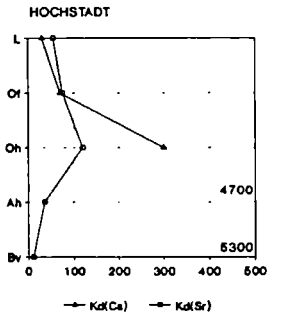


Fig. 2: Soil properties of the site Hochstadt





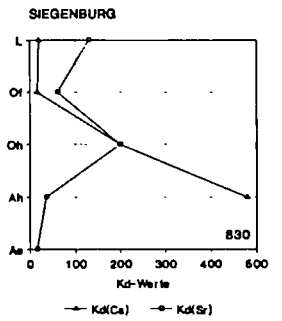


Fig. 3: Kd-Values for Sr 85 and Cs 134 found in different soil horizons of the sites Garching/Alz, Hochstadt and Siegenburg in 1990

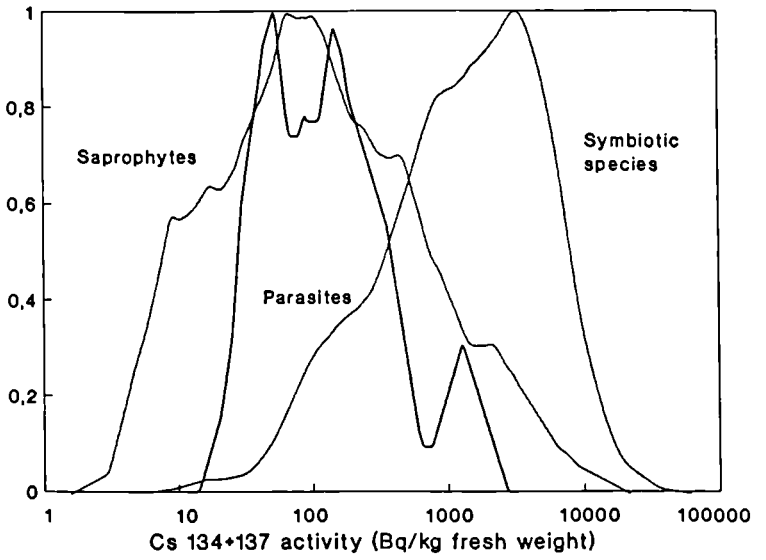


Fig. 4: Frequency distribution of Cs 134+137 activity in symbiontic species, saprophytes and parasites, combined over all years and sites

Head of Project 2: Ir. Guillitte

II Objectives for the reporting period

The objectives were chiefly methodological ones:
-choice of the sampling sites;
-choice and standardization of sampling techniques and methods between the different proposals of each team;
-test of these techniques in our sampling sites;
Several objectives on soil-plant transfers were also approached:
-influence of the rooting or the mycelium depth in soil;
-influence of the ecological micro-sites under forest canopy.

III Objectives for next period

We 'll continue the research on the both last objectives.
We 'll approach too:
-the action of the microflora in soil on radionuclides retention;
-the action of mycorrhyzas on radionuclides transfers from the soil to forest-trees;
-the relationship between humus decomposition and the turn-over of the radionuclides.
Theses approaches will be carried out principally under controlled conditions in laboratory.

IV Progress achieved including publications

We achieved our main sampling in two forest sites for which we had many radioecological data since 1986.The fisrt one is settled under deciduous trees(beeches) in Grand-Duchy of Luxemburg at Mersch.The second one is settled under coniferous trees(spruces) in belgian South Ardennes at Florenville.The sampling techniques and methods were tested in the both sites.
We completed these settlements by 5 secondary sampling sites with contrasted soils.These sites are closed to the main ones.
In each sites,we collected in summer ,soil samples and different parts(roots,stems and leaves) of trees and herbaceous plants growing above sampled soils. Fruitbodies of the main mycorrhizic and saprophytic species were collected in autumn.
We had the opportunity to achieve a similar sampling under very contaminated pine-stand at the north of Gomel in White Russia.
On the whole,356 samples of plants,mushrooms and soil were carried out in our sites.Half of them are analysed for their content in Cs134,Cs137 et K40.
First results point out the importance of micro-sites under forest-canopy on radionuclides transfers .These micro-sites influence the radionuclides availability and the rooting of plants(or the mycelium localisation)in the different soil horizons .They influence also largely levels of radionuclides deposits on soil , concentrations in the different soil horizons and density of herbaceous plants.This heterogeneity complicates the sampling .

List of our publications edited in 1990 and in relationship with our CEC-project:

GUILLITTE O.,KIRCHMANN R.,VAN GELDER E. & HURTGEN C.;1990.
Radionuclides fallout on lichens and mosses and their leaching by rain in a forest ecosystem.
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Contract: Bi7-016 Sector: A25

Title: Behaviour of Cs and Sr in natural ecosystems and the potential radiation exposure of their extensive use.

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2	Fraiture	Univ. Liège
3	Palo Univ. Umeå Agricultural Sciences	
4	Nimis	Università degli Studi di Trieste
5	Bergman	Swedish Defense Res. Establ.
6	Wickman	Univ. Umeå
7	Melin	Nat. Inst. of Rad. Protection (SSI)

I. Summary of Project and Global Objectives

Forest ecosystems are of considerable importance to man by providing wood, paper, recreation, berries, mushrooms and game animals. In Sweden, consumption of game animals and berries are the major pathways of radionuclides from forest ecosystems to man. The moose (Alces alces) constitute the most important hunting bag in Sweden and about 130 000 animals were shoot in 1990. This correspond to about 20 x 10^6 kg meat for consumption. Because of this importance, the understanding of factors governing uptake, time dependence and population distribution of in particular Cs-137 in this animal became of public concern. The diet of moose, in different regions seasons and individual variations are poorly known. This incomplete knowledge is especially serious when considering uptake of environmental pollutants such as Cs-137 by moose. By focusing on the ecology of this large herbivore it is possible to achieve information which is applicable to plant-animal transfer in general.

Head of Project 3: Dr. Palo

II Objectives for the reporting period

The work during the first year has focused on diet composition and collection of muscle samples from killed animals during the hunting season in September. The Cs-137 activity concentration muscle tissue in relation to deposition, diet, age and sex has been analysed. Sampling of vegetation for biomass, nutritional and radionuclide analyses has been performed. Trapping of voles in different forest environments, with different vegetation has been done, as well as sampling of muscle tissues from a major predator on voles, the red fox.

III Objectives for next period

The next period of research will focus on individual variations in diet composition of moose and population effects on the distribution of radiocaesium between animals. We will focus on regional differences in Caesium-137 uptake in moose, not expected from deposition data and modelling of Caesium-137 transfer in relation to above factors. Analyses of the food chain; ground vegetation-voles-fox will continue. Comparative studies of a related forest ecosystem in Italy (P.L. Nimis) and in Sweden will be performed.

IV Progress achieved including publications

Some of the results concerning the uptake of radiocaesium by moose in relation to deposition, sex, age and year of collection are presented by Palo et al. 1991. The major factors important for variation in radiocaesium activity in moose tissue, using a multivariate linear model, are deposition at collection site and year of collection. Age showed a negative correlation coefficient suggesting higher levels in calves. Sex of animals have only low explanation to Caesium-137 activity concentration in moose. Results covering the years 1986-1990 for vegetation, voles and fox are in preparation.

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EC Research Project N B 17 0016-C (MB)

University of Trieste, Department of Biology

ANNUAL REPORT 1990

The research activity of the group of the Trieste University during the year 1990 was focused on two main problems:
1) Study of the radiocesium content in all plant species occurring within a natural ecosystem.
2) Study aiming at the selection of an optimal bioindicator of radiocontamination in mountain areas.

Study on the distribution of radiocesium in plants

This study aims at investigating the distribution of radiocesium in all plants present in a natural forest, in order to analyze the main factors responsable for radiocesium transfer within the ecosystem.

The study site is located in the Carnic Alps, at P.so Pura (Ampezzo, UD), in a seminatural mixed *Fagus sylvatica-Abies alba* forest developed on limestone as parent material.

For each of the following species, leaves, stems and roots have been sampled and measured separately:

Vascular plants

Abies alba, Acer pseudoplatanus, Adenostyles alliariae, Anemone trifolia, Aposeris foetida, Aquilegia atrata, Aruncus dioicus, Asperula odorata, Asplenium viride, Athyrium filix-foemina, Cardamine trifolia, Chaerophyllum hirsutum, Cirsium erisithales, Daphne mezereum, Dentaria enneaphyllos, Dryopteris austriaca, Dryopteris filix-mas, Fagus sylvatica, Galeobdolon luteum, Gentiana asclepiadea, Geum rivale, Homogyne sylvestris, Hieracium sylvaticum, Lonicera alpigena, Lonicera coerulea, Luzula nivea, Lycopodium annotinum, Moehringia muscosa, Oxalis acetosella, Phyteuma spicatum, Polygonatum multiflorum, Prenanthes purpurea, Ranunculus lanuginosus, Ranunculus platanifolius, Ribes alpinum, Rosa pendulina, Rubus idaeus, Rubus saxatilis, Salix appendiculata, Saxifraga rotundifolia, Saxifraga hostii, Sorbus aria, Sorbus aucuparia, Thelypteris phegopteris, Vaccinium myrtyllus, Veronica urticaefolia

Furthermore, the followng cryptogams have been sampled and measured:

Lichens

Bryoria capillaris, Cladonia digitata, Cladonia furcata, Cladonia pyxidata, Evernia divaricata, Peltigera leucophlebia, Peltigera praetextata, Platismatia glauca, Pseudevernia furfuracea

Bryophytes

Ctenidium molluscum, Dicranum undulatum, Hylocomium splendens, Isothecium alopecuroides, Mnium marginatum, Plagiochila porelloides, Plagiomnium cuspidatum, Pleurozium schreberi, Polytrichum juniperinum, Rhytidiadelphus triquetrus, Rhizomnium punctatum, Tortella tortuosa.

Mushrooms

Amanita muscaria, Hygrophorus erubescens, Lactarius blennius, Lactarius salmonicolor, Lactarius scrobiculatus, Lycoperdon perlatum, Sarcodon imbricatum, Tricholoma saponaceum, Tricholoma terreum, Tricholoma vaccinum.

All the material was weighted immediatly after collection, dried, and weighted again. The difference between the fresh and dry weight allows to express the contamination in Bq/l (only for vascular plants) in order to avoid the error due to the different water content of different species, or of different parts of the same species. Furthermore, for all vascular plants the average depth of the root systems has been estimated (measurements carried out on 5 soil profiles for each different species). This datum will be important to study the relations between total contamination of the plants, and their root absorption from different soil layers.

All the data have been stored in a databank. A first statistical elaboration, limited to ca. 40 % of the total number of measurements, showed a very high degree of correlation between radiological data and the ecology of the species within the forest ecosystem. The statistical elaboration of all the data will be part of the work foreseen for 1991.

Study on bioindicators

This study aims at finding a suitable bioaccumulator for a rapid production of deposition maps in mountain areas. We consider this as an important point, given the high variability of deposition rates in areas with a rugged morphology, and the necessity of knowing the amount of actual deposition at a given site for most radioecological studies.

The organisms considered were: higher plants, mushrooms, lichens, bryophytes. The study has been carried out at P.so Pura (Carnic Alps, Province of Udine 1400 m), within a seminatural mixed *Abies-Fagus* forest.

Among these organisms, bryophytes appeared to be the most suitable bioaccumulators; the contamination of higher plants is influenced by the depth of the root systems and by the degree of lignification; the contamination of epiphytic lichens is highly affected by the micromorphology of the trunks (rain-tracks on the trunks, position of the lichen on the tree crown, etc.); furthermore, both higher plants and lichens make an estimate of deposition expressed on a surface basis very difficult. On the contrary, some bryophytes have several features of an ideal bioaccumulator: they are widely distributed in mountain areas, they lack epidermis and cuticle, they are not lignified, they have no organs for uptake of minerals from the soil, there is poor transport of the radionuclides within the plant due to the lack of vascular tissues, they tend to form carpets whose area can be easily measured, they have a very high absorbing capacity.

The test study was carried out on 9 species: *Ctenidium molluscum, Dicranum undulatum, Hylocomium splendens, Isothecium alopecuroides, Mnium marginatum, Plagiochila porelloides, Plagiomnium cuspidatum, Rhizomnium punctatum, Tortella tortuosa.*

The absorbing capacity for each species has been measured in the laboratory.

For each species, 5 samples have been collected on a) horizontal, b) inclined, c) vertical surfaces.

For each inclination range and for each species 5 samples have been collected a) near the trunks, b) under the canopy at 2.5 m from the trunks, c) at the margin of the canopies.

The results were as follows:

1) no significant difference in radiocontamination was detected among species with the same growth form; significant differences exist among groups of species with the same growth form.

2) the highest absorbing capacity is found in mosses of the *Ctenidium*- growth form; they have also the highest contamination values.

3) the highest contamination was measured in samples collected near the trunks, otherwise there was no statistically significant correlation with the position of the samples under the canopy

4) the contamination, expressed in Bq/m^2, tends to decrease with increasing slope; this is related to the fact that horizontal surfaces have the largest deposition area.

Also studied were the relations between contamination and thickness of the moss carpets.

To reduce the data spread the contamination value of a mixture of at least 5 individual carpets was compared with the distribution of the values obtained in all horizontal samples: the former value falls at the centre of a bell-shaped curve. The sampling strategy adopted for mapping is as follows:

1) Mosses of the *Ctenidium*-type will be sampled since they have the highest absorbing power, 2) Sampling should be not carried out near the trunks, 3) Only carpets growing on horizontal surfaces will be sampled, 4) The samples should have a thickness of at least 1 cm, 5) A sample should be a mixture of at least 6 different individual carpets.

[redacted]lopted during 1991 for the production of a deposi[redacted]

Trieste, April, 15, 1991

The scientifi[redacted]nsable

Prof. Pier Luigi NEMIS

Head of Project 5: Dr. Bergman

II Objectives for the reporting period

1) quantitative analyses of the site specific deposition pattern in the ecosystems under study; 2) radioecological evaluation of the time dependent levels of radioactive caesium in the biotic components, as compared to the site specific deposition, in order to quantify the uptake, retention and transfer in food-chains of expected significance for the internal exposure to man, and in terms of exposure to certain groups as well as the population on a regional basis; 3) field investigations with regard to soil-plant interactions as well as for the determination of loss of Cs-137 by run off from a catchment area.

III Objectives for the next period

1) Time dependent distribution of Cs-137 in a boreal forest ecosystem. An analysis based on comparisons of the distribution of Cs-137 in the boreal forest ecosystem a short time after a deposition (results from measurements on the deposition after the Chernobyl accident) with that after relatively long time of turnover (results concerning the behaviour of Cs-137 deposited as fallout from nuclear weapons test),
2) "Secondary" sources and losses of CS-137 in the forest ecosystem. Analysis of the importance of compartments (mosses, lichens,trees) of expected slow turnover of caesium and of losses by run off respectively for the levels observed or predicted for important food-chains to man.

IV Progress achieved during the present period.

The behaviour of radioactive caesium in a boreal forest ecosystem

Ronny Bergman The national defence research institute, Umeå, Sweden

SOURCES OF INPUT AND LOSS

The study concerns transfer of radioactive caesium in a boreal forest ecosystem and its bearings on the exposure to man in various time perspectives. We therefore focus on such soil, vegetation and animal processes which are known or expected to affect the distribution and transport of caesium in the system and ultimately its transfer to man.

Some studies have been made at a regional level that covers mainly the district of Västerbotten and the northern part of the district of Västernorrland. These deal with gammaspectrometric measurements of the ground deposition and the concentration of radioactive caesium, i.e. Cs-134 and Cs-137, in certain key-plants important for many herbivores, as well as for the forest food-chains over berries and moose to man (Bergman et al 1989).

The local study area is situated at the Forest Research Station at Svartberget, 50 km west of Umeå 64 16'N, 19 48'E in the district of Västerbotten.

Deposition of Cs-137 before the Chernobyl accident.

The amount of "old" Cs-137, i.e. due to atmospheric nuclear weapons test, remaining in the boreal forest at the time of the Chernobyl accident has been estimated based on :

* the average cumulative deposition at the latitude band 65' N (UNSCEAR 1977), which amounts to 1.2 kBq/m^2;

* the cumulative deposition, 1.8 kBq/m^2 (DeGeer et al. 1977, Bergman et al. 1989), according to the measurements of particulate airborne radioactivity at the sampling site in Lycksele (60 km SW of the study site at Svartberget); and

* the fraction of "old" Cs-137 according to our measurements of Cs-134 and Cs-137 in area-defined samples of soil and lichens from the study site at Svartberget, and the known ratio of Cs-134:Cs-137 activity in the air at Umeå (DeGeer, private communication) during the period of the main deposition of "Chernobyl"-caesium. Preliminary results yields a level of 2.5 +/- 0.5 kBq/m^2.

The distribution pattern of old caesium in samples of e.g. soil and ground vegetation taken in the period 1986-1990 from the same pine stand, as well as in comparisons of results between adjacent localities at the study site, exhibit variations, which are large in comparison to the respective average value. These site specific measurements (Bergman et al.1991) indicate the uncertainties involved in extrapolations from one site to another even over short distances. We base our analyses of radioactive caesium in the pine stand and the catchment area, (where detailed field studies are performed at Svartberget), on the local data: 2.5 +/- 0.5 kBq/m^2 of Cs-137 from atmospheric nuclear weapons tests, which does not significantly differ from the estimate based on the Lycksele data.

Deposition of "Chernobyl"-caesium at the local study site

Direct deposition (i.e. not including resuspension) due to release of radioactive caesium from the reactor in Chernobyl occurred mainly within some weeks after the accident (IAEA 1987, Hull 1987, Laaksonen 1987)). At Svartberget the mean deposition of caesium-137 released from the reactor in Chernobyl amounted to 15 +/- 3 kBq/m^2 in 1986 according to our gammaspectrometric measurements reported elsewhere (Bergman et al. 1988). A detailed mapping based on extensive sampling of soil (Nylen, techincal report) peformed 1989-1990 indicates that the levels have been higher than apparent from the field gamma measurements, and were 20 +/- 5 kBq/m2 at the local study sites within the Svartberget area about four years after the accident .

Waterborne transport

The content of radioactive caesium in water has been studied with regard to run off from a catchment area and groundwater at different depths at the forest research site (Nyle'n and Grip 1989). Based mainly on waterflow and radioactive concentration i 1986, 1989 and 1990, the loss of radioactive caesium by water leaving the terrestrial compartment has been estimated.

The measurements on ground-water from different depths show 0.1 Bq/l at the most superficial level and no significant concentrations of Cs-137 below that depth in 1986. Whether the transport to deeper layers of the ground-water will increase or not several years after the deposition is not known at present. However, the profiles in soil and concentration in the run off water of "old" Cs-137 appear consistent only with relatively small losses by transport to the ground water. This possible loss should be negligible concerning the main feature of the Cs-137 distribution in this system even in a long time perspective.

At the time (April 29th 1986), when the deposition of Chernobyl caesium became significant, the depth of snow was about 0.6 m at Svartberget (Degermark 1986). One week later nearly all snow had disappeared. The amount of Cs-137 discharged from the studied 0.5 km^2 catchment during this peroid was about 600 MBq with a maximum concentration in the water of 21 Bq/l (Bergman et al 1988). During summer to winter 1986 the concentration decreased from around 5 Bq/l to 0.1 Bq/l. In the following years only low levels of radioactive cesium was detected in the stream. The values are summarized in table 1 (for details cf. Bergman et al. 1988, Nylén and Grip 1989).

Table 1. The activity concentration of Cs-137 in run off water from the forest catchment at Svartberget over the period april 1986 to may 1990.

Year		Season	Concentration of Cs-137 Bq/l
1986	29/4-31/ 5	spring	1 - 21
1986	1/6-17/ 8	summer	0.2 - 5
1986	18/8- 1/11	autumn	0.1 - 1
1986	2/11-31/12	winter	0.08- 0.1
1989	14/4- 7/5	spring	0.1 - 0.4
1990	11/4- 2/5	spring	0.06- 0.2

The deposition remaining in the catchment is unhomogenously distributed, being higher on the water dividers and lower in the discharge areas (Bergman et al 1991). This indicates that the main source to activity in the stream has been the discharge areas. The total ground-depgsition in the catchment, determined by soil and water sampling, is $8.10^9 + 0.6.10^9$ Bq (i.e. the sum of the remaining deposition and the loss by run off). The loss by run off from the catchment was thus about 7% or 1200 Bq/m2 in 1986. During the subsequent years this leekage decreased to about 0.2% per year. Under the assumption that only the discharge areas are responsibel for the release of activity to the stream, the total loss there of Cs-137 during 1986 was 6000 Bq/m2. This corresponds to an early fractional loss of about 30 % from such sites under the "Chernobyl" conditions.

THE CAESIUM DISTRIBUTION IN VARIOUS TIME PERSPECTIVES

The content of radioactive caesium in "key"-plants: annual and seasonal response

Pine (pinus silvestris), birch (betula alba) and billberry (vaccinium myrtillus) constitute key-plants in the diet of moose and other herbivoreus game animals (Bergman et al. 1988; Nyle'n and Ericsson, 1989; Nelin and Palo, 1989; Palo et al. 1989; Danell et al. 1989).

Since the injection of radioactive caesium in 1986, only small changes in its concentration (based on the average content over a year) are observed in most of the vegetation under study , but for the period of relatively rapid decline from the initial levels, which took place during the spring and summer seasons in 1986. However, there are some exceptions:

- in pine needles directly exposed to the fallout a further significant decrease of about 30% occurred till June 1987, as compared to the level in November (i.e. late autumn) 1986 (Bergman et al 1988). The time dependence for the decrease of the Cs-137 content we observe in the tree canopy at Svartberget for the period, early june 1986 to june 1987, is similar to that reported for a site close to Chernobyl (Myttenaere, private communication), although in our case it concerns the effects in a pine stand after wet deposition, in contrast to the situation at Chernobyl dealing with dry deposition over a decidious forest;

- a small but significant decrease has also occurred for bilberry (Nelin and Palo, 1989; Palo et al. 1989) during the whole period.

Apart from the annual variation in average concentration, typical seasonal patterns of variations are evident particularly for birch and bilberry (Bergman et al. 1988). The variations in caesium concentration between individual plants are smaller than the annual seasonal variability, which implies that seasonal allocation and translocation in plants are important events for the observed dynamics in the redistribution of Cs. The reoccurrence of a similar pattern from year to year indicates that ecological conditions are responsible for the main feature of the observed variations in concentration of caesium with time.

The long term perspective

Twigs sampled in 1984 from three different pine stands growing on either very poor soil (the old group of trees, 58 y) or normal soil (the two groups of younger trees, 5 and 9 y) indicate that twigs from pine trees born in the fifties have more than one order of magnitude higher levels of Cs-137 as compared to plants of the same size, but less than 10 years of age in 1986 (Bergman et al. 1988). Whether the difference in concentration of Cs-137 mainly depends on the soil characteristics, on effective retention after deposition directly on old trees, or on cumulative retention as a function of the age of the tree is not clear.

Preliminary results from a detailed study of the cycling of radioactive caesium in a catchment area show, however, that the distribution between different parts of a pine tree: first year shoots; different year classes of pine needles; small twigs; branches and trunk , is almost the same for old and Chernobyl caesium already within the first five years after the accident (Nylén unpublished material).

These results has bearings on the dynamics of the turnover of caesium in the forest ecosystem.

secondary sources of Cs-137

The relatively fast redistribution of the "mobile" part of the Cs-137, (i.e. excepting those fractions with a known slow turnover, such as the content in lichens, mosses and the inner core of large trees), is interpreted as a reflection of the role of the total biomass in the

boreal environment with regard to a gradual dilution of a recent input of caesium into the common biomass pool.

The fractions with a particularly slow turnover (cf. above) constitute secondary sources (and in the case of the tree, the trunk during its lifetime practically operates as a sink) of considerable importance for the long term behaviour of Cs-137. The actual levels of Cs-137 in these sources estimated from measurements on samples from different biotopes within the Svartberget area are shown in tables 2 and 3 (Bergman et al.1988, Nylén and Ericsson 1989, Nylén unpublished results).

Table 2 The specific activity of Cs-137 in potential secondary sources in boreal forest pine stands within the Svartberget area 5 years after the deposition due to the Chernobyl accident.

Compartment	Pine canopy	Pine trunk	Mosses	Lichens
Specific activity	1-3 (kBq/m2)	50 (Bq/kg)	3-15 (kBq/m2)	15-25 (kBq/m2)

The activity concentration of Cs-137 in lichens and mosses five years after the Chernobyl accident is still relatively high as compared to the levels a short time after the deposition in 1986, when corrected for physical decay. The importance of these secondary sources is illustrated in table 3 as a function of how much of the ground surface that is covered by mosses or lichens, as well as the fraction of the total biomass contributed by coniferous trees in a particular biotope .

Table 3: Fraction of the total deposition over typical boreal forest ecosystems residing in secondary sources 5 years after the Chernobyl accident.

The assumed fraction of the ground surface covered by mosses or lichens range from 10% to 90%, and the ratio of the biomass (per unit surface area) in the trees to that in the other ground vegetation (including litter and organic matter in the upper soil horizons) range from 10:1 to 100:1.

Compartment	Fraction of the total deposition
Tree canopy	0.01 - 0.1
Tree trunks	0.02 - 0.2
Mosses/Lichens	0.1 - 0.7

The importance of such secondary sources with slow feedback of Cs-137 to circulation in the ecosystem is presently analysed by the use of compartment models.

The transfer of Cs-137 from the tree canopy to the ground vegetation, due to litter fall and through-fall of the precipitation has been studied since 1986 (Nyle'n and Grip 1989) in pine and mixed coniferous stands in the Svartberget area. Three phases are apparent from the measurements on litter fall and through-fall: 1) the intense leakage from the canopy during 1986; 2) the period during 1987-1989, while directly exposed pine needles yield relatively high transfer of Cs-137 by litter fall; 3) the period starting 1990 , i.e. when litter fall from pines contains mainly

year classes of needles not directly exposed to the fallout in april and may 1986.

THE IMPORTANCE OF THE PRODUCTS FROM THE BOREAL ECOSYSTEM WITH REGARD TO INTERNALLY ABSORBED DOSE TO MAN

The input to the human population of radioactive ceasium from forest products has been analysed with regard to the situation a short and long time after the deposition in the ecosystem (Bergman et al.1988, Bergman and Johansson 1989). There is not yet sufficient knowledge about several possibly critical ecological processes for accurate assessments. Above all the effect on the caesium distribution from external factors, such as the acid deposition, cannot at present be described adequately. The assessment may be done under the conditional assumption that the past and future effect on the behaviour of ceasium in the forest ecosystem from these external factors will be the same. In that case the conclusion is that moose and berries probably will contribute as much caesium-137 to the intake in the Swedish population as the agricultural components meet, milk and milk products, when added over a period of about twenty years.

This conclusion is based on the analyses of the contribution from agricultural products and forest products the first year after the Chernobyl accident; the total contribution over subsequent years from meat, milk and milk products (Holmberg 1986, Holmberg et al. 1988); and our estimate of the total contribution from forest products (i.e. moose meat and wild berries) (Bergman et al 1988, Bergman and Johansson 1989) over a period of the order of the physical half-life of Cs-137.

SUMMARY

The caesium budget for a terrestrial boreal forest ecosystem has been analysed. Physical decay governs the change in the content of Cs-137. Thus, as a consequence this applies to an even higher degree for Cs-134. A significant output occurs by water leaving the catchment area. Although small (5-10%) in comparison to the loss of Cs-137 by physical decay, that fraction transported out of the terrestrial compartment constitutes a considerable source with regard to the input to the aquatic recipients. This concerns in particular the possible causes to the present levels of radioactive caesium in fresh water fish as well as the levels expected in the future.

The redistribution of a primary deposit of radioactive caesium between the boreal forest compartments participating in the caesium turnover is seemingly fast. Similar proportions of the "old" (nuclear weapons-) and "new" (Chernobyl-) Cs-137 content within the pine tree (different year classes of needles, small twigs, branches, bark and superficial parts of the trunk), perennial plants and the upper organic horizons in the soil is obtained in less than five years after the Chernobyl accident. i.e. excepting those fractions of the system with a known slow turnover, such as the content in lichens, mosses and the inner core of large trees. The latter fractions constitute secondary sources of considerabel importance for the long term behaviour of Cs-137.

The ratio between the concentration of Cs-137 in moose meat and the cumulative ground deposition per unit area is not significantly different in 1985 from that in the period 1986-1990 (when corrected for decay). It is probable that the slow release of Cs-137 from the secondary sources

discussed above is an important factor concerning the observed relatively high availability of Cs-137 in the food-chain over vegetation to moose two decades after its introduction in the ecosystem .

The potential contribution to the collective dose to the Swedish population from forest products, such as moose and berries, is at the same level as meat, milk and milk products from the agricultural area. This estimation relies on the assumption that cesium will behave similarly in the future as in the past two decades. However, such an assumption is far from proven, and there are several factors, among other the environmental pollution by the depostion of nitrogen- and phosphorous compounds as well as the decreasing pH in the soil due to acid rain, that probably will affect the behaviour of caesium and many other elements in the future. The net result on the behaviour of caesium of the complex interaction between various possible effects is far from clear. For example, an increased mobility for caesium may lead to increased loss from the system by run off, as well as to a higher uptake in those plants that are important links in the food-chains to man.

Consequently there is a need for a better understanding of the basic cause - effect relationships for accurate assessments and validation of applied models. Our current study is directed towards this question, particularly with regard to the processes governing the distribution between soil and vegetation and the transport of radioactive caesium over important food- chains.

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UNSCEAR 1977

Head of Project: Dr. Wickman

II Objectives for the reporting period

Study of radiocaesium in Swedish moose. Different methods for the assessment of the body content of radiocaesium in critical groups and the general population in a boreal forest area.

III Objectives for next period

See II.

IV Progress achieved including publications

Introduction

Our contribution to the project is mainly the study of the last link in the ecological transport chain of caesium in the boreal forest to man including methods for the assessment of the body content in different critical groups and in the general population.

In collaboration with the University of Agricultural Sciences in Umeå we are studying caesium in moose. The concentration of caesium in moose meat gives a significant contribution to the account of caesium in man. The mean consumption in the studied area is about 8 kg per year and person. Our department started already at the beginning of May 1986 to cooperate with the police in the county of Västerbotten to get samples from moose killed in traffic accidents. Totally about 300 moose samples have been obtained in this way. This gives the seasonal variation of the activity concentration in mean. During the hunting seasons 1986-1990 a total of about 7 000 moose samples were collected and measured. A detailed picture of the local activity concentration variation could therefore be obtained. At present 300-500 samples are collected every year from selected hunting teams in order to follow the caesium concentration in moose with time. Our results can be found elsewhere (1,2).

Whole body measurements have long been the traditional way to assess the internal contamination of gamma emitting radionuclides in man. This is the best method to use when individuals or smaller groups are considered, however for a larger group the method has the disadvantage that whole body counters are sophisticated, heavy and expensive. The number of samples that can be taken from a population must be severly limited due to practical and economical reasons. Furthermore, a practical disadvantage is that the individual being measured must be located in the whole body counter during the measurement. Since the measurement involves participation of volunteers the sampling can be distorted by refusals. There is an obvious risk that

individuals ignoring the hazards by eating critical foodstuffs will be underrepresented. Alternative methods for the assessment of the body burden in a population might therefore be considered such as:

- Analysis of human tissues from autopsy or surgical procedures
- Measurement of activity concentration in excreta

Tissue Samples from Medico-legal Autopsies

Centres for medico-legal autopsies normally covers a well defined geographical area. Specimens from a great number of individuals can be selected from their material in order to get a representative sample of the population. The selection includes an adjustment with respect to age and sex, and a deselection of individuals such, as abusers that can be suspected of being malnutrited before their death. The psoas muscle was used for measurements of Cs-137 activity concentration.

The yearly internal absorbed dose from Cs-137 may be obtained by using the conversion factor 4.6 μGy/year per Bq/g (K) which is obtained by applying data from Snyder et al (1975).

For comparison with whole-body activity, information is needed about the total mass of muscle and the fractional uptake in the muscle. Such conversion factors may be obtained applying data from a metabolic model developed by ICRP (1988) and the reference man data (see ICRP 1975, 1988).

A set of measurements were carried out to check this procedure (Wickman et al, 1989). The weight, total body content of Cs-137 activity and total body content of potassium as well as the muscle activity concentration were measured for 3 individuals. From these data it is possible to calculate the potassium content per muscle mass. Values of 3.8 ± 0.3 to 4.2 ± 0.7 g of K per kg muscle is obtained. Applying the ICRP reference man data 3.3 g/kg is obtained*. The three experimental determinations are in very close agreement with one another and agree also fairly well with the calculation using ICRP data.

Autopsy measurements may be used for the purpose of obtaining a sufficiently large, randomly distributed sample of a large population. The result must however be corrected with regard to the age and sex distribution.

Measurement of Excreted Activity

If the biological half-life is known, the body burden, under equilibrium ingestion and excretion, can be determined by measurement of the daily excreted activity. The group of 29 adult male and female Laplanders whose whole-body content is measured regularly, were asked to collect their 24-hour urine the day before the whole-body measurement was performed. This routine was done at two occasions.

Figs. 1a and b present the relation between daily urinary excretion and total body activity measured simultaneously. Since extreme cases may deviate for reasons not relevant to the study only those individuals who has a whole body content below 80 kBq and an urinary excretion rate less than 600 Bq/day have been included. This excludes four individuals from the first occasion and one from the second one.

If steady state is assumed, i.e. daily intake equals daily excretion, the slope of the fitted line is proportional to the average half-time of Cs in the studied population.

*The ICRP reference man has a mass of 70 kg, a muscle tissue mass of 28 kg and a body content of 140 g potassium, see ICRP (1975). 65 % of the body potassium is assumed to be located in the muscle tissue, ICRP (1988).

The observed slope leads to an effective half life of 100 ± 5 days. About the same slope was obtained in May and December, why the mean is stated. It also presumes that 10 % of Cs taken up from the GI-tract is excreted rapidly and thus gives an insignificant contribution to the total body activity.

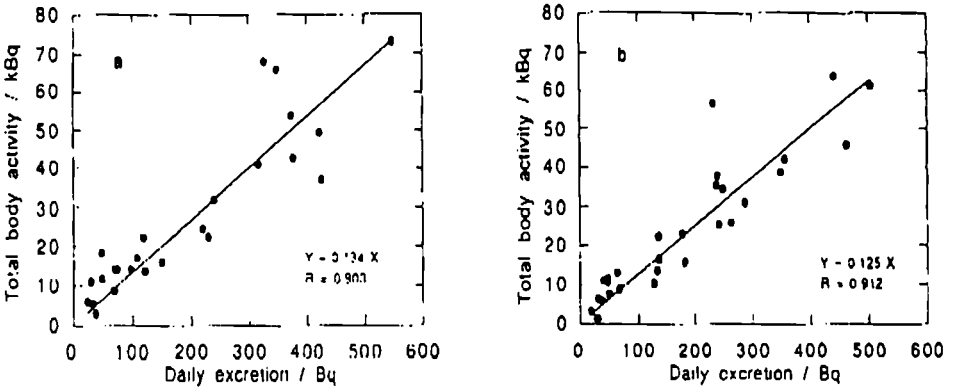

Figure 1. Measured relation between daily excretion in urine, X, and total body activity, Y, of ^{137}Cs. a) May 1988; b) December 1988. The regression lines shown are forced through the origin.

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Project Title: Retention and distribution of cesium and strontium in boreal forest ecosystems.
Contract nr : B17-0016-C (MB)
Name of contractor : Judith Melin/Swdish Radiation Protection Institute

Progress report for the period 1 st of April 1990- 31 st of March 1991

The objective is to investigate parameters of relevance in order to model the dynamic and retention of cesium and strontium in a forest environment. The studied involves the interaction of nuclides in different soil systems and the accumulation and redistribution of cesium and strontium in trees and understory vegetation.

Three locations have been chosen for the study.

1. In order to study the partial equilibrium of cesium and strontium in a forest environment a mixed stand of spruce and pine was selected . The stand was exposed to the fallout from the nuclear bomb tests during the fifties and sixties but not to the deposition from the Chernobyl accident. The sampling was accomplished before the Chernobyl accident in such a way that a balance of cesium and strontium content in the environment (trees, understory vegetation and and soil) could be realized. In order to obtain information on the dynamic of cesium and strontium within the season sampling was conducted on several occasions during the year.

Besides the study on the dynamic of cesium and strontium in the environment the relationship between the cesium and potassium content in different compartments in the trees are studied by K-40 measurements.

All sampling, sample preparation and most of the measurements of cesium and potassium as well as the radiochemical analyses of strontium have been accomplished during the year.

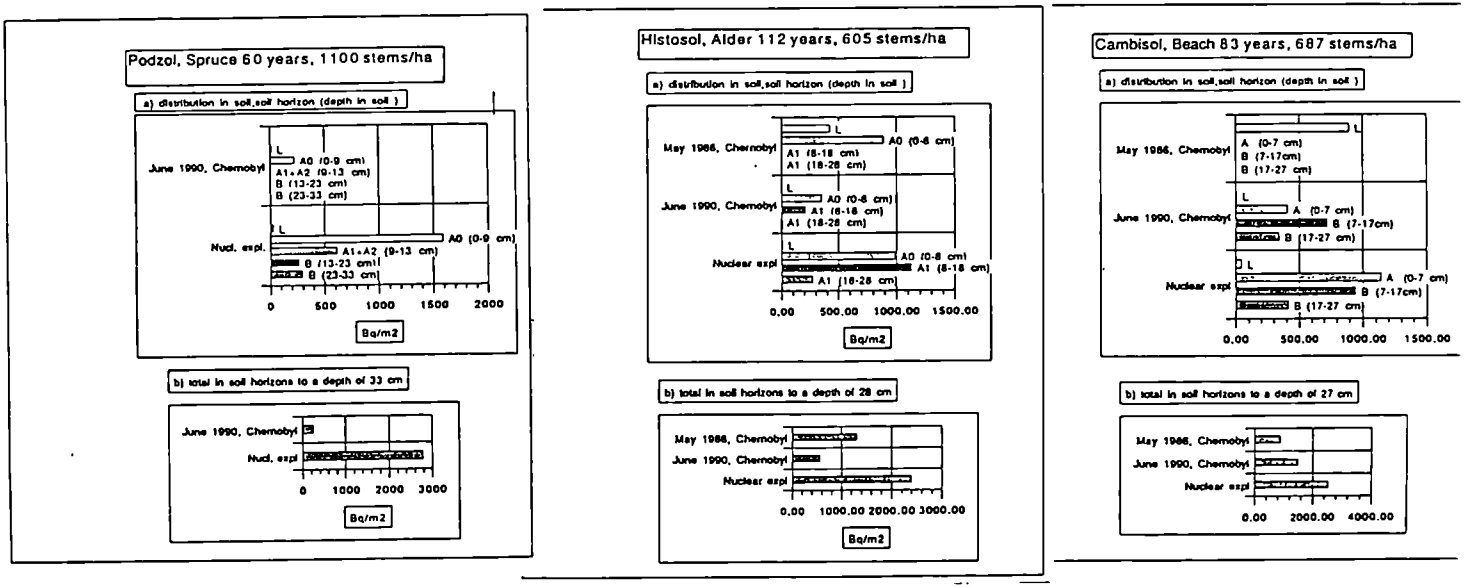
2. The interaction of cesium with soil constituents are studied in eight soil systems with different stand and soil caractersitics. The soils investigated in this study have been classified as cambisols, podsols and histosols below stands of pine, spruce, birch, beach alnus and oak. Sampling have been conducted in order to study the partial equilibrium of cesium from the fallout during the fifties and sixties in the different soil systems. In addition the interaction and distribution with time of cesium deposited in 1986 are studied by sampling the area in 1986 and in 1990.

During the year all sampling and some of the measurements have been conducted. The areas have been caracterized in respect of , soil classification, clay and organic matter content, pH, catioexchange capacity.

Example of the results obtained are presented in Fig 1.

3. The dynamic of cesium in a forest environment during the period immediately after the fallout from the Chernobyl accident is studied in a heavily contaminated Scot pine stand. Sampling soil and vegetation has been performed immediately after the Chernobyl accident and repeated four years after the accident. Measurements of the samples are in progress. During the current year a biomass study will be performed to achieve a cesium balance for the ecosystem (soil, understory vegetation and trees).

Figure 1. Distribution of cesium-137 in soils classified as Podzol, Histosol and Cambizol respectively.
Deposition of Cs-137: nuclear expl. 2000-3000 Bq/m2
Chernobyl 2000-3000 Bq/m2
Sampling date: May 1986, June 1990
Data on distribution of cesium from nuclear expl. are calculated from measurements on samples collected in May 1986.



Progress Report

Contract: Bi7-044 **Sector: A25**
Title: Radioecology of seminatural ecosystems.

1	Colgan	NEB
2	Horrill	NERC
3	Aarkrog	Risø National Laboratory
4	Johanson	Univ. Uppsala Agricult.Sciences

I. Summary of Project and Global Objectives

Member States of the European Community have suffered fall-out both as a result of nuclear weapons testing and again following the Chernobyl accident. Radiocaesium from both these sources is present in soils in measurable amounts and has remained available for recycling in upland ecosystems for a longer period of time than was predicted by models available at the time of the Chernobyl accident. These models were largely based on data from studies of radionuclide movement in lowland agricultural ecosystems. Current work has also shown that radiocaesium concentrations in different species, from the same habitat, can vary by an order of magnitude. This could be due to the physical composition of the plant, or to specific physiological factors.

The levels of radiocaesium present in upland vegetations and herbage is therefore often enhanced when compared to that grown on mineral-rich lowland soils. Semi-natural ecosystems are also the natural habitat of domestic and wild animals such as sheep, red deer, moose and reindeer and so a direct pathway into human food chains can be identified.

The mechanisms of recycling and transfer in these ecosystems are poorly understood, and the necessary data with which existing models can be revised or new models developed are not available. The research projects described in the following sections have been formulated with a view to using similar techniques to evaluate a number of different ecosystems where elevated levels of radiocaesium activity have already been identified, both in plant species and in grazing animals. Soil characteristics, plant composition, plant physiology and biological activity are all being examined and their effect on soil-plant transfers in acidic soils assessed. In addition, the seasonal variation in dietary composition of grazing animals and the subsequent effect on their radionuclide burden is being evaluated. Where appropriate, critical groups will be identified and annual dose committments assessed. All data collected will be made available for modelling purposes.

The research contract involves work on blanket bogs and montane peatlands (Ireland), upland heaths (UK), Northern Boreal forests (Sweden) and more extreme habitats as found in the Faeroe Islands (Denmark). A contrasting Mediterranean ecosystem grazed by sheep has recently been included in the programme.

The research contract is co-ordinated by the Nuclear Energy Board and a number of intercomparison exercises to ensure uniformity of sampling and measurment by all participants is an essential part of the work programme.

Head of Project 1: Dr. Colgan

II. Objectives for reporting period

1. Collection of soil, vegetation and faeces at 4 pre-selected sites. Analysis of samples for radiocaesium and preliminary assessment of results.

2. Comparison of radiocaesium in sheep by in vivo monitoring with activity in faeces taken from the grazing area.

3. Establish methodology for collection and analysis of soil water from upland peat soils.

4. Identification of seasonal variability in the diet of mountain sheep.

5. Co-ordination of programme to include laboratory intercalibration and a comparison of soil sampling methodologies.

III. Objectives for next period

1. Development of a sampling strategy for upland soils and vegetation by assessment of site variability.

2. Field experiments to determine radiocaesium returned to the ecosystem via plant litter and standing dead material.

3. Laboratory based leaching experiment to assess mobility of caesium in peat soils. Results will be compared with field samples to assess radiocaesium losses from the ecosystem.

4. Complete interpretation of soil-plant transfer of caesium using existing data.

IV. Progress Achieved.

Soils and Plants. The 1990 research programme involved study of four farms located in upland areas of Ireland. Deposition to these farms from Chernobyl radiocaesium fallout was high relative to other Irish ecosystems. Sheep are the main agricultural product of these areas, and are the principal potential route for radionuclide transfer to man. Sheep that graze the four research farms had previously been shown to have radiocaesium activity levels in excess of 1,500 Bq/kg. Plant species which were common on three of these farms were sampled on five occasions between April and November in order to follow any seasonal pattern in radiocaesium content. A fourth farm was sampled for vegetation on a monthly basis between May 1990 and May 1991. Calluna vulgaris, Erica tetralix, Erica cinerea, Vaccinium myrtillus, Eriophorum vaginatum, Eriophorum angustifolium, Scirpus caespitosus, Myrica gale, Pteridium aquilinum, Juncus squarrosus, Polytrichum commune, Sphagnum cuspidatum, Molinia caerulea, Cladonia impexa and Potentilla erecta were sampled where present.

The highest radiocaesium activities, up to 3,500 Bq/kg dw, are found in the new shoots of Calluna vulgaris and Scirpus caespitosus and in Pteridium aquilinum. All other species have concentrations less than 1000 Bq/kg throughout the period of sampling. Seasonal variability is most marked in Scirpus caespitosus and, for some of the graminoid species, an increasing Cs-137:Cs-134 ratio is observed during the growing season. It is hoped to correlate this with the caesium ratio in the underlying soils as an indication of rooting depth and the source of caesium uptake within the soil profile.

Soil samples were collected and profiled from three farms to assess total deposition, spatial variability and vertical migration of radiocaesium. Samples collected in April 1990 from deep peat sites have shown that caesium-134 has already penetrated below the 30 cm horizon, indicating an annual migration rate in excess of 7 cm.

Faeces and Sheep In vivo monitoring of a minimum of 50 ewes grazing upland pasture was carried out at one site from May 1990 to April 1991. At the same site between 2 and 4 samples of fresh faeces were collected from designated locations within the grazing area of the flock. An additional sample of faeces was also collected from a nearby coniferous forestry area on each occasion.

The seasonal variability in measured radiocaesium levels in ewes is shown in Figure 1. Table 1 shows the corresponding activity in faeces collected monthly up to December 1990. Greater variability observed in the radiocaesium content of faeces early in the season is attributed to animals returning to upland grazing over a period of 2-3 weeks. The radiocaesium activity in samples taken from the forestry area is 2-3 times higher from September onwards and this may possibly be due to the consumption of fungi. A comparison of the fungal spore count in faeces from both inside and outside the forestry area is being considered.

Soil Water Preliminary soil water investigations have been carried out on upland organic soil sites. Using porous clay pots in which a vacuum is set up, soil water has been extracted in situ. Other methods of collection of soil water were also tried. Water has been collected from channels in these sites and river water from the upland catchment have been analysed. A method was devised of soil water uptake in situ using semipermeable membranes.

All of these methods have yielded similar results and generally the radiocaesium content of soil water and channel water has been of the order of 20 mBq. This result is comparable with those achieved in Cumbria by the NRPB. One site has been found which yielded water of 240 mBq/l and will be used for comparison with laboratory studies during the next 12 month period.

Diet Evaluation Using the method developed by Martin (1955, Trans. Proc. Bot. Soc. Edin., 36 : 278 - 288), the cuticular fragments of vegetation which constitute the faeces samples were identified and quantified. This analysis gives an indication of the diet of the sheep and how it changes throughout the year. This dietary analysis was carried out on two farms and detailed information was collected in the field on the vegetation available and the radiocaesium content of each species. Comparing dietary information with available vegetation in the field reveals diet selectivity and the maintenance by sheep of a constant diet over the study period in spite of seasonal change in vegetation availability. The relative importance of each component of the diet shifts when their contribution to radiocaesium intake is considered. The radiocaesium content of each vegetation species and the diet analysis allows a prediction of faecal radiocaesium concentrations. The actual radiocaesium content of the sheep faeces at these two sites was measured and compared to the predicted levels and also to in vivo sheep measurements of radiocaesium. The same trends throughout the year was observed in all three although the predicted faeces activity was low compared with the actual situation.

Programme Co-ordination The principal areas of collaborative work were as follows:

- laboratory intercomparison of radionuclide determination in soils and plants
- laboratory intercomparison of measurement of soil physical and chemical parameters
- field exercise to compare different methods of sampling of organic soils.
- comparison of radiocaesium concentrations in vegetation species collected from semi-natural ecosystems.

The performance of each participating laboratory was assessed by counting two soil and two plant samples which were homogenised and distributed to all laboratories. Each sample was then measured by each laboratory and results for Cs-137, Cs-134 and K-40 were reported. A normal probability plot of the pooled data from all laboratories showed only 10 out of 161 readings to be obvious outliers and seven of these ten were attributed to one laboratory. One laboratory consistently overestimated relative to the global mean, another laboratory consistently underestimated while the other two showed positive and negative bias dependent on isotope. Both bias and measurement errors were found to increase in the order Cs-137, Cs-134, K-40

Different methodologies for soil sampling were compared as part of a field intercomparison. Analysis of the results is in hand and more information is included in the attached report of Dr. Aarkrog.

A comparison of radionuclide concentrations in vegetation species common to all 4 ecosystems has commenced and will be completed in 1991.

TABLE 1

Radiocaesium in Ewes and Faeces April - December 1990

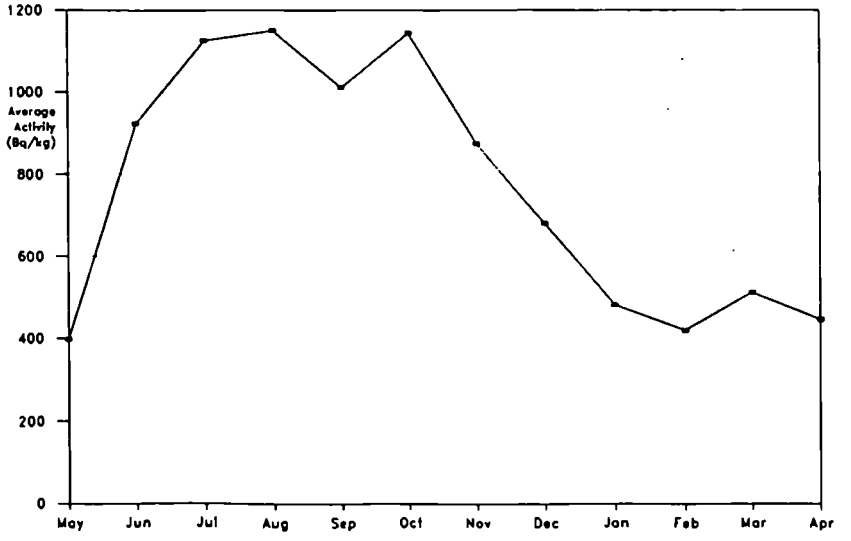
MONTH	EWES*		FAECES-Pasture*		FAECES - Forestry**	
	(Bq/kg fw)	n	(Bq/kg dw)	n	FORESTRY**	n
Apr	-	-	886±246	4	1185±47	1
May	398±193	70	1045±404	4	-	1
Jun	923±250	56	1336±264	3	776±31	1
Jul	1126±385	54	1392±166	3	-	1
Aug	1150±310	57	1104±73	3	873±32	1
Sep	1012±358	59	1234±104	3	2883±107	1
Oct	1144±316	59	932±91	3	3820±129	1
Nov	875±211	70	595±66	2	2102±88	1
Dec	681±171	57	780±88	3	1423±65	1

* ± SD

** ± 2SD counting error

FIGURE 1.

Seasonal Variation in Radiocaesium Levels in Mountain Sheep May 1990 - April 1991.



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2. B Rafferty, E.J. McGee and P.A. Colgan. Comparison of diet selectivity and availability of natural fodder in free ranging mountain sheep. (in press)

3. E.J. McGee, P.A. Colgan and M. Keating Intercalibration of soil and plant samples among four european radiation research laboratories (in prep)

Head of project 2: Dr A D Horrill

II Objectives for the reporting period

1. To identify chemical and physical characteristics soils from three upland heaths - Sillathwaite, Ennerdale (NY065128), Corney Fell (SD150897) and Ardverikie (NN533902).

2. To take part in four inter-laboratory comparison studies (see section IV).

3. To supply Riso Laboratory with a summary of radionuclide concentrations found in tissues of United Kingdon wild animal populations and an estimate of their food potential for man.

4. To grow heather plants for a transpiration experiment.

III Objectives for next period

1. To assess how the chemical and physical characteristics of soil from the three study sites have affected radiocaesium concentrations down soil profiles, and in *Calluna vulgaris*, *Vaccinium myrtillus*, *Molinia caerulea* and *Polytrichum commune* growing on those soils.

2. To co-ordinate the writing of a paper to assess the effects of different analytical methods used by participating laboratories on measured physical and chemical soil characteristics.

3. To conduct an experiment to study the effect of transpiration rate on radiocaesium uptake by *Calluna vulgaris*.

4. A pilot study to measure biological activity in soils using an infra red gas technique, and to investigate whether there is corrrelation between radiocaesium concentrations in plants and the level of biological activity in the soils that they are growing on.

IV Progress achieved including publications:

1. Upland heath study

Upland heath sites selected for this study were known to have received relatively high inputs of Chernobyl radiocaesium:

Sillathwaite, Ennerdale, (NY065128, north west England) - altitude 275m, acidic peat grassland, sheep grazing only form of management.

Corney Fell (SD150897, north west England) - altitude 400m, acidic peat grassland, sheep grazing only form of management.

Ardverikie (NN533902, central north Scotland) - altitude 320m, heather dominated deep wet peatland, low density sheep and deer grazing only form of management.

Samples of soil and vegetation have been collected from all three sites and analysed for radiocaesium concentrations and for a range of nutrient elements and physical characteristics. This data has been tabulated and is ready for statistical analysis.

Inter-laboratory comparison studies

i. A study of the effect of different sampling techniques on measured concentrations of radiocaesium down soil profiles.

Samples were collected on 18 September 1990 from Ennerdale and Corney Fell as part of the Group meeting at Merlewood Research Station, Grange-over-Sands, Cumbria. These have been analysed. Tabulated results and a summary of sampling technique have been sent to Riso Laboratory, Denmark to be incorporated into a paper assessing the effect of sampling method on measured radiocaesium concentrations down soil profiles.

ii. A study of possible bias in measured radiocaesium concentrations in soil and vegetation due to laboratory analytical technique.

Homogeneous samples of *Pteridium* and *Calluna* litter have been provided for all member countries of the group, to be analysed for radiocaesium. These samples, together with the soil samples provided by the Nuclear Energy Board (NEB) Dublin, have been analysed. Tabulated results have been sent to the NEB for an assessment of the effect of laboratory analytical methods on measured radiocaesium concentrations.

iii. An assessment of radiocaesium uptake by plant species common to each country involved in the contract.

The following samples have been collected and analysed for radiocaesium:

a) *Molinia*, *Calluna vulgaris*, and *Polytrichum commune* and their associated soils from Ardverikie (NN533902).
b) *Erophorum angustifolium* and *Polytrichum commune* and their associated soils from Devoke Water (SD160975).
c) *Calluna vulgaris* and *Vaccinium myrtillus* and their associated soils from Ennerdale (NY132128).

Tabulated results have been sent to NEB to be included in an ecosystem intercomparison study (see Table 1 as an example of results sent to group leaders co-ordinating inter-laboratory comparison studies).

iv. A study of the effect of different analytical methods on the measurement of physical and chemical soil characteristics.

Soil samples collected for inter-laboratory comparisons of sampling techniques and ecosystems have been analysed for organic matter, clay, silt and sand; extractable potassium, phosphate, nitrate and ammonia; total phosphorus and nitrogen, and for cation exchange capacity.

3. Radiocaesium concentrations in UK wild animal populations

Riso Laboratory, Denmark has been supplied with a summary radionuclide concentrations measured in tissues from red deer, foxes brown hares, blue hares, black grouse and red grouse, after the Chernobyl accident. A rough estimate of the worst case consumption of contaminated flesh by the local human population has also been given.

4. Transpiration experiment

About 30 heather plants have been propagated in sand ready for a transpiration experiment.

TABLE 1 ECOSYSTEM INTERCOMPARISON SAMPLES

Sample type	^{134}Cs Bq kg^{-1}			^{137}Cs Bq kg^{-1}			^{40}K Bq kg^{-1}		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
1. ARDVERIKIE (NN533902)									
Molinia	161± 2.6%	126±2.7%	118± 3%	1212±3.6%	988±3.5%	901±3.6%	340±15%	316±13%	325±4%
Associated soils									
0- 5 cm	208± 2.6%			1561±3.5%			108±30%		
5-10 cm	66± 3%			594±3.6%			199±16%		
10-20 cm	5±11%			62±4%			529± 6.5%		
Calluna	346± 1.8%	316±1.8%	304± 2%	2671±3.5%	2438±3.4%	2364±3.5%	202±13.5%	197±13%	164±20%
P. commune	132± 2.4%	102±3%	164± 2.5%	999±3.5%	777±3.6%	1255±3.5%	338±12.5%	219±25%	183±19%
Associated soil									
0 - 2.5 cm	160± 2.8%	138±2.6%	165± 3%	1047±3.6%	969±3.5%	1144±3%	983±54%	590± 5.6%	1178± 6%
2.5- 5 cm	62± 3.6%	37±4%	98± 4.9%	478±3.6%	288±3.7%	663±4%	593±57%	494± 6%	<80
5 - 7.5 cm	31± 4.4%	14±4.4%	60± 9%	259±3.8%	126±3.7%	391±5%	<80	487± 4.3%	<80
7.5-10 cm	14± 5.7%	7±9%	37±11%	136±3.9%	76±4.3%	266±5%	<80	<80	<80
10 -12.5 cm	12± 9%	5±9.5%	22±12%	112±4.4%	58±4.2%	190±5%	<80	<80	<80
12.5-15 cm	6±17%	ND	20±15%	70±4.8%	32±5%	142±6%	<80	<80	<80
15 -20 cm	4±14%	ND	13±14%	47±5%	186±4%	125±5%	<80	<80	<80
2. DEVOKE WATER (SD160975)									
E. angust.	54± 3.7%	32± 4.9%	15± 7%	438±3.7%	263±3.9%	121±4.5%	234±18%	189±21%	194±22%
Associated soils									
0- 5 cm	84± 4.5%	93± 3%	87± 4%	689±3.8%	724±3.6%	685±3.8%	1570± 6%	322±28%	142±58%
5-10 cm	34±14%			325±5%			<80		
10-20 cm	22±10%			231±4.7%			<80		
P. commune	309± 2%	57± 4%	136± 2.7%	2447±3.5%	459±3%	1033±35%	140±28%	250±20%	188±25%
Associated soils									
0- 5 cm	20± 5%	19± 4.5%	19± 5%	181±3.9%	180±3.7%	176± 3.9%	117±36%	123±29%	104± 4.4%
5-10 cm	ND	3±17%	3±17%	39±5%	37±5%	39± 5%	553± 5.5%	520± 5.5%	484± 5.6%
10-20 cm	ND	ND	ND	24±5%	23±5%	23± 6%	128±28%	<80	134±20%
3. ENNERDALE (NY132138)									
Calluna ag	159±2%	72±2%	179±2%	1426±3%	636±3.5%	1543±3.5%	184±25%	<80	125±36%
Vaccinium leaves	95±3%	90±3.5%	95±3.5%	823±3.6%	775±3.6%	744±3.7%	327±17%	280±21%	320±20%
Vaccinium cyg	75±3%	86±3%		621±3.6%	754±3.6%		157±23%	797± 5%	
Vaccinium oyg	68±3.1%	56±2.5%	50±3.5%	581±3.6%	477±3.5%	388±3.7%	145±23%	158±17%	90±33%
Associated soils									
0 - 2.5 cm	108±3.1%	104±3.1%	103±3.3%	875±3.6%	851±3.6%	833±3.7%	<80	85±50%	96±56%
2.5-10 cm	10±8%	9±7%	10±7%	155±4%	149±3.8%	176±3.8%	261±14%	236±11%	328±10%
10 -15 cm	ND	SS	SS	33±5%	SS	SS	1012± 4%	SS	SS

KEY

Calluna	*Calluna vulgaris*
Vaccinium	*Vaccinium myrtillus*
P. commune	*Polytrichum commune*
E. angust.	*Eriophorum angustifolium*

±%	confidence limits of counting
R	replicate samples
ND	not detected
SS	sample too stoney for analysis
ag	aerial growth
cyg	current year's growth
oyg	other years' growth

Head of Project 3: Dr. Aarkrog.

II. Objectives for the Reporting period

1. Collection and analysis of Faroese samples and calculation of transfer factors.

2. Participation in the intercalibration programme comprising analysis of soils, bracken and heather and intercomparison of soil-sampling techniques.

III. Objectives for the next Reporting Period.

1. The comparative study of radiocaesium in a number of selected vegetation species from Ireland, United Kingdom, the Faroe Islands, Sweden and Greece will be accomplished in 1991.

2. An estimate of doses received from radioceasium in foodchains in seminatural ecosystems will be carried out. The doses (individual and collective) will be compared with those received by the general population in the E.C.

IV. Progress achieved.

1. Faroese Samples

(a) Methodology. Three locations in the Faroes have since 1962 been used for collection of milk (monthly), lambs meat (annually), potatoes (annually) and drinking water (quarterly). One further location was added and from these four locations samples of soil, grass, milk, lambs meat and fresh water were collected in July 1987, 1989 and 1991. Precipitation data have since 1962 been available from two stations. The samples are analyzed for Sr-90 and radiocaesium (Cs-134 and Cs-137).

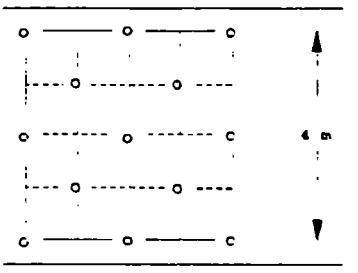
(b) Results Since 1982 (when fresh direct fallout from nuclear weapons testing essentially had ceased) the effective half life of Sr-90 from global fallout in Faroese milk has been 3.2 years and that of Cs-137 from global fallout 2.2 years, whereas the effective half life of Chernobyl Cs-137 (1987-1989) was 1.2 years. In lambs meat Cs-137 from global fallout decayed with a 4.3 years half life and Chernobyl Cs-137 with a 1.2 years half life. In Faroese potatoes Cs-137 from global fallout showed a half life of 2.8 years and Chernobyl Cs-137 showed 2.3 years.

(c) Discussion The observation of Chernobyl caesium in the Faroese environment has until now been too short to conclude that Chernobyl debris in the long run should behave differently from global fallout. We believe that the effective half lives of caesium from the two sources will approach each other with time. When these environmental half lives have been determined, it will be possible to calculate transfer factors and consequently doses from unit deposition (kBq m^{-2}) of global fallout and Chernobyl fallout in a seminatural ecosystem as that in the Faroese Islands.

2. Intercomparison of gamma-spectrometric analyses and soil-sampling techniques.

(a) Methodology. Samples were distributed among the project participants (soil samples from NEB; bracken and heather samples from ITE). The samples were prepared in advance (drying and homogenizing). The soil samples received weighed 100-200g and the vegetation samples 20-60g. The samples were measured in 200ml cylindrical containers on Ge detectors connected to standard electronics and placed in 10cm lead shields. The detectors are calibrated with water solutions of radionuclide standards, and the results are corrected for coincidence losses and self absorption in the sample. The recorded gamma spectra were analyzed by non-commercial software developed at Riso. Minerals and texture parameters were determined from the soil samples (pH, organic matter, clay content, N, P, K, NH^{+}_{4} , and cation exchange capacity by Hedeselskabet according to the official Danish methods for soil analysis.

The joint soil-sampling intercomparison was carried out in Cumbria, UK, the 18 September 1990 where the participants used their own techniques for taking soil samples at depths 0-5cm, 5-10cm and 10-20cm at two locations (Ennerdale and Corney Fell). The Riso procedure uses a 65mm diameter stainless steel corer for the sampling. A total of 13 cores is taken from 4 X 4 grid as shown in the figure below.



The corer is sharpened at the cutting edge which is rotated and pressed down to collect the desired depth segment in each of the 13 locations. The 13 sub-samples thus obtained are composited to yield one sample for each depth segment. Grass and roots are kept in the samples. In the laboratory the samples are comminuted, dried, crushed, sieved, blended, and aliquots are taken out for gamma-spectrometric analysis.

(b) Results The results of the gamma-spectrometric analyses of the intercomparison samples (Bq kg^{-1} of Cs-134 and Cs-137) are sent to the Nuclear Energy Board in Dublin. The results of the analyses of the samples from the soil-sampling intercomparison are given in the Table 1 in terms of becquerels of Cs-137 and Cs-134 per square metre for each site sampled. Full statistical analysis of the results are presently being undertaken and will appear in print in due course.

Table 1
Best Estimate of Radiocaesium Deposition
(0 - 20 cm)

	Cs-137 kBq/m^2		Cs-134 kBq/m^2	
Laboratory	**Site 1**	**Site 2**	**Site 1**	**Site 2**
1(n=3)	15.6±6.5	15.98±2.6	1.14±0.34	1.73±0.3
2(n=5)	16.97±3.2	18.72±3.8	1.24±0.21	1.72±0.29
3(n=3)	11.4±0.3	21.55±7.6	0.88±0.15	1.68±0.61
4(n=1)	11.31	19.54	1.26	1.35
5(n=1)	6.94	13.08	-	0.95

Errors are reported as ± 1 SD
n= number of observations.

Head of Project 4: Prof. Johanson

II Objectives for the reporting period.

1 To obtain an understanding of the behaviour of Cs-137 in the forest ecosystem; in soil, the soil-plant transfer and the plant-animal transfer.

2 To investigate possible methods to reduce the transfer of Cs-137 to game animal and further to man.

III Objectives for the next period.

1 To continue investigations on the transfer of radiocaesium to roe deer by evaluation of diet.

2 To study counter measures in the Swedish forest ecosystem to reduce individual and collective doses to man.

IV Progress achieved including publications.

Soil-plant transfer. The effects on the uptake of Cs-137 to heather have been studied after adding potassium fertilizer to a forest ecosystem. There was a 50% decrease in the Cs-137 activity concentrations in heathers in plots where 50 or 250 kg KCl was added per ha. The decrease occurred within a period of about two months. Without fertilizer a Cs-137 transfer factor (soil-heather) between 0.25 and 1.0m^2 kg^{-1} was found.

The soil-plant transfer was studied for the three most common and important berries in Sweden - lingonberry, bilberry and cloudberry. Samples of berries were collected at various locations in the northern and middle in Sweden. At the same site samples of soil were also collected and used for determinations of deposition of 137Cs deposition and soil chemical parameters. The mean transfer factors (Bq kg^{-1} D.M. per Bq m^{-2}) were found to be 0.129 for cloudberry, 0.032 for lingonberry and 0.041 for bilberry. For lingonberry and bilberry the transfer factor decreased when the exchangeable potassium or pH of the soil decreased. For cloudberry no such correlation was found.

An assay system for studies of the Cs-137 transfer capacity for various soils from the forests has been established. Small soil samples are used for pot experiments with sheep's fescue (Festuca ovina) as test plant. At the site of collecting soil samples eleven samples of plants, usually bilberry, was collected and a short description of the plant community is given.

The frequencies of fruitbodies of mushrooms were studied at 4 plots (20x20m) in 1990 from middle of July to early October. All fruitbodies in the plots were sampled with about 2 weeks intervals. There was one peak in late July beginning of August and a second peak in late September. Since mushrooms contain high activity concentrations of Cs-137 and is an important food for roe deer the abundance of mushrooms will affect the levels of Cs-137 in roe deer. A mean Cs-137 transfer factor for all mushrooms collected in our investigation area is between 1.0 and 1.2m^2 kg^{-1}.

Plant-game animal transfer. Based on our previous studies on the seasonal variation of the Cs-137 activity concentrations in roe deer the authorities in Sweden decided to try a hunting period of roe buck in May 1990. The normal hunting period for roe buck in Sweden is in August and September. The mean Cs-137 activity concentrations in row bucks harvested in May was about 4-5 times lower compared to those harvested in August and September. In May the mean activity concentration was 1,250 Bq kg^{-1} and in August and September nearly 5,000. A simple counter measure such as changing the hunting period may thus give quite large reduction in the transfer of Cs-137 to man. Aggregated transfer factors (soil to roe deer) ranging from 0.04 to 0.2m^2 kg^{-1} were found. The variation depends on seasonal variations.

The Cs-137 activity concentrations in about 250 samples from harvested moose from our research area each year have been determined. An aggregated transfer factor (soil to moose) of about 0.02$m^2 kg^{-1}$ has been found. During the 5 hunting periods after the Chernobyl accident there seems to be no trend in the development of the Cs-137 activity concentrations. The variation from year to year is rather large and today no significant decrease in the levels of Cs-137 in moose can be seen. This indicates a very long ecological half life of Cs-137 in the forest ecosystem.

Based on the results of our studies as well as measurements of the local Health Boards in some communes we have calculated the total Cs-137 transfer to man by moose meat. In for example Gavle commune with nearly 90,000 inhabitants about 125 million Bq of Cs-137 was transferred to man by 938 moose harvested in 1989 by about 1,500 hunters. The corresponding collective dose is about 1.5manSv.

References

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2. Johanson, K.J., Kardell, L. Radiocaesium in lingoberry, bilberry and cloudberry in Sweden. Presented at meeting of the Union of Nordic Agricultural Scientists, Beitostolen, Norway 1989. Revised manuscript 1991.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-B-325-F

Commissariat à l'Energie Atomique
CEA, CEN de Cadarche
B.P. n° 1
F - 13108 Saint-Paul-lez-Durance

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 42.25.70.00

Title of the research contract:

Conséquences sur l'environnement d'un accident majeur sur centrale nucléaire: réhabilitation des sols et des surfaces (Programme RESSAC)

List of projects:

Conséquences sur l'environnement d'un accident majeur sur centrale nucléaire: réhabilitation des sols et des surfaces (Programme RESSAC)

Title of the project no.:

Conséquences sur l'environnement d'un accident majeur sur centrale nucléaire : Réhabilitation des Sols et des Surfaces après Accident (programme RESSAC).

Head(s) of project:

Dr André GRAUBY

Scientific staff:

Henri MAUBERT
Philippe FACHE
Pierre RONGIER
André JOUVE
Michel MOUTIER
Michel HAMONIAUX
René ESCARIOT

I. Objectives of the project:

La mission du projet RESSAC est d'étudier les moyens techniques et leur mise en oeuvre pour une intervention dans les zones contaminées par un accident nucléaire majeur en vue de réhabiliter les surfaces pour les rendre à un usage normal. L'impact à court et long terme de l'accident doit être évalué, même dans les zones déjà soumises à réhabilitation. Cette prévision doit être basée sur la connaissance du comportement des radionucléides dans les sols et sur leurs transferts à la végétation en fonction du temps.

II. Objectives for the reporting period:

Une concertation avec nos collègues Européens devait permettre de choisir un ensemble de sols devant être échantillonnés. En France 4 sites devaient être sélectionnés de façon à préparer les prélèvements.

Les performances du système de régulation du potentiel hydrique du lysimètre 1800 devaient être évaluées de même que le coût et les conditions de la réalisation et du transport des lysimètres.

Une expérience de contamination d'herbe et de fourrages devait être conduite en 1990 de façon à fournir des matériaux contaminés au centre nucléaire de MOL et à l'université de Piacenza.

La construction du bâtiment devait débuter.

III. Progress achieved:

Les quatre sites sélectionnés en France sont : TRICASTIN, BELLEVILLE, CATTENOM et FLAMANVILLE. Les réunions de concertation avec nos collègues Européens ont permis de préciser les contraintes de prélèvements des lysimètres ainsi que leur coût. Les caractéristiques et les performances climatiques du bâtiment RESSAC ont été détaillées. La date d'un prélèvement de démonstration a été fixée au mois de Juin 1991.

Le système de régulation du potentiel hydrique des lysimètres a été testé. Ses performances sont satisfaisantes. Sur une durée de six mois la consigne (humidité du champ) a été suivie à moins de 15 hPa près. Ce système peut être considéré comme qualifié. Les participants aux réunions du Comité Programme RESSAC ont pu visiter cette installation en Novembre 1990.

L'expérience de contamination à l'aide des aérosols générés par le four POLYR s'est déroulée de façon satisfaisante. Les équipes de Gembloux, Mol et Piacenza ont pu utiliser les matériaux contaminés dans le cadre de leurs travaux. Le terme source POLYR est maintenant opérationnel.

La construction du bâtiment a débuté. Elle devrait s'achever fin 1992.

Plus généralement, un rapport final sur les acquis expérimentaux du programme RESSAC a été rédigé. Il porte sur les points suivants :

Les principaux types de sols d'une importance économique sensible autour des sites électronucléaires français ont été recensés et échantillonnés. Après plusieurs sélections il a été possible d'en retenir un nombre réduit (5) couvrant une large gamme de caractéristiques pédologiques influant sur le comportement des radionucléides. Un programme expérimental a été conduit sur les sols issus de cette sélection.

La répartition d'un dépôt éventuel entre le couvert végétal cultivé et le sol (facteur d'interception) a été déterminé pour le cas d'aérosols microniques secs. Des courbes permettant de déterminer le facteur d'interception en fonction de la biomasse par m^2 sont disponibles pour plus d'une dizaine de cultures.

Les facteurs de transfert sol-plante du césium et du strontium ont fait l'objet d'une étude systématique pour huit cultures sur les cinq types de sols déjà cités. Des résultats expérimentaux un modèle a été déduit permettant de prévoir le degré de contamination des récoltes en fonction de la radioactivité résiduelle dans les sols. Ce travail fondamental a été appliqué à la prévision des résultats de contre-mesures telles que l'ajout d'engrais pour diminuer la contamination des végétaux.

Un logiciel permettant de calculer la migration de 8 radionulcéides dans les sols a été créé. Des données en mémoire autorisent des calculs par défaut, ce qui est adapté à une situation de crise. La possibilité d'entrer au clavier des valeurs spécifiques en étendent l'emploi à des cas particuliers, ou à un usage plus centré sur la recherche. Ce logiciel est disponible en français et en anglais.

Un ensemble important de travaux a été consacré à la connaissance du terme source accidentel. La forme physico-chimique des radionucléides relâchés lors d'un accident majeur était mal connue en 1985. Depuis des études bibliographiques (notamment sur l'accident de Tchernobyl), des travaux théoriques menés à Fontenay-aux-Roses et la mise au point d'un simulateur, le four POLYR, ont permis d'accroître considérablement notre savoir sur ce point particulier. Dans le futur, le programme RESSAC, et plus largement les études expérimentales en matière de contamination radioactive accidentelle, pourront se dérouler avec un terme source représentatif.

Afin de disposer des conditions expérimentales les plus représentatives possibles, une technique de prélèvements de monolithes de sols de grandes dimensions (5 m^3) a été mise au point. Les blocs des sols ainsi échantillonnés, appelés lysimètres, disposent d'un fond permettant de réguler les flux d'eau et ainsi de ne pas modifier les échanges hydriques ayant normalement lieu entre le sol et les couches sous-jacentes.

L'ensemble des lysimètres et du système de contamination sera placé dans un bâtiment spécialement conçu à cet effet, ou différents climats pourront être simulés. Les études de cet outil expérimental sont achevées et la construction a débuté.

Enfin la collaboration entre RESSAC et d'autres équipes de la Communauté Européenne est entrée dans une phase active.

VI. Publications:

P. RONGIER ; H. MAUBERT ; Expérience POLYR 03/90 réalisée pour la CCE les 09 et 10/05/1990. Note technique RESSAC 15/90. Mai 1990.

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JOUVE A. ; Essai de modélisation du facteur de transfert sol-plante du strontium 85. Note RESSAC 14/90. Avr. 1990.

MOUTIER M. ; Réalisation d'un lysimètre. Préparation d'un site, prélèvement et transport d'un monolithe. Note RESSAC 44/90 ; Oct. 1990. En anglais sous le titre : Manufacturing of a lysimeter. Preparing the site, sampling and ferrying the monolith.

RONGIER P. ; MOUTIER M. ; Réalisation d'un lysimètre. Acquisition des données climatiques et hydriques sur un site de prélèvement. Note RESSAC 56/90 Nov. 1990. En anglais sous le titre : Preparation of a lysimeter. Acquisition of climatic and hydrous data on a sampling site.

RONGIER P. ; Descriptif simplifié du bâtiment RESSAC et de ses fonctions. Note RESSAC 03/91. JAN. 1990. Traduction en Anglais en cours.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Risø National Laboratory
DK - 4000 Roskilde

Contract no.: BI6-B-326-DK

Head(s) of research team(s) [name(s) and address(es)]:

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Title of the research contract:

Design and Development of a Skim and Burial Plough for Reclamation of Contaminated Land

List of projects:

Design and Development of a Skim and Burial Plough for Reclamation of Contaminated Land

Title of the project no.:

BI6-B-326-DK

Design and Development of a Skim and Burial Plough for Reclamation of Contaminated Land.

Head(s) of project:

Jørn Roed

Scientific staff:

Kasper G. Andersson

H.L. Gjørup

Jørn Roed

I. Objectives of the project:

To design and develop a plough that can remove a contaminated top-layer of about 5 cm soil and bury it beneath some 50 cm soil without inverting the 5-50 cm horizon, in order to avoid less fertile subsoils to be brought to the surface and allow normal tilling procedure to be carried out without mixing the contaminated buried material with the ≈ 30 cm layer of tilled soil.

II. Objectives for the reporting period:

III. Progress achieved:

1. METHODOLOGY

Development Strategy

The outline plan for development of the skim and burial plough was as follows

1. to discuss different design possibilities with experts in agricultural machinery
2. to build a small demonstration model of the selected design
3. to build a 1/10 scale working model and test it
4. to modify and improve the 1/10-scale model
5. to build a full scale model for field tests under real conditions, and to modify as necessary
6. to build the first full scale prototype based on knowledge gained from earlier tests
7. to test the prototype and modify it to achieve the objective

Design Considerations

Various possibilities for construction of a skim and burial plough were considered.

The first consideration was to modify a trench plough so that it no longer inverts the layer between the trench bottom and the surface layer, but places the topmost 5 cm soil layer into the bottom of the trench. A large trench-plough was located and modifications were discussed with experts in deep-ploughing. It was concluded that the modifications suggested might achieve the objective but the suggested modifications were rather drastic and a purpose built device would be better.

The second possibility was a machine that lifts a half meter layer of soil to a height of about 60 cm from its original position while simultaneously placing the uppermost 5 cm under

the lifted soil. This construction guarantees that the uppermost layer really is placed in the bottom of the furrow and that the rest of the layer is not inverted. However, this design is expensive in terms of energy consumption since the soil is lifted up over the surface when the skim coulter is placing the uppermost 5 cm layer in the trench.

The third option was to design an entirely new type of plough and this is what we have done. In this new design, a skim coulter places the uppermost layer of soil (~ 5 cm) in a trench made by the main ploughshare which in one movement digs the trench and places the soil lifted in the former trench on top of the thin layer of topsoil in the bottom of the trench of the former run. In this way the 5-50 cm soil layer is lifted only about 10-15 cm and therefore energy consumption is minimiser. However, the probability that the layer from 5-50 cm will be partially inverted and that not all of the uppermost layer will be placed in the bottom of the trench exists.

Design phase

Initial design and construction was carried out at the Risø Laboratory. A small scale model was built in the laboratory and taken to Bovlund Plovfabrik in Jutland, Denmark, a company with long experience in the design and construction of large trench ploughs.

The first assignment for Bovlund Plovfabrik was to build a 1/10 scale model using the small demonstration model designed at Risø.

A series of field tests, each followed by adjustments and modifications, eventually lead to a sufficiently good performance for the construction of a full-sized version.

This full-sized version was then tested in the field and a number of problems were found. The plough was then returned to the factory and modified. This procedure was repeated twice before the performance of the model was acceptable. The tests were conducted in a former dung yard where the topmost 30 cm layer of soil was very dark and heavy and the soil in the bottom was gravelly. These different soil horizons gave good visual indication of the performance of the model plough.

The modifications left us with a model consisting of bits and pieces welded on the original model.

The Fullscale Prototype

Based on the much modified full-sized model, a prototype fullscale plough was built and tested on land which consisted of a 30 cm, layer of light soil overlying a sandy type of soil.

This land was specially chosen as a stern test of the performance of the plough on a light soil. The tests showed that it was necessary to build a higher and broader shield in order to avoid the sandy soil falling into the furrow before the uppermost layer was placed in the bottom of the furrow.

Further testing and modification resulted in a plough worthy of extended and intensive trials, such trials have been conducted at the Risø National Laboratory.

2. RESULT

Field test at Risø

The plough was tested on two different types of land.

1. permanent pasture that had not been tilled for 40 years and

2. tilled soft soil

The tests took place in April 1991.

The plough was drawn by a normal farm tractor.

The plough produced a furrow 60 cm wide and 50 cm deep in both types of land. The skim coulter, which is adjustable, was set to skim off the topmost 5 cm soil horizon.

In order to determine the new positions of the original soil layers after ploughing, small metal rifled cylinders (2 cm height and 1 1/2 cm diameter) were placed in vertical columns to a depth of 56 cm and spaced at 20 cm intervals. See Figure 1.

Each column of cylinders was painted in a different colour and each cylinder in a column was marked with a number indicating its original depth under the surface. After ploughing, the cylinders were relocated by use of a metal detector. The new positions of the cylinders were then found by excavation and compared to their original positions. (See figure 2.) The results of the tests on pasture land are given in Figures 4 - 10 and for tilled land in Figures 11 - 16. In the Figures, the lines connect the original positions of the cylinders with the positions where they were found after ploughing.

Some cylinders were never relocated. This was due to the skidding tractor wheels throwing some of the surface cylinders to considerable distances. The skidding of the tractor wheels was due to the very wet ground: continuous rain fell during the tests.

For the second test, the number of surface cylinders was trebled, making it much easier to relocate surface cylinders after the test.

Had the plough performed perfectly, the cylinders would have been redistributed as indicated in Figure 7.

3. DISCUSSION

Some of the figures show that the 5-25 cm soil layer had become inverted. This is immaterial since this layer becomes new tilth.

Figure 1. Excavation for finding positions of metal cylinders after ploughing.

Figure 2.

Cylinder set-up.

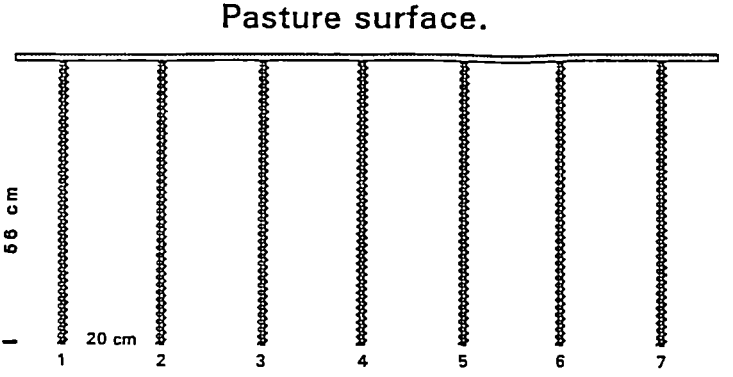


Figure 3.

Key to figures 4 - 16.

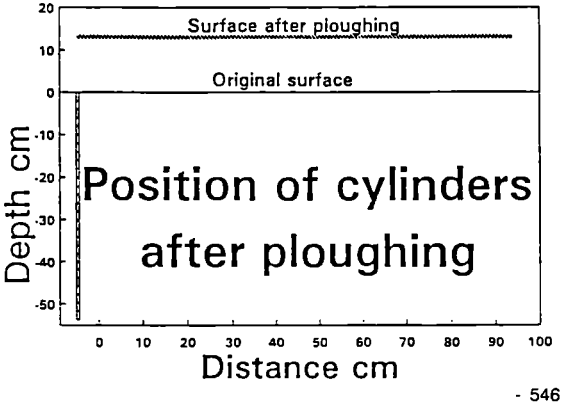
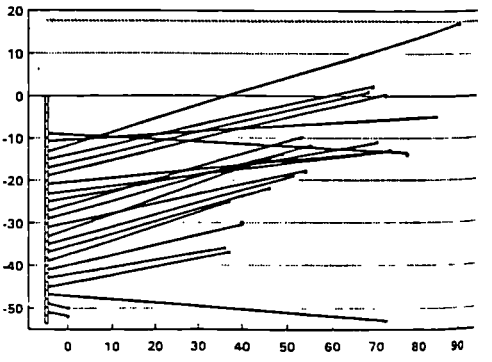


Figure 4.

Grey series. Pasture.



White series. Pasture.

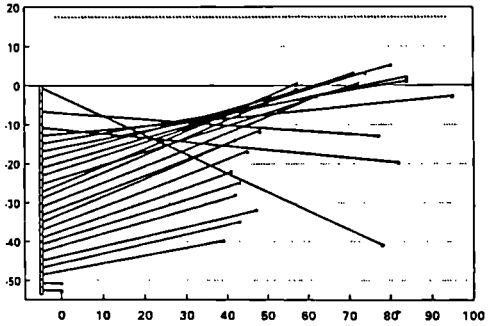


Figure 5.

Yellow series. Pasture.

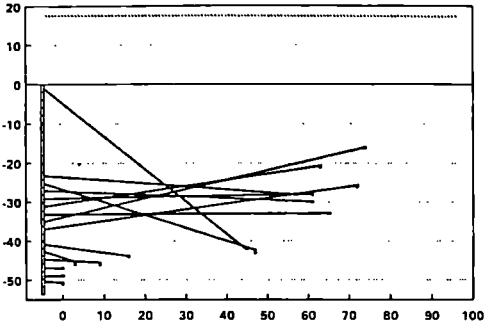


Figure 6.

Red series. Pasture.

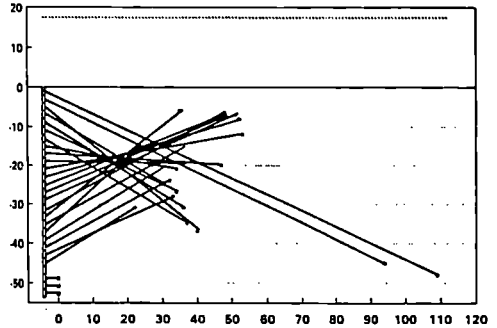


Figure 7.

Green series. Pasture.

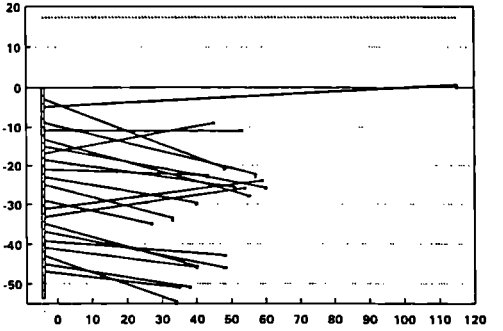


Figure 8.

Blue series. Pasture.

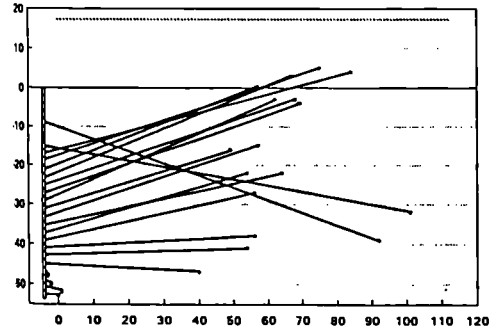


Figure 9.

Light blue series. Pasture.

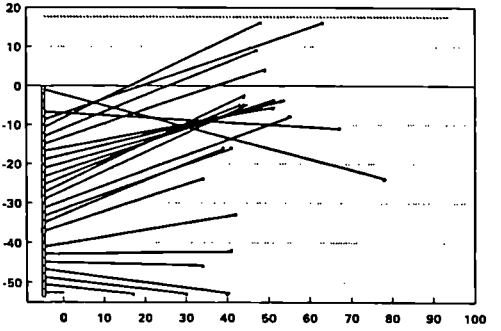


Figure 10.

White series. Tilled land.

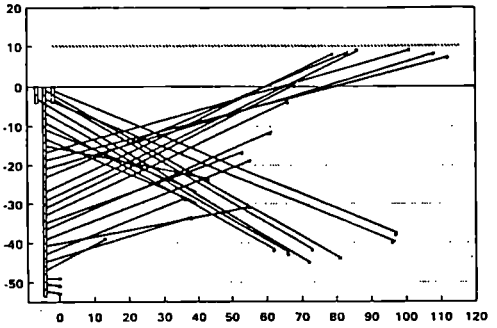


Figure 11.

Grey series. Tilled land.

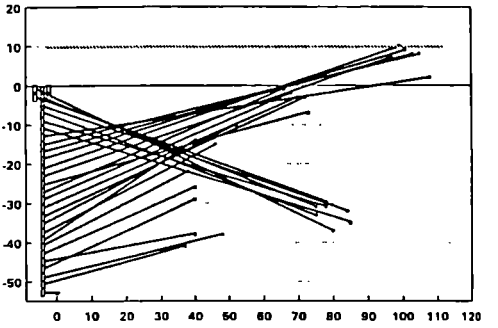


Figure 12.

Yellow series. Tilled land.

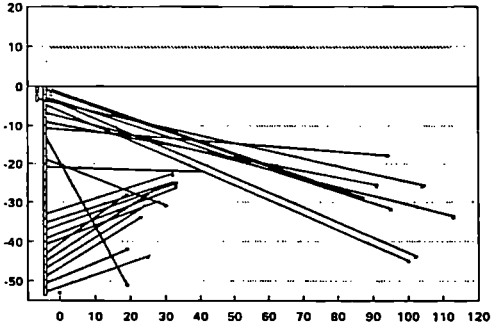


Figure 13.

Red series. Tilled land.

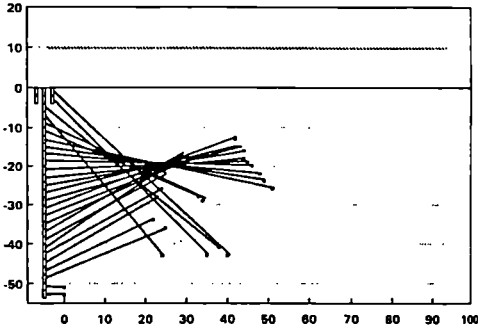


Figure 14.

Blue series. Tilled land.

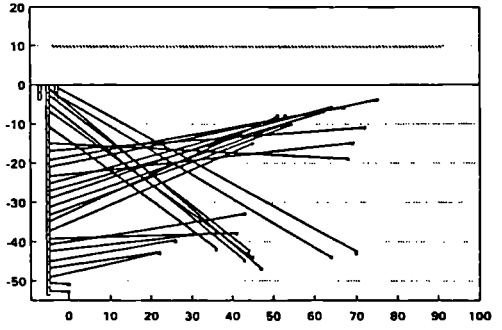


Figure 15.

Light blue series. Tilled land.

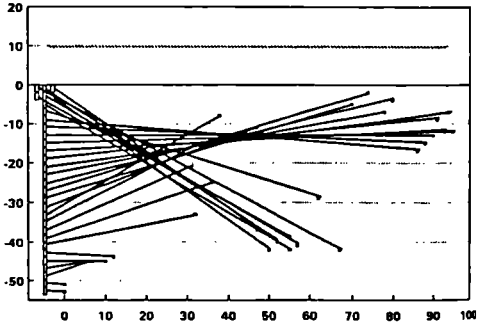


Figure 16.

The overall performance of the plough was very satisfactory considering the extremely wet conditions, for the tests both on the pasture land and on the very soft tilled land.

If the tests were to be repeated under ideal ploughing conditions, there is no doubt that the plough would perform even better.

Observations of the tests confirmed the results obtained from analysing the cylinder positions namely that
1. the topsoil fell into the bottom of the furrow and
2. that the deeper soil layer did not come to the surface.

A weakness of the plough at present is that the skim coulter is in rigid connection with the main ploughshare so that the slice cut by the skim coulter varies with the depth of main ploughshare. A separate skim coulter would permit selection of the thickness of the soil layer to be cut totally independent of the depth of the main plougshare.

Conclusion.

A full sized plough has been constructed which skims off the uppermost layer of soil (thickness about 5 cm) and buries it at a depth of about 50 cm without inverting the intermediate layer. The deeper less fertile soil is not brought to be surface as is the case with deep ploughing. The plough can be drawn by a normal farm tractor.

There is little doubt that the plough can be improved further by making the skim coulter separate from the main plougshare.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-B-327-B

Faculté des Sciences Agronomiques
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Passage des Déportés 2
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Title of the research contract:

Study of the transfer of accidentally released radionuclides in agricultural products with the aim of developing appropriate countermeasures

List of projects:

Study of the transfer of accidentally released radionuclides in agricultural products with the aim of developing appropriate countermeasures

Title of the project no.: BI6-B-327-B

Study of the transfer of accidentally released radionuclides in agricultural products with the aim of developing appropriate countermeasures.

Head(s) of project: Dr. R.Kirchmann & Prof. J.Deltour
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O.Burton

I. Objectives of the project:

The objective of the study is to define the countermeasures to be applied in order to improve the radiological quality of agricultural products after a major nuclear accident at a NPP (PWR type).
In order to reach this goal, one should consider two main phases :

1) identification of sensitive soils and agricultural products in the near and intermediate fields;
2) experimental research to define the parameters and the methods to reduce the transfers along the soil-plant-animal foodchain and to investigate the possible use of industrial processes to reduce the level of radioactivity in the end-products of the plant and animal production.

II. Objectives for the reporting period:

- General survey, around the relevant NPP, of the soils types and of agricultural production (plant, animal). This survey is performed in the framework of a contract with the Belgian "Service de Protection contre les Radiations Ionisantes".
- Identification of the "sensitive" soils and crops, in relation with the major radionuclides considered (Cs, Sr) (databank, field sampling and soil analysis).
- Experiment on selected soils to study the behaviour of the PWR simulated source-term (produced in Cadarache) in soil-to-plant and plant-to-animal transfers of these radionuclides (comparison of the aerosol and ionic forms).

III. Progress achieved:

A. SURVEY AND IDENTIFICATION OF SENSITIVE SOILS AND CROPS.

1. Methodology.

The identification of the sensitive soils and crops from the radioecological point of view lies on the confrontation on the one hand of the results of the soils and agricultural products surveys, and on the other hand of the soil-to-plant transfer factors.

Theses surveys were realized on the belgian territory, in a radius of 15 km around the NPP of Tihange, Chooz, Doel. For each of them, it was proceeded as follows.

a) The soils classification requires the knowledge of the parameters that influence the transfer of radionuclides from soils to plants .
The most important of them (texture, hydric conditions, presence of lime), as far as agricultural ecosystems are concerned, are taken into account in the Belgian Soil Map published by I.G.N.
Gathering the cartographic units, six soil types, based on these three parameters, were retained :
- sandy soils
- stony loamy soils
- loamy soils (loess)
- clayey soils
- calcareous stony loamy soils
- hydromorphic soils.

b) The question of the agricultural products was achieved according to two ways.
The first one is a statistic one. It was carried out by counting in each locality around the relevant sites, the areas devoted to specific crops (grass, cereals, root crops, fodder, ...) as well as farm animals (cows, pigs, fowls, sheeps). The original documents are provided by agricultural statistics of the Belgian I.N.S.
The second one was performed by using remote sensing (Landsat image) which allowed to localize the agricultural areas but also to compare these ones with the soil map produced at the same scale.

c) That stage allows the determination , in each point around the NPP, of the soil-plant association. Then the superposition with transfer factors was made, in function of the qualitative and quantitative results of the surveys.
The transfer factors values used were provided by the data bank worked out by the IUR's Working group on soil-to-plant transfer factors.

2. Results.

For the three nuclear sites, the sensitive soils and crops to Cs and Sr were identified. The most important result is that for Cs like for Sr, permanent pastures appear to be the critical pathway in the soil-plant foodchain, because of both their abundance in the country and their high transfer factor values.

Furthermore, in the case of Cs, special attention has to be drawn on potatoes cultivated on sandy and loamy soils.

3. Discusssion.

The objectives planned were reached and a practical knowledge of the environment of the NPP was gained; the radioecological sensitivity of the soil-to-plant transfer has been identified.

B. SOIL-TO-PLANT AND PLANT-TO-ANIMAL EXPERIMENTS.

1. Methodology.

In the framework of the RESSAC programme, a close cooperation was foreseen with CEN/SERE of Cadarache and CEN/SCK of Mol. An experiment on permanent pasture (critical pathway) was designed to estimate the transfer factors from soil (Mat) to grass and from grass to milk, for radionuclides of biological importance released in case of a nuclear accident (Cs,Sr). The transfer of the radionuclides released as aerosols (by a simulated PWR source-terme : Polyr furnace) was compared with that of ionic solutions of well known chemical form, in order to realize a comparative assessment of the bioavailability of these two different source-terms.

- Plant and soil material.

Surface horizons of soil (mat layer) with standing vegetation were collected at Dion (near Beauraing and Chooz) by the FSA (Faculté des Sciences Agronomiques) Gembloux. These soils, from an old permanent pasture, are representative of the region. They are stony loamy soils with a schistous charge (FAO classification : eutric cambisols).
12 mat layers (100 x 70 cm), about 10 cm thick, were placed in polyethylene trays (100 x 70 x 24 cm) laterally and underneath sustained by a wooden frame. The mat was laid on glass-wool and white sand to reach a total height of 20 cm. The plastic trays were provided with a funnel at their bottom to allow percolation of the water through the soil.

- Contamination of soils and standing vegetation.

** Aerosols.*

Four trays were transported to the CEA-Cadarache and exposed to the Polyr furnace on May 9th, 1990. Radioactive aerosols with physico-chemical characteristics similar to those of the particles released in the case of an accident in a nuclear reactor, were produced by burning in a crucible 10 stable elements (Te, Ce, I, Cd, Sn, In, Ag, Ni, Cr, Fe) associated to 37 MBq of ^{134}Cs and 111 MBq of ^{85}Sr under the form of carbonates, at a temperature of 2650 °C. The radioactive aerosols formed during the heating phase were deposited on aerial parts of the grass.
A total of 3734 ± 277 kBq ^{134}Cs and of 4158 ± 246 kBq ^{85}Sr were deposited on each soil container with standing vegetation. The fraction of the deposition retained by the vegetal cover amounted to 73 % , (for a DW of

about 120 g/tray), regardless of the radionuclide considered.

* Ionic solutions.

Four other trays were exposed at Mol to wet deposition on May, 17th 1990. Solutions of $^{134}CsCl$ and $^{85}SrCl_2$ were sprayed onto vegetation and soil of four trays. The last four trays were used to perform control trials on the grass before the contamination. The concentrations and volumes of the radioactive solutions were adapted to reach concentration on grass and deposition onto the soil comparable to those obtained by particles deposition.
A total of 9288 ± 1474 kBq ^{134}Cs and of 8877 ± 1252 kBq ^{85}Sr were deposited on each soil container with standing vegetation. The fraction of the deposition retained by the vegetal cover amounted to 68 % and 73 % (for a DW of about 220 g/tray), for Cs and Sr respectively.

- Soil-to-plant experiment (F.S.A.Gembloux).

After their contamination and removing of the contaminated grass, all trays were disposed in an open air plot at Gembloux in order to compare the bioavailability of both source-terms by studies on the radionuclide uptake by grass from the mat horizon (0-5 cm soil layer) and on migration.
Each tray was divided in four parts to allow grass growing between two cuts (± 1 month) and the execution of one sampling a week.
Until November, the newly growing vegetation was collected at regular intervals, and the corresponding soil cores taken up, divided and gathered into one cm layers.
All samples were dried, weighed and prepared for gamma spectrometry analysis.
Owing to the very dry summer, all trays were sprayed several times; twice, they were fertilized with nitrogen (NH_4NO_3 form).
The rare excess water drained into a collector connected to the bottom of the trays was also collected. CsCl and $SrCl_2$ were introduced as carriers in the collectors.

- Plant-to-animal experiment (CEN-SCK/Mol).

Grass exposed to the deposit of aerosols or ionic forms were collected on the next day, cut with scissors into pieces of 2 cm length and dried in open air. 480 g dry weight of grass contaminated with aerosols were obtained yielding concentrations of 21.6 and 18.7 kBq/g DW in ^{134}Cs and ^{85}Sr respectively. 880 g dry weight of grass contaminated by deposition of ionic forms were available, with concentrations of 27.7 and 23.9 kBq/g DW in Cs and Sr respectively.
This material was used to prepare 120 doses (4 g DW) from both type of contaminated grass. The grass material was poured into a sandwich to facilitate the administration to the animal. Each of them was counted to ensure a constant daily dosing.

Four lactating cows were divided in two groups. During 30 days, the cows from the first group received twice a day one sandwich with vegetation contaminated by radioactive aerosols, while the cows from the second group were given in a similar pattern grass contaminated with ionic forms. The daily diet of the cows in the two groups was similar (hay, concen-

trates and beet pulp).
After one month of chronic contamination, administration of radioactivity was stopped to follow the biological elimination of the absorbed activity during two more months.
Milk samples from the four cows were collected daily during the whole contamination period and the first 26 days of the decontamination period, and at least every two days afterwards.
Urine and faeces were collected daily during the first fortnight of the contamination and decontamination period and at larger intervals afterwards.
The various samples were prepared by Mol and sent to Gembloux for gamma spectrometry analysis.

2. Results.

- Soil-to-plant experiment (F.S.A.Gembloux).

The initial retention by vegetation of the radionuclides deposited was higher for ionic strontium than for ionic caesium, most probably due to stronger interaction with the electric charges on the leaves surfaces for a divalent element than for a monovalent element.
Retention of caesium and strontium as aerosol particles was similar, rather due to physical interactions between particles and leaves surfaces than to chemical interactions.
The relationship grass yield/interception factor is quite good for aerosol deposition but less obvious for ionic deposition.
Taking into account that the grass yield for ionic forms was almost twice (1.8) as high as for aerosol forms, it seems that the direct retention by the leaves for aerosols forms is more effective, for Cs like for Sr. This can be explained by the wet form of the ionic deposition.

For aerosol forms, the mat activities range from 767 to 1359 kBq/tray and from 873 to 1436 kBq/tray for Cs and Sr respectively.
For ionic forms, these activities ranged from 1235 to 3841 kBq/tray and 975 to 3170 kBq/tray for Cs and Sr respectively; a larger heterogeneity of the deposition was observed, owing to the fact that these trays were not contaminated together, i.e. in identical conditions.
Because of the large variability of the measurements performed on soil and grass samples (even into the same trays), the mat contamination was estimated on the basis of the average activities observed in each quarter of the trays.

The concentration ratios ($Bq.kg^{-1}$ plant DW/$Bq.kg^{-1}$ mat DW) were calculated and their evolution with time followed for both source-terms and radionuclides :

- Regardless of the source-term and the radionuclide considered, these concentration ratios were very high on the first cut (plant-base contamination), but decreased quickly over a period of about 20 days by effect of dilution by growth and weathering processes.
Beyond this period, one can consider that indirect contamination by the root system or translocation processes from the plant-base prevails on direct contamination, and that concentration ratios are really transfer factors (T.F.).
- For both source-terms, the uptake appears to be higher for Sr than Cs, by a factor ranging from 2 (early days after contamination) to 10 (last days of sampling), which means that the grass contamination decreases more quickly for Cs than Sr.
- The availability of both radionuclides as ionic forms becomes higher than as aerosol forms, as soon as indirect transfer is predominant, i.e. about 30 days after contamination. Beyond this period indeed, the differences between the whole sets of aerosol and ionic T.F. values are very highly significant for ^{134}Cs like for ^{85}Sr even if some heterogeneity can appear between samples collected at the same date (fig. 1, 2).
The time evolution of the ratios (ionic T.F./aerosol T.F.) was calculated. The average value of these ratios amounted to 3.3 ± 1.2 and 2.6 ± 1.0 for Cs and Sr respectively, confirming a smaller availability for grass of both radionuclides as aerosols particles.

No migration was noticed in the soil during the observation period. The activities detected below the mat layer (0-5cm) always remained < 2% of the total soil activity, but were higher for Sr than Cs under both forms.

From May to November, the percolated water was collected only six times, in spite of the water suppplies by spraying; from June 12th to September 6th, no water was found in the collectors. The total activities of the collected water were low in the early days of the experiment, but increased gradually at each sampling, regardless of the source-term and the radionuclide considered. For all trays, the ratio "Total percolated activity over the period of observation/total soil deposition" was calculated. For aerosols forms, this ratio amounted to $0.2\ 10^{-4} \pm 0.1\ 10^{-4}$ and $17.9\ 10^{-4} \pm 3.8\ 10^{-4}$ for Cs and Sr respectively.
For ionic forms, this ratio amounted to $0.4\ 10^{-4} \pm 0.3\ 10^{-4}$ and $17.5\ 10^{-4} \pm 8.2\ 10^{-4}$ for Cs and Sr respectively.
Nevertheless, these results show a much higher mobility of Sr (almost 2 orders of magnitude) but no significant difference between the two source-terms.

One can also conclude that the loss of activity through the trays stays very low over the experimental period.

- Plant-to-animal experiment (CEN-SCK Mol).

The concentrations of both radionuclides in milk increased rapidly after the beginning of the contamination period and reached a plateau after about 10 days. The transfer factors to milk were estimated on the samples collected from day 27 to day 30 - corresponding to larger samples (2 l in Marinelli beakers instead of 225 ml in 250 ml vials). The values obtained for the transfer of radiocaesium were similar for both source terms, respectively 0.0064 ± 0.0012 and 0.0063 ± 0.0002 d/l for ionic and aerosol forms, and are in agreement with the Fm reported in the litterature. The values calculated for radiostrontium amounted to 0.00040 ± 0.00002 and 0.00027 ± 0.00005 d/l for ionic and aerosol forms respectively and are significantly different.
For both source terms and both radionuclides, the evolution of the radiocontamination levels in milk during the elimination phase indicates the existence of at least two compartments. The determination of the parameters characteristic of these compartments was performed using a non-linear least square method. For radiocaesium, the estimation of the characteristic parameters are given in table 1.
The biological half-times estimated for the two compartments are respectively similar regardless of the chemical form administered suggesting, as expected, that the rate of secretion of the absorbed fraction is not dependant on the source term.

An accurate estimation of the parameters characteristic of the two compartments describing the evolution of the secretion of radiostrontium into milk during the decontamination phase has not been possible due to the low contamination levels measured in the samples and the large variability associated to those results. Meanwhile, the excretion patterns of ^{85}Sr from both source terms are comparable and present a first rapid decrease with a biological half-time comprised between 1 and 2 d followed by a period of time during which the contamination levels in milk remain rather constant.

The excretion patterns of both radionuclides during the decontamination period with urine and faeces also indicate the existence of two compartments. The parameters of the two exponential curves describing the two compartments of urinary and fecal excretions are given in tables 2 and 3 for radiocaesium and radiostrontium respectively.

			IONIC Cs	AEROSOL Cs
1st Comp.	$T_{1/2}$	(d)	2.0 ± 4 %	2.1 ± 5 %
	C_o	(10^{-3} d/l)	5.3 ± 2 %	5.6 ± 2 %
2nd Comp.	$T_{1/2}$	(d)	19.7 ± 6 %	24.9 ± 9 %
	C_o	(10^{-3} d/l)	1.1 ± 6 %	0.9 ± 8 %

Table 1. Parameters of the compartmental analysis for radiocaesium secretion in milk.

			IONIC Cs	AEROSOL Cs
URINE				
1st Comp.	$T_{1/2}$	(d)	1.5 ± 13 %	1.6 ± 10 %
	C_o	(10^{-3} d/l)	14.7 ± 8 %	15.9 ± 6 %
2nd Comp.	$T_{1/2}$	(d)	16.8 ± 16 %	20.0 ± 16 %
	C_o	(10^{-3} d/l)	3.9 ± 12 %	3.3 ± 12 %
FAECES				
1st Comp.	$T_{1/2}$	(d)	1.4 ± 5 %	1.0 ± 4 %
	C_o	(10^{-3} d/l)	82.2 ± 4 %	143.8 ± 3 %
2nd Comp.	$T_{1/2}$	(d)	18.6 ± 8 %	17.4 ± 8 %
	C_o	(10^{-3} d/l)	11.7 ± 7 %	11.8 ± 6 %

Table 2. Parameters of the compartmental analysis for radiocaesium excretion with urine and faeces.

			IONIC Sr	AEROSOL Sr
URINE				
1st Comp.	$T_{1/2}$	(d)	1.1 ± 18 %	1.5 ± 43 %
	C_o	(10^{-3} d/l)	2.0 ± 14 %	1.0 ± 28 %
2nd Comp.	$T_{1/2}$	(d)	32.0 ± 34 %	38.1 ± 59 %
	C_o	(10^{-3} d/l)	0.4 ± 15 %	0.3 ± 25 %
FAECES				
1st Comp.	$T_{1/2}$	(d)	1.3 ± 4 %	0.8 ± 2 %
	C_o	(10^{-3} d/l)	176.4 ± 4 %	330.8 ± 2 %
2nd Comp.	$T_{1/2}$	(d)	62.4 ±137 %	43.9 ± 33 %
	C_o	(10^{-3} d/l)	1.0 ± 56 %	1.7 ± 16 %

Table 3. Parameters of the compartmental analysis for radiostrontium excretion with urine and faeces.

3.Discussion.

Except for the contamination stage, the results obtained confirmed the higher mobility of strontium than caesium, even under aerosol form.
Direct retention by the grass leaves is quite effective for radiocaesium as well as for radiostrontium released as aerosol form, meaning that some physico-chemical interactions between particles and leave surfaces could occur.
As to indirect transfer, the statistical comparison of the behaviour of caesium and strontium under both source-terms shows a permanent superior availability for grass of these two radionuclides under ionic forms.
The time evolution of the ratio ionic T.F./aerosol T.F. confirms this fact and indicates that radiocaesium and radiostrontium released as aerosols would be about three times less available for grass than under ionic form.

The calculated transfer factors to milk (Fm) show that the radiocaesium under both (ionic and aerosol) physico-chemical forms is equally available for the secretion into milk. Moreover, similar transfer factors to blood were estimated (0.0014 and 0.0013 d/l), that indicates that both forms are absorbed to the same extent through the gastro-intestinal barrier.
Strontium as aerosol particles appears to be 30 % less available than ionic strontium. A similar respective behaviour in milk, urine and faeces, of both radionuclides regardless of their origin during the decontamination period suggests that the fraction absorbed joins pools specific for these nuclides independently of the initial physico-chemical forms that were investigated in this study. This assumption will probably be true for Cs from other source terms but could not for strontium which is likely to form stable organic complexes susceptible to cross the intestinal wall as such.

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Figure 1.

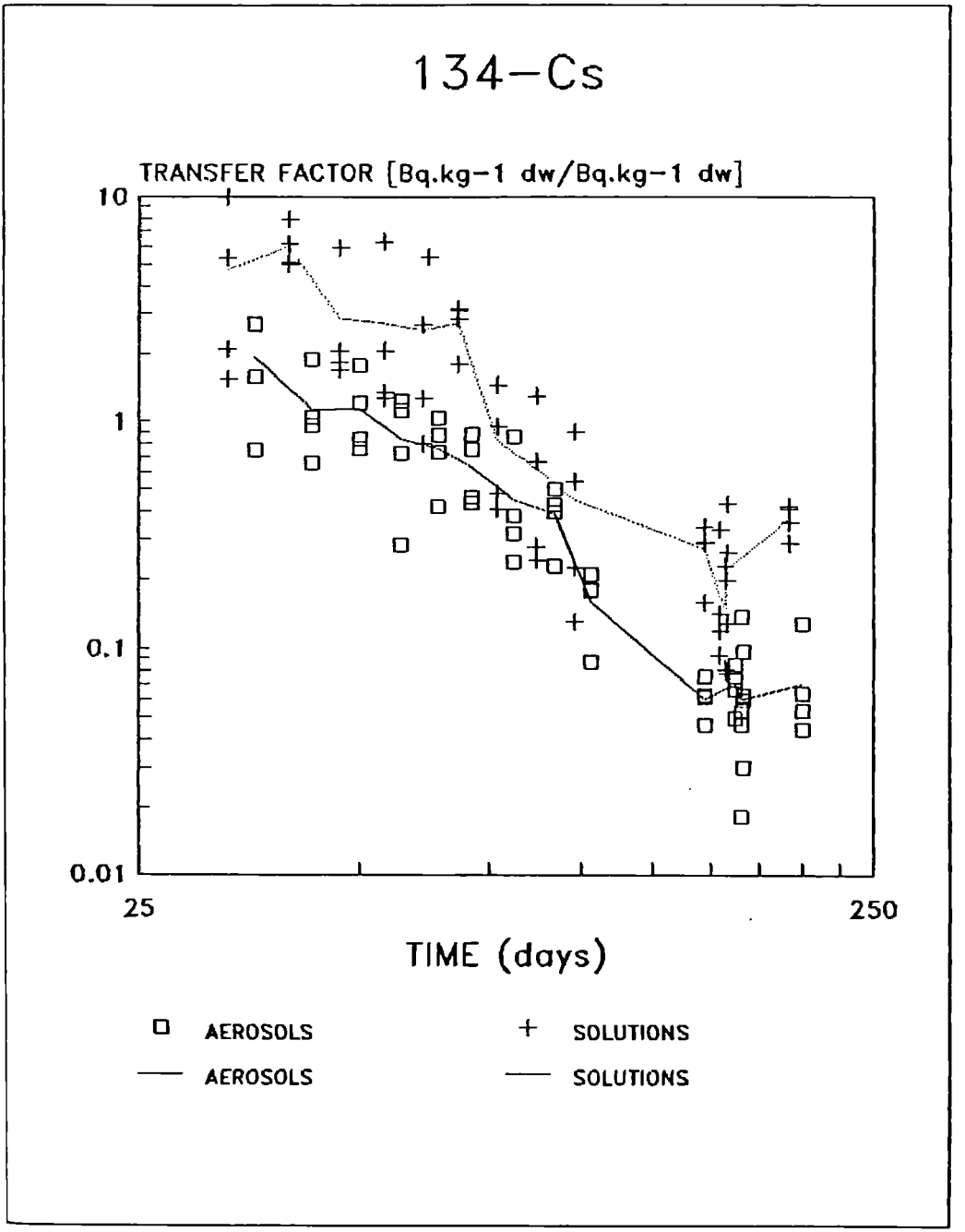
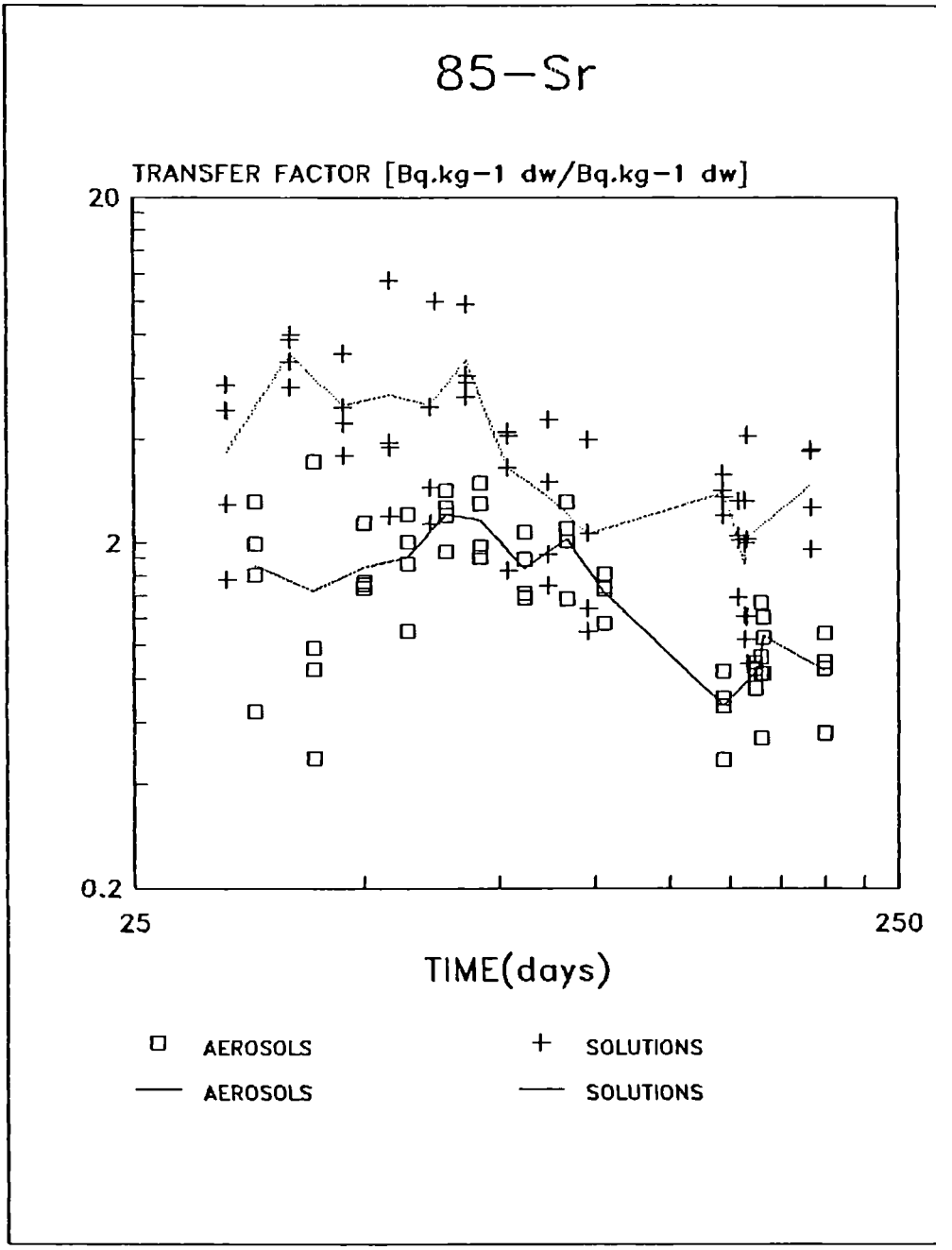

134-Cs
TRANSFER FACTOR [Bq.kg-1 dw/Bq.kg-1 dw]
10
1
0.1
0.01
25
250
TIME (days)
AEROSOLS
SOLUTIONS
AEROSOLS
SOLUTIONS

Figure 2.


85-Sr
TRANSFER FACTOR [Bq.kg-1 dw/Bq.kg-1 dw]
20
2
0.2
25
250
TIME(days)
AEROSOLS
SOLUTIONS
AEROSOLS
SOLUTIONS

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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V. Publications:

- Rapport d'avancement S.P.R.I.-C.C.E. (01/01-31/12/1989).
- Progress report for the period 01-01-90/30-06-90.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-B-329-I

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Title of the research contract:

Chemical treatments to reduce the transfer of caesium radioisotopes to the human foodchain after a serious nuclear accident

List of projects:

Chemical treatments to reduce the transfer of caesium radioisotopes to the human foodchain after a serious nuclear accident

Title of the project no.:

Chemical treatments to reduce the transfer of cesium radioisotopes to the human foodchain after a serious nuclear accident

Head(s) of project:

Prof. S. Silva

Scientific staff:

S. Silva, V. Cappa, F. Carini, P. Bani, M. Montruccoli, P. Vazhapilly

I. Objectives of the project:

The aim of the project is to produce a reduction of the transfer factors of radiocesium in plants, milk and meat owing to a deposition of radioactive products following a severe nuclear accident.

The project includes two distinct working departments (A and B).

The first (A department) studies the possibility to reduce the translocation of radiocesium from the aereal parts to the edible products of crops.

The second (B department) studies the possibility to reduce the radiocesium in the milk and in the meat as a consequence of the feeding with contaminated fodder.

II. Objectives for the reporting period:

A Department: After the fixing of the salient points of the methods, particularly of those concerning the contamination of plants in field by nebulization of radiocesium, the possibility has been investigated to reduce the radiocesium concentration in wheat and barley grain and in tomato fruits through foliar fertilizations with potassic salts at various concentrations.

B Department: With the researches carried out on sheep and dairy cows we wanted to evaluate the amount of radiocesium (Cs-134 and Cs-137) that, administered to the animals as contaminated feedstuffs, was excreted in milk, feces and urine or accumulated in the organs, as well as the effect of feeding Ammonium-Iron (III) Hexacyanoferrate (II) (AFCF) on bentonite on their contamination level.

III. Progress achieved:

A Department:

1. Methodology

Two cereal crops, wheat and barley, and a horticultural crop, tomato, were cultivated in open field in 1989 and 1990. At different growing stages they were contaminated through leaves with Cs-134 with the aim of simulating a contamination from radioactive fallout. 2 ml of a watery solution of Cs-134 chloride carrier free were nebulized directly into plexiglass boxes based on the ground and containing the crop to be treated.

The aerosol particles were obtained by means of a medical aerosol nebulizer and had a size of about 1 um. The attempt to produce dry aerosol by passage of this one through a column containing silica gel has failed owing to the high percentage of moisture inside the box, due to the plant evapotranspiration. The boxes dimension varied according to the growing stage and the kind of plant.

The activity of Cs-134 nebulized for each barley and wheat plot varied from 115 to 200 kBq.m^{-2}, whereas for the tomato varied from 415 to 500 kBq.m^{-2}.

24 hours after the nebulization, a solution of KCl was sprayed over the plants at increasing concentrations of potassium: 0-100-300 mg of K.l^{-1} in 1989 and 0-300-900-1800 mg of K.l^{-1} in 1990. Such rates have been increased in the second research year to obtain a more significant reduction of radiocesium. Each treatment was effected on three plots.

The crops interception capacity was determined at different growing stages, by nebulizing Cs-134 on a few plots, mowing them a few hours later and measuring their radioactivity.

The products were harvested at ripening and submitted to radiometric analysis through GeHp semiconductor for Cs-134 determination.

2. Results

Wheat and barley

In order to assess whether potassic foliar fertilizations can produce a reduction in the rate of radiocesium translocation from the leaves to the grain, barley and wheat were contaminated with Cs-134 and later sprinkled with KCl at various potassium concentrations: 0, 100, 300 mg of K.l^{-1} in 1989 and 0, 300, 900 mg of K.l^{-1} in 1990. At the rate of 900 mg of K.l^{-1} the treatment was effected also with addition of a surfactant agent at the rate of 0.5 per thousand. The corresponding translocation factors expressed as a percentage ratio between the Bq.m^{-2} present in the grain and the Bq.m^{-2} intercepted by the plant are reported in table 1.

From the analysis of the data it appears that potassic foliar fertilizations

do not produce any decrease in the Cs-134 concentration in the grain. Besides the treatment effected by adding a surfactant agent to the KCl solution, while is uninfluential for the barley, causes an increase of the translocation factor in the wheat, as if it would increase the penetration ability of Cs-134. Translocation factors for the wheat vary between $0.2 \cdot 10^{-2}$ and $0.5 \cdot 10^{-2}$, whereas for the barley they vary between $1.0 \cdot 10^{-2}$ and $1.8 \cdot 10^{-2}$. The cause of the higher translocation factor in the barley in comparison with the wheat is due to the fact that the barley grain is covered by the glumes that have an high radioactive concentration, whereas the wheat grain is naked.

It is known that the radioactivity intercepted by the plant and translocated to its edible parts depends on its biomass, particularly on the foliar surface exposed to the fallout, so therefore it is affected by the growing stage of the plant at the time of contamination. This attitude was analysed as regards wheat growing.

The wheat was contaminated with Cs-134 at different growing stages in order to assess the activity variation intercepted by the plant and translocated to the grain. The corresponding Cs-134 average activities expressed in $kBq.m^{-2}$ or, for the grain, in $kBq.kg^{-1}$ of dry weight, are shown in table 2. In the same table are also shown the percentages of activity intercepted by the plant as compared with the nebulized one. It can be noticed how this percentage drops from the stem elongation stage (34.4) to that of lacteal and physiological maturity (13.1 - 14.3). The close connection between the interception capacity of the plant and its growing stage was checked by calculating a linear regression, which turned out to be 5% significant.

Even though the activity intercepted by the plant is rather elevated (25 - 50 $kBq.m^{-2}$), the one translocated to the grain is very low: it varies, in fact, from 0.06 to 0.3 $kBq.m^{-2}$ and, expressed as percentage of the intercepted activity, it drops of about three times when the period of the contamination gets near the maturity.

Finally, with a wiew to checking whether the different plant fractions reacted to radiocesium in the same way, the percentage translocation factors

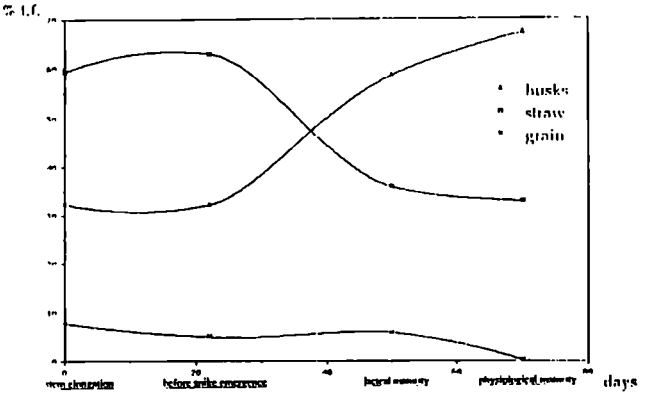
were calculated in the grain, straw and husks of wheat contaminated with Cs-134 at different growing stages (graph 1). It is interesting to notice how the factor increases for the husks, whereas in the grain and straw the factors decrease as the radiocontamination treatment gets near to the maturity stage. Thus the particular structure of this fraction turns out to be as a biological barrier to the radiocesium translocation from the leaves to the grain.

Table 1. Wheat and barley. Average values of the Cs134 translocation factors into grain, expressed in $\frac{\text{Bq of the grain} \cdot m^{-2}}{\text{Bq of the plant} \cdot m^{-2}} \cdot 10^2$, obtained in 1989 and 1990 by administering potassium at various concentrations

Treatment Year, plant and its growing stage	Cs134 + K 0	Cs134 + K 100	Cs134 + K 300	Cs134 + K 900	Cs134 + K 900 + surfaccant agent
1989 Wheat at the ripening stage	0.22±0.04	0.30±0.09	0.46±0.17	-	-
1990 Wheat before spike emergence	0.45±0.06	-	0.49±0.08	0.40±0.07	2.98±0.47
1990 Barley at end of flowering	1.17±0.30	-	-	1.78±0.20	1.01±0.31

Table 2. Wheat. Average activity of nebulized Cs-134, intercepted by the plant at different growing stages and translocated to the grain, expressed in $kBq.m^{-2}$ or in $kBq.kg^{-1}$ of dry weight

Cs-134 / Growing stage	Nebulized activity $kBq.m^{-2}$ A	Intercepted activity $kBq.m^{-2}$ (% of A) B	Translocated to the grain activity $kBq.m^{-2}$ (% of B)	$kBq.kg^{-1}$ d.w.
Stem elongation	114.8±0.8	39.5±13.0 (34.4)	0.29± 0.09 (0.71)	0.60±0.19
Before spike emergence	194.2±5.7	-	0.30± 0.03 (0.44)	0.63±0.05
Flowering	193.5±5.7	47.2± 5.5 (24.4)	-	-
Lacteal maturity	194.3±15.1	25.4± 3.8 (13.1)	-	-
Physiological maturity	200.0±8.2	28.2 (14.3)	0.06±0.02 (0.23)	0.13±0.07



Graph 1 - Wheat. Per cent translocation factors into grain, straw and husks of crops contaminated by Cs-134 at different growing stages

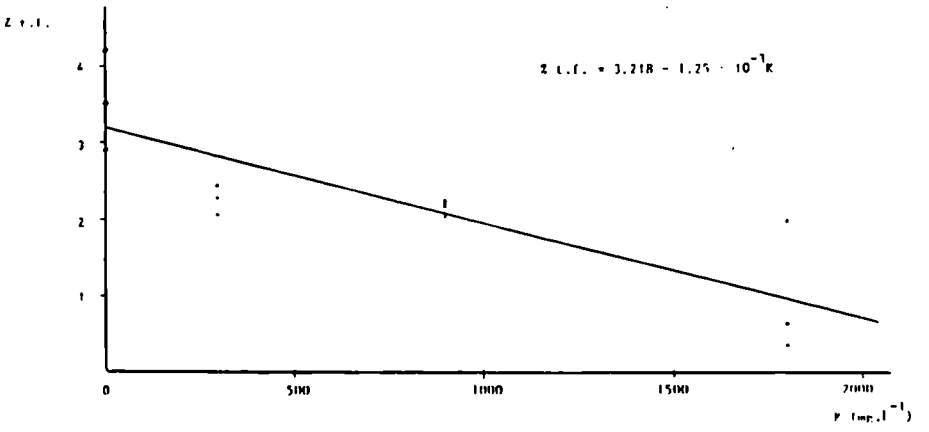
Tomato

The tomato crops were contaminated at turn of colour and at late flowering in 1989, and only at turn of colour in 1990, since the translocation factors obtained in the first year didn't present significant differences between the two growing stages. The treatments with potassic salts in order to decrease the radioactivity translocated to the fruits were effected at the rate of 0, 100, 300 mg of $K \cdot l^{-1}$ in 1989 and of 0, 300, 900, 1800 mg of $K \cdot l^{-1}$ in 1990. At the dose of 900 $mg \cdot l^{-1}$, KCl was also sprayed in association with the surfactant agent. The corresponding translocation factors expressed in: (Bq present in the fruits$\cdot m^{-2}$/Bq intercepted by the plant$\cdot m^{-2}$)$\cdot 10^{2}$ are shown in table 3. The data obtained in the first year don't present any significant difference among the plots treated with potassium at the rates of 0, 100, 300 $mg \cdot l^{-1}$. Only in the second year, with the increasing of potassium doses - 900 and 1800 $mg \cdot l^{-1}$ - a significant reduction of the translocation factor of about 3.5 times is achieved. The surfactant agent, also in this case, doesn't produce any significant reduction of the translocation factor. The test of multiple comparisons carried out by Tukey's procedure shows a significant 5% difference between translocation factors obtained with a 1800 $mg \cdot l^{-1}$ potassium solution and the ones obtained with 0 mg of $K \cdot l^{-1}$. Also the reverse linear regression calculated between the per-

Table 3. Tomato. Average values of the Cs434-translocation factors into tomato fruits, expressed in $\frac{\text{Bq of the fruits} \cdot m^{-2}}{\text{Bq of the plant} \cdot m^{-2}} \cdot 10^{2}$ obtained in 1989 and 1990 by administering potassium at various concentrations

Treatment / Year and growing stage	Cs434 + K 0	Cs434 + K 100	Cs-134 + K 300	Cs434 + K 900	Cs434 + K 900 + surfactant agent	Cs434 + K 1800
1989 Turn of colour	1.94± 0.14	3.22± 0.58	3.36± 0.25	-	-	-
1989 Late flowering	2.60± 0.04	2.54± 0.71	2.72± 0.61	-	-	-
1990 Turn of colour	3.52± 0.38	-	2.24± 0.20	2.16± 0.05	1.84±0.62	0.99±0.49

centage translocation factors and the potassium levels is 1% significant and the corresponding line is shown in graph 2. The equation thus calculated describes the quantitative ratio between the percentage of radioactivity translocated into the tomato fruits and the concentration of potassium salts sprinkled over the plants. Therefore it can be employed as a predictive equation of the amount of potassium to be employed to drop the translocation factor down to a prearranged value. This anticipatory model needs an experimental check in a higher activity range (around and above 600 $Bq \cdot kg^{-1}$) and a research for further values in the range from 900 to 1800 mg of $K \cdot l^{-1}$.



Graph 2 - Tomato. Regression of the per cent translocation factor of Cs-134 into fruits, on the amount of potassium administered through leaves, expressed in $mg.l^{-1}$

In table 4 are reported the average activities of nebulized Cs-134, intercepted by the plant and translocated to the fruits. The activity nebulized per surface unit is about twice as big as the one employed for the cereal crops, as the tomato interception power (1.5-2.9%) is considerably lower as compared to the ones of wheat and barley (13-35%). Instead the activity translocated to the fruits is as high as 2.3-3% and it is on the average seven times as high as the one translocated to the grain (0.23-0.73%).

Table 4. Tomato. Average activity of nebulized Cs-134, intercepted by the plant and translocated to the fruits in 1989 and 1990, expressed in $kBq \cdot m^{-2}$ or in $kBq \cdot kg^{-1}$ of fresh weight

Cs-134 / Year	Nebulized activity $kBq.m^{-2}$ A	Intercepted activity $kBq.m^{-2}$ (% of A) B	Translocated to the fruits activity $kBq.m^{-2}$ (% of B)	$kBq.kg^{-1}$ f.w.
1989	500.9±2.1	14.6±0.06 (2.9)	0.34±0.01 (2.3)	0.11±0.01
1990	414.9±4.1	6.1±1.7 (1.5)	0.18±0.05(3.0)	0.05±0.02

3. Discussion

Potassium foliar fertilizations can pull down radioactivity significantly in the edible parts of tomato plants, but not of cereal ones. The result is probably due to the kind of these crops: in fact tomato, differently from wheat and barley, is a plant rich in potassium, which, if it is suitably administered, can compete with cesium owing to their chemical affinity. Consequently, it is possible to draw predictive curves for the pulling down of radiocesium contamination in the event of a serious nuclear accident. As regards cereals, they will be treated during the next year by employing ammonium salts, instead of potassium salts, for radioactivity pulling down. As regards the interception capacity and the translocation factor for the wheat, they are affected by the growing stage of the plant at the time of the contamination.

Moreover the employing of the surfactant agent has the only effect to increase or leave unchanged the translocation of radiocesium to the edible parts of the plants.

METHODOLOGY

Sheep trial (1st year)

The research was carried out using contaminated hay from radiocaesium (14207 Bq/kg DM; 3077 Bq from Cs-134 and 11130 Bq from Cs-137) in consequence of the Chernobyl accident. This hay was fed to four lambs (already weaned, mean body weight of 28 kg) at the rate of 55 g DM/$kg^{0.75}$ l.w./d for 55 days. From the 28th day onwards, the four animals were kept in individual metabolic cages and, after a week of adaptation, two lambs were fed 0.32 g/head/d of Ammonium Iron (III) Hexacyanoferrate (II), hereafter indicated as AFCF. This product was equally divided into two portions and was fed in two meals a day as the hay. Water was always available. The quantitative and individual collection of faeces and urine was started three days before the beginning of AFCF administration and went on for three weeks. On samples of urine and of dried faeces radioactivity was measured. Another lamb from the same flock was fed a very low contaminated hay (133 Bq/kg DM) for the whole experimental period. At the end of the trial all animals were slaughtered and the radiocontamination in some organs and tissues was measured after freeze-drying.

Sheep trial (2nd year)

For the trial 10 adult female Sarda sheep of 42±8.7 kg average weight were fed with radiocaesium "uncontaminated" hay pellets supplemented with concentrates containing minerals and vitamins. After two weeks of "uncontaminated" hay pellets feeding the animals were subdivided into 5 groups of two animals each and four groups received radiocaesium contaminated hay pellets from Chernobyl accident at the rate of 50 g DM/$kg^{0.75}$ l.w. in two equal meals administered in the morning and in the evening for 20 days. The total radioactivity of hay pellets was 5077 Bq/kg DM (661 and 4416 Bq from Cs-134 and Cs-137 respectively). Besides the hay pellets, one group received 200 g/head/d of pelleted concentrate feed containing 1 g of AFCF in two equal meals as hay pellets; a second group received the same concentrate at the same rate but in one meal during the morning feeding; a third group received 250 g/head/d of pelleted concentrate containing 50 g bentonite (cation exchange capacity:79 mEq/100 g) administered in two equal meals as the first group; the fourth group received a normal concentrate without AFCF or bentonite at the rate of 200 g/head/d in two meals. The fifth group continued to receive the "uncontaminated" feeds as before. All animals of the first four groups were put in individual metabolic cages 4 days before the start of contaminated hay pellets feeding for total collection of urine and faeces.

After 20 days of contaminated hay pellets feeding all animals were slaughtered and some organs and tissues were collected. On samples of urine, faeces, organs and tissues the radioactivity was measured as in the first trial.

Dairy cow trial (2nd year)

Eight milking Friesian cows of our Institute were subdivided into 4 groups of two animals each (one of high and the other of low milk production). All animals received the same type of ration consisting corn silage, hay, pelleted concentrates but supplied according to milk production. All 8 animals received 40 g of soybean meal artificially contaminated with Cs-134 (provided by the Institute de Protection et de Surete nucleaire, Cadarache) in two equal meals administered in the morning and in the evening for 14 days. The total radioactivity was 4957 Bq/d. Besides the contaminated soybean meal, group 1 received 5 g/head/d of AFCF in two equal meals as soybean meal; group 2 received the same amount of AFCF but in one meal in the morning; group 3 received 300 g/head/d of bentonite in two equal meals as the group 1, while the group 4 received neither AFCF nor bentonite. Daily doses of contaminated soybean meal, AFCF and bentonite were mixed respectively to 360, 200 and 1000 g of normal concentrate and pelleted which substituted equal amount of normal concentrate in the ration of the animals in the respective groups.

Total milk production for each animal was measured daily and milk samples were collected before and during the feeding of contaminated soybean meal for measuring the radioactivity.

RESULTS

Sheep trial (1st year)

No difference was noted between Cs-134 and Cs-137 for excretion or retention. Therefore data of total radioactivity from both isotopes are only given. Radiocaesium excretion for the whole period of the trial is in Fig. 1. Faecal excretion increased markedly, while urinary excretion decreased in the AFCF group with respect to the control group from the 1st day of AFCF administration. In the last week of the trial mean radiocaesium excretion was 96.05% of intake in the AFCF group and 78.49% in the control group; urinary output was 1.61% and 11.90% respectively. Consequently the retention was 2.34 and 9.61% of intake in respective groups.

The figures of radiocontamination of organs and tissues are shown in Tab. 1. Highest concentration is found in the kidnies of control group

Fig.1 - Excretion of Cs 134+137

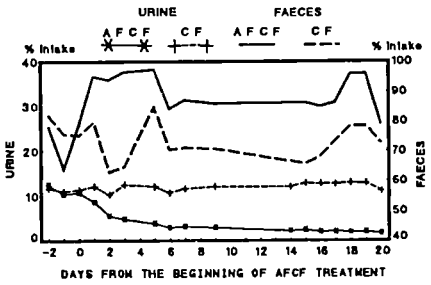


Table 1 - Radioactivity (Bq/kg DM) from Cs 134+137 in some organs and tissues at the end of the 2nd sheep trial:mean of two animals and in parenthesis difference with the mean

TREATMENT	CONTAMINATED HAY + A F C F	CONTAMINATED HAY	UNCONTAMINATED HAY
THIGH MUSCLES	1772 +/- 625	12440 +/- 682	271
LOIN MUSCLES	1577 +/- 444	10940 +/- 365	224
SHOULDER MUSCLES	1447 +/- 420	11482 +/- 37	250
HEART	337 +/- 114	7112 +/- 680	143
LIVER	288 +/- 73	6635 +/- 574	96
KIDNEY	818 +/- 154	17290 +/- 1173	406
TESTICLES	622 +/- 95	12917 +/- 738	306
SMALL INTESTINE	195 +/- 8	756 +/- 186	28
LARGE INTESTINE	357 +/- 45	1699 +/- 221	87

followed by testicles, muscles, heart, liver, large intestine and small intestine. In the AFCF group the order is changed; highest concentration is in the muscles follwed by kidnies, testicles and other organs. In the animal received the "uncontaminated" hay the concentration of radioactivity follows the same order that found in the control group, but at a very low level. Although both groups received similar amount of radiocaesium in all organs and tissues the level of radiocaesium was evidently much lower in AFCF group with respect to control group. Transfer coefficient of radiocaesium to fresh meat was $2.56*10^{-1}$ for control group (contaminated hay only) and $3.4*10^{-2}$ for AFCF group. In the animal receiving "uncontaminated" hay transfer coefficient was very high ($4.2*10^{-1}$).

Sheep trial (2nd year)

Excretion of radiocaesium (134 + 137) in the faeces and urine during the whole period of the trial is shown in Fig. 2. Mean excretion for the whole period was highest in the group receiving AFCF twice a day (88% of intake) followed by AFCF once a day and bentonite twice a day. The control group (contaminated hay only) had the lowest excretion (73%). There was no big difference between the three treated groups.

For the urinary excretion of radiocaesium just the contrary of the faecal excretion was verified. The urinary excretion was 0.45, 0.52, 2.56 and 8.90% of intake for AFCF 2/d, AFCF 1/d, bentonite 2/d and control group respectively. The control group had highest excretion and the bentonite group had evidently higher excretion rate with respect to AFCF group.

Level of radioactivity in the organs and tissues are reported in the Tab.2. Among groups receiving contaminated hay the lowest level of contamination was found in all organs and tissues of AFCF 2/d group. AFCF 1/d had higher values than AFCF 2/d, but lower than bentonite group. The control group had the highest values in all organs and tissues. Mean radioactivity in the tissues and organs were significantly negatively correlated ($r=0.996^{***}$) with faecal excretion and positively ($r=0.997^{***}$) with urinary excretion of radiocaesium. The presence of radiocaesium in the group with "uncontaminated" hay was very low. In the hay of this group neither of the isotopes could be detected.

Fig. 2 : Radioactivity from Cs 134+137 in faeces and urine of sheep fed contaminated hay

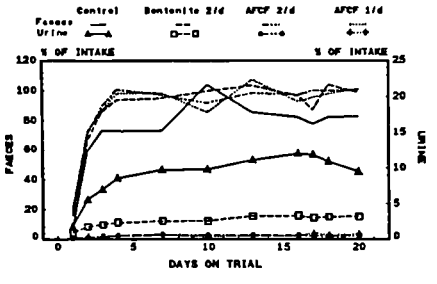


Table - 2: Radioactivity (Bq/kg DM) from Cs 134+137 in some organs and tissues at the end of 2nd sheep trial: mean of two animals and in parenthesis the difference with the mean

GROUP	CONTAMIN. HAY ONLY	CONT.HAY BENT.2/d	CONT.HAY AFCF 2/d	CONT.HAY AFCF 1/d	UNCONTAM. HAY ONLY
THIGH MUSCLES	2042 (381)	378 (3)	64 (6)	110 (0)	9 (1)
LOIN MUSCLES	1758 (258)	325 (3)	52 (8)	91 (0)	10 (3)
HEART	2070 (440)	504 (37)	49 (4)	120 (18)	5 (5)
LIVER	1537 (222)	365 (18)	35 (4)	78 (5)	7 (7)
KIDNIES	4268 (596)	1024 (91)	124 (3)	278 (33)	12 (4)
SMALL INTESTINE	1368 (43)	319 (16)	35 (1)	101 (20)	1 (1)
LARGE INTESTINE	1215 (410)	314 (73)	61 (19)	111 (21)	7 (7)

Mean radioactivity in the fresh meat was 14, 25, 89 and 443 Bq/kg for AFCF 2/d, AFCF 1/d, bentonite 2/d and control group respectively and the transfer coefficients for the respective groups were $3.5*10^{-3}$, $5.9*10^{-3}$, $2.09*10^{-2}$, and $1.186*^{-1}$ of intake.

Dairy cow trial (2nd year)

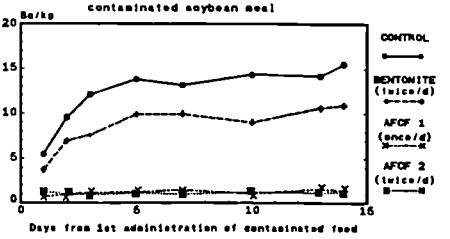
The results of dairy cows trial are in Tab.3 and in Fig.3. In the control group the radioactivity from Cs-134 sharply increased the day after the administration of the contaminated soybean meal and was still rising two weeks later. The maximum radioactivity was reached at a level of 18.1 Bq/kg, with a consequent transfer coefficient (TC) of $3.12*10^{-3}$.

Tab. 3 : Mean milk production and radioactivity parameters in dairy cows trial.

Treatments		Control	Bentonite	AFCF 1/d	AFCF 2/d
Daily intake	(Bq/d)	- - - - - - - - -	4957	- - - - - - - - -	
Milk yield	(kg/d)	21.27 +/- 5.22	22.02 +/- 2.29	23.09 +/- 8.06	20.91 +/- 14.04
134-Cs output in milk (*)	(% intake)	6.80	4.80	0.65	0.54
Transfer coefficient (*)		3.1E-03	2.2E-03	2.8E-04	2.4E-04

(*) Mean of the two animals of each group at the 14th day of the trial

Fig. 3 : ^{134}Cs activity in the milk of cows fed contaminated soybean meal



The bentonite treatment proved to be quite efficient; the level of Cs-134 activity in the milk of this group of animals was as high as two thirds of the control group's activity during the whole trial. The final level (14th day) was 10.92 Bq/ kg, and the TC $2.2*10^{-3}$. On the contrary no major variation were seen in the four cows fed the AFCF in addition to the contaminated feed. Nevertheless, in the animals receiving the AFCF once a day there was a slight increase in the milk radioactivity during the fortnight of the trial (from 0.66 to 1.38 Bq/kg), while in AfCF twice fed group the level remained more constant from the first day of the trial to the last day (1.29 and 1.23 Bq/kg respectively). The transfer coefficient at the end of the trial was $2.8*10^{-4}$ and $2.47*10^{-4}$ respectively for the two groups.

DISCUSSION

During the two years of research, trials on sheep and dairy cows were carried out in order to check the effect of the oral administration of two additives (AFCF and bentonite) on the transfer of radiocaesium from feeds to meat or milk.

The AFCF showed the highest blocking capacity, reducing the absorption of caesium in thedigestive tract.

When compared to the control group, the mean reduction achieved was over 30 times with AFCF 2/d and about 5 times with bentonite 2/d for the meat, 11 and 1.5 times respectively for the milk for the two additives (results of 2nd year trials). In addition, when AFCF was administered twice a day instead of once a day, its efficacy was almost doubled.

The levels of radiocaesium in fresh meat were over the limits established by the EEC in the control group (contaminated hay only) of the first trial when lambs had ingested 780 Bq/kg$^{0.75}$ l.w./d for two months, but below these limits in the 2nd trial of ewes fed 250 Bq/kg$^{0.75}$ for three weeks. Nevertheless the radioactivity in fresh kidnies was over 600 Bq/kg also in the 2nd trial. But in all trials meat and all other organs (kidnies, liver, heart) of treated groups were below the official limits.

Milk radioactivity was always much lower than the official EEC limit in all groups.

In conclusion, our results show that, in order to reduce the transfer of radiocesium from forages to meat and milk, both AFCF and bentonite are efficacious but at a different degree. Moreover for a better result their immediate use is recommended in case of a pollution from nuclear accident.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

V. Publications:

1.

- F. Carini, M. Montruccoli, I. Anguissola Scotti, S. Silva, 1989 - Radio cesium contamination in vegetable food products in relation to foliar treatments with potassic salts. I. Ann. Fac. Agr. UCSC, 29, 123-132.

In press:

- F. Carini, M. Montruccoli, S. Silva, 1991 - Foliar treatments with potassic salts of cereals contaminated by radiocesium, Ann. Fac. Agr. UCSC, 32.

- F. Carini, M. Montruccoli, M. Vincini, I. Anguissola Scotti, S. Silva - Trattamenti fogliari con sali potassici di piante agrarie contaminate da radiocesio, Atti del IV Congresso Nazionale SITE, Arcavacata di Rende (Cosenza), 28 ottobre-1 novembre 1990.

- V. Cappa, P. Bani, P. Vazhapilly, S. Silva - Accumulo di radiocesio nei tessuti di agnelloni alimentati con fieno radioattivo. Zoot. Nutr. Anim. 1991.

Contract: Bi7-046 **Sector: A26**
Title: Transfer of accidentally released radionuclides in agricultural systems (TARRAS)

1	Cancio	CIEMAT
2	Maubert	CEA - Cadarache
3	Rauret	Univ. Barcelona
4	Colle	CEA - Cadarache
5	Derwent	AEA Technology Harwell Lab.
6	Grandison	Univ. Reading
7	Gutierrez	CIEMAT

I. Summary of Project and Global Objectives

The aim of this project is to contribute to the reliability of radiological assessment methods and establish a scientific base for the design of post-accident countermeasures.

Three main aspects are considered in this project:

1.- A simulated accidental source term is used and the behaviour of aerosol deposits containing Sr, Cs and Ag isotopes are followed in some European soil-crop systems.

The research has started with lettuce (Lactuca sativa), one of the most universal plants in vegetable gardens and wheat (Triticum aestivum), one of the most important cereals in Europe.

Two types of soil are used, a French and a Spanish one. Both types of soils were chosen considering that they will also be used in the CEC RESAC program.

Several distinct fractions, including the fractions intercepted by plants, adhered to leaves and absorbed directly, as well as the dynamics of leaf washout, root uptake, migration and speciation in soil, have been studied in different growth stages of the lettuce plants. The soil-plant dynamics and the activity transfer to mature grain will also be described for wheat sown in soil contamined by aerosol deposits.

2.- The modification of radionuclide transfer rates through the food chain by well established food processing techniques is studied for Sr, Cs, Co and Ru.

The factors in food processing that modify the radionuclide content in food and the potential contribution of those factors for dose control in the event of an accident will be studied.

The processes to be studied include freezing, drying, canning, milling, juice extraction, dairy processing and normal culinary operations.

3.- Finnally, the project includes a study on the specific mediterranean diet and transfer data that will be compared with currently used generic parameters. The relevant data that are lacking will be identified and some soil/plant transfer factors for Sr and Cs isotopes will be obtained.

Head of Project 1: Dr. Cancio

II Objectives for the reporting period

General coordination of the whole project and contribution to the aerosol transfer data analysis.

A reorganization of the project was authorized by CEC and a subcontract has been established with the University of Barcelona that has made it possible to increase their contribution to the excution of the experimental work optimizing thereby the human resources in the Spanish part.

III Objectives for next period

- Overall coordination
- Participation in the interpretation of experimental data concerning the interceptation by plants of the aerosol, root uptake, derivation of parameters and modelling

IV Progress achieved including publications

Experimental aerosol deposits in soil-crop systems.

The two first experiments consisted in the contamination of 24 mature lettuces planted on two different soils. The soil characteristics are explained under project 3. The data produced have been statistically analyzed and filtered in order to obtain relevant information.

For each soil type four independent experimental 'lisymeters' were used. The four lysimeters were contaminated at the same time with a high temperature activated aerosol simulating the aerosols produced by a severe PWR nuclear accident. The aerosol characteristics are shown under Project 2.

The total activity released on the plants is determined by the contamination of a pellet. The two pellets that were used were very different and they produced a very different release in the two experiments performed.

After the maturation period the total biomass of lettuces was 3.039g ±80g on the sandy-loam soil and 3.523g ± 20g on the sandy soil. However, in the sandy-loam soil the proportion of biomass in leaves in relation to the biomass in the whole body of the plants was higher. For this reason the lettuces in sandy-loam soil have a specific surface s_{eff} higher than the lettuces growing in sandy soil:

s_{eff} = 53.5 cm^2/g in sandy-loam soil, and
s_{eff} = 45.9 cm^2/g in sandy soil.

This may have influenced in the higher interception capacity R detected in the former lettuces.

The total crop surface was 8640 cm^2 in both cases. This implies a yield factor Y:

Y = 0.35 Kg/m^2 for lettuces in sandy-loam soil, and
Y = 0.41 Kg/m^2 for lettuces in sandy soil.

Four lettuces were extracted at each sampling (one from each experimental lysimeter) at six times along a period of 14 days (9 days for sandy soil) starting after the initial contamination. The sampled lettuces were transported to Barcelona University, for processing and measurement of caesium, strontium and silver isotopes.

Interception factor analysis.

The interception factor R is a parameter that tries to quantify the expected fraction of air deposition that is intercepted by a macroscopic crop. The complementary 1-R parameter quantifies the fraction of deposition arriving onto the soil.

The concentration and the individual mass of the lettuces have been used to obtain the total contamination on the vegetation.

The total contamination of the soil has been obtained extrapolating eight measurements carried out at the initial and final times at four sampling in the lysimeters. The soil was extracted in these points until a depth that varies between 2 and 3 cm. The soil density is known, being 1.46 (sandy-loam soil) and 1.54 (sandy soil).

The table 1 shows the interception factors obtained and the total error propagated from the experimental measures (2σ) is in all cases ± 0.01.

The measurements in sandy-loam soil made it possible to obtain a value for the interception factor of 0.91 $\pm$ 0.03, but only data of Cs-137 have been used in this case due to the low activity released.

	R0	R
Cs-134	0.80	0.80
Sr-85	0.79	0.79
Ag-110m	0.80	0.80

TABLE 1
Interception factors of the lettuce crop on the sandy soil for the three nuclides

The calculations under the title "R0" were performed using exclusively the samples extracted inmediately after the contamination. Given that the washout of contamination was negligable, it is possible to calculate the R parameter using the whole set of lettuces extracted at different times. The results obtained by this method are shown under title "R". The results are coincidents in both methods.

Wash-Out analysis.

The plants were irrigated five times over the 14 days period (9 days for sandy soil) after the contamination in order to detect washout effects.

The contamination observed in individual lettuces have been normalized to the total contamination released in order to make comparable the three nuclides behavior (fig. 1).

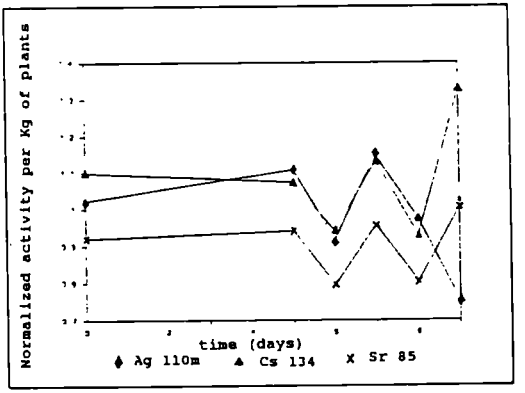


Figure 1 Normalized average concentration in lettuce samples

It can be observed that the normalized concentrations of the three nuclides in plants extracted at the same time is quite similar but it varies widely between different times. The

individual interception of contamination is very sensitive to the particular characteristics of the plant. However we can take advantage of the lower variability of the soil measurements to calculate the washout observed from the activity getting into the soil.

The results obtained for lettuces on sandy soil are:

Nuclide	λ0(d-1	λ(d-1)
Cs-134	0.010	0.011
Sr-85	0.003	0.003
Ag-110m	0.001	0.001

TABLE 2
Washout rates for the lettuces on the sandy soil.

The rate λ0 has been obtained considering only the lettuces extracted at the starting time and extrapolating the expected concentrations to the rest of the lettuces in the same lysimeter. The rate λ has been calculated using the whole set of data on lettuces.

The related residence time is over 60 days for the three nuclides. The experience time has been 14 days on sandy-loam soil and 9 days on sandy soil. Since 14 days is a typical time of harvest after a lettuce has become mature, it is meaningless to consider the washout as a process removing contamination from the surface at least for the yield factors defined before. The rain and sprinkling washout rate should be considered neglegible for lettuce crops of high yield density.

Other experimental results are included under project 3.

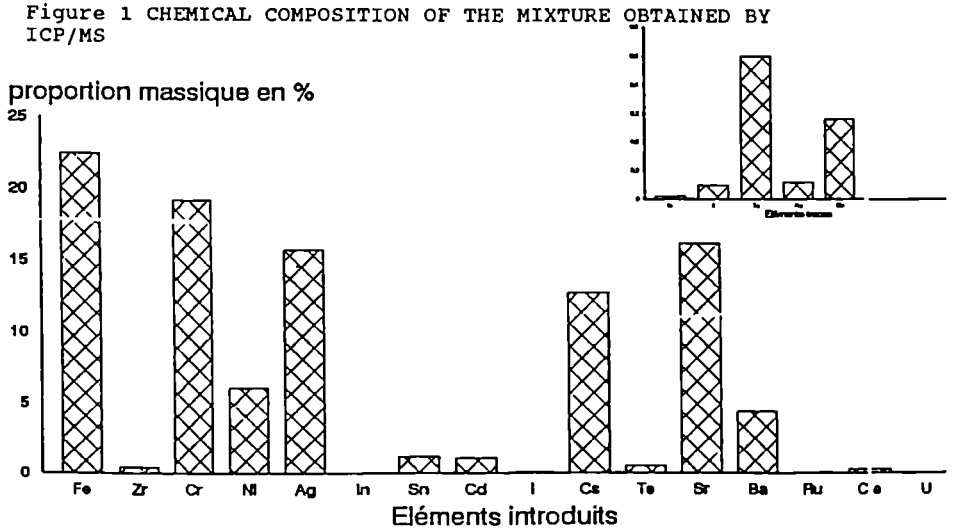
Head of project 2: Dr Maubert

II Objectives for the reporting period
The aim of these tests is to study the transfer of radioactive aerosols onto two types of plants in relation to two types of soil and to the degree of maturity of the plants. In order to do so, we had to adapt our system of radioactive aerosol release to the types of experiment that were planned, and to carry out contamination on the crops to be studied.
In collaboration with our partners, we determined the plant species to be studied, that is to say lettuce and wheat; the radioelements to be used: Cs134, Sr85, Ag110m; the two types of soil. We set up a schedule for the different releases to be performed as well as for the measurements to be made. We have now carried out the various tests as planned.

III Objectives for next period
During the next period, we have to finish performing the experiments required four our objectives. These will end in November as regards the contamination proper, and in March-April so as to obtain the results of the various measurements.
There will follow a period of data processing and the writing of the final report.

IV Progress achieved including publications
In the frame of the TARRAS project, the "Service d'Etudes et de Recherches en Environnement" of Cadarache is responsible for:

1. The generation of heat generated polymetallic aerosols that are representative of a real accidentel release:
This involves a mixture of 16 elements representing fuel and cladding, structural materials, control rods and of course, fission products. The mixture is heated to 2750° in the graphite tube of an electrically heated furnace. The aerosols thus produced are then scattered into a tight containment in wich we can set up cultivation. We can also introduce radioactive elements into such a mixture; their measurement can later be made by means of gamma spectrometry.
Figure 1 below gives us the chemical composition of the mixture that is used.
Figure 1 CHEMICAL COMPOSITION OF THE MIXTURE OBTAINED BY ICP/MS

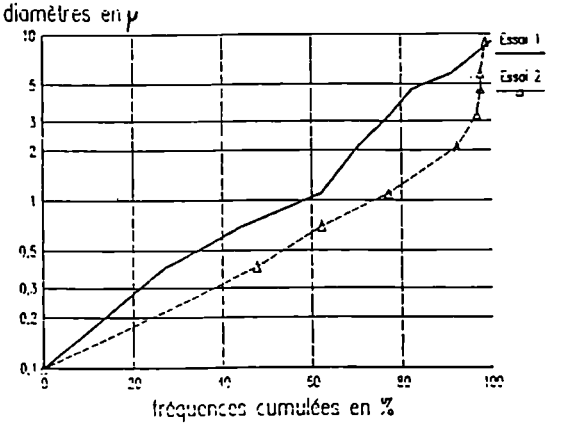


2. Characterization of the aerosols thus produced:

2.1. Physical characteristics:

We estabished the particle size curve of the aerosols which gives us particle size distribution. The measurements were made with Andersen impactors. Figure 2 below represents the distribution frequency in percentage in relation to the diameter of the particles in microns, for two tests. The mean geometrical diameter is obtained from the experimental particle size distribution; we can see that it is 0.75μ in the first test and 0.4μ in the second. The D25 to D75 diameter range is of 0.35 to 3μ for the first test and of 0.26 to 1.1μ for the second.

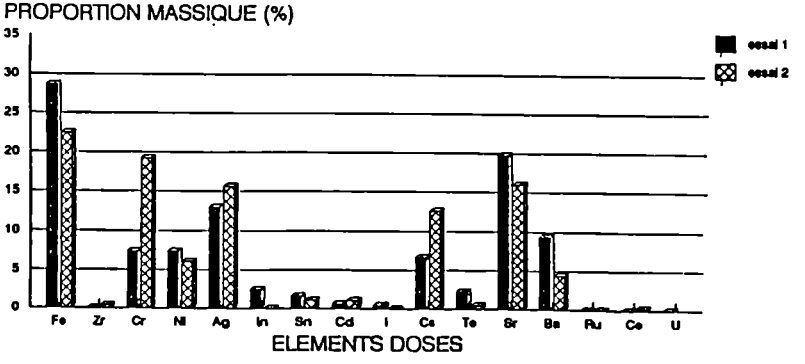
Figure 2: PARTICLE SIZE DISTRIBUTION CURVES



2.2. Chemical Characteristics:

In order to globally analyse the chemical composition of the aerosols, we used the same method as for the composition of the mixture, that is to say ICP/MS mass spactrometry. Figure 3 below shows the chemical composition of the aerosols during the two tests. It is to be noted that all the elements were released, and if these results are compared with the calculated theorical values, we can see that certain elements are released in greater quantity, such as iron, chrome, nickel, strontium and barium. On the other hand, others are released in smaller quantites or found in the aerosols, such as zirconium, indium, tin, cadmium, iodine, tellurium and ruthenium. The proportions of silver, cesium and cerium found in the aerosols are, however, close to the theorical values.

Figure 3: CHEMICAL COMPOSITION OF THE AEROSOLS



The determination of the chemical species was obtained by X diffraction, a method that only enables cristalline phases to be distinguished. These measurements allowed, in particular, the identification of non oxidized metal Ag,of metal Sr that rapidly changes into Sr(OH)2 in the air, and metal Cs that also rapidly changes into cesium hydroxide.
The aerosols then underwent observation through an electronic scanning microscope coupled to a retrodiffusion probe. These two methods enable a visual evaluation of the samples to be made, in particular of their geometrical form. Retrodiffusion ables the elementary composition of a particle to be determined. We were thus able to observe Ag particles in the form of spherical globules of 1 to 4μ, Sr alone or more often associated with other elements, as well as other associations. All these measurements and analyses thus enabled us to characterize the aerosols produced by our facilities and to clearly show the similitude betwen them and the modelling.

3. The contamination of chosen plant species:
Different contaminations or "shoots" have already been carried out in collaboration with partners.
We have performed "shoots" over the two chosen soils on fully grown lettuces, on bare soil just sown with wheat on one hand and lettuce on the other hand. We are soon to contaminate lettuce seedlings and half-grown lettuce plants, so as to cover the different vegetative stages.

Head of project 3: Dra. Rauret.
II. Objectives for the reporting period.

1st. Characterization of the soils.
2nd. Study of the interception by mature lettuce.
3rd. Study of the radionuclide migration during irrigation.(washing-out)
4th. Soil speciation.
5th. Deposition on leaf surface: successive extraction.

III. Objectives for the next period.

1st. Study of the lettuce cover interception in different stages of plant growth.
2nd. Study of root uptake by lettuce and wheat and its relationship with the phase in which radionuclides occur in the soil.
3rd. Influence of plant growth stages and type of soils on plant contamination.

IV Progress achieved including publications.

1) Characterization of the soils.

Two soils has been selected to carry out the plot experiments with contrasted properties to compare radionuclides behaviour. Main characteristics are summarized in table 1. Sandy texture correlates with low organic matter content and both properties are responsible for low cation exchange capacity with an outstanding low K^+ level. Sandy-loam "terra-rossa" soil has higher adsorption capacity and cation content: owing to the presence of lime, pH of this soil is slightly alkaline and the exchange complex is Ca^{2+} saturated.

Table 1. Characteristics of the soils

	SOIL 1	SOIL 2	Exchangeable cations ($cmolq \cdot Kg^{-1}$)	SOIL 1	SOIL 2
pH H_2O	7.5	6.7	Ca^{2+}	9.15	1.56
pH KCl	7.1	6.0	Mg^{2+}	1.85	0.19
% OM	2.40	0.22	Na^+	0.35	0.11
% $CaCO_3$	5.86	0.00	K^+	1.54	0.10
TEXTURE	Sandy-loam	Sandy	NH_4^+	1.65	1.86

2) Study of the interception by mature lettuce.

Mature lettuce plants grown on both soils were polluted in Cadarache with radioactive aerosols. Soil and plant samples were analysed to know ^{134}Cs, ^{85}Sr and ^{110m}Ag activity. Due to the low levels of measured activity for the experiment carried out with sandy-loam soil results are only refered to sandy soil experiment.

Radionuclide canopy interception was very high (80-90 %), mainly in the sandy-loam soil due to the crop covered almost completely the soil surface.

3) Study of the radionuclide migration during irrigation.

After pollution, plants have been irrigated with 250 cm^3 of distilled water on alternate days during two weeks (12 days), sampling a lettuce before each irrigation and soil at the beginning and at the end of the experiment.

Five irrigations were carried out. The leaching effect of lettuce plants was overlapped by the high variability of the radionuclide deposition.

Soil profiles were sampled at the end of the experiment. Radionuclide distribution in sandy soil layers (fig. 1) shows a significative increase of the soil activity after irrigation for the three radionuclides. It can be noticed, despite the short period of study, a slightly radionuclide migration according to the sequence: Sr > Ag > Cs (fig. 2). Nevertheless, the major part of each radionuclide remained in the first layer.

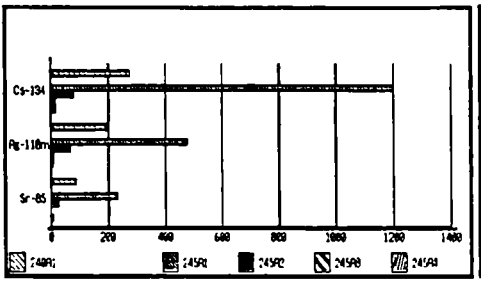


Fig. 1

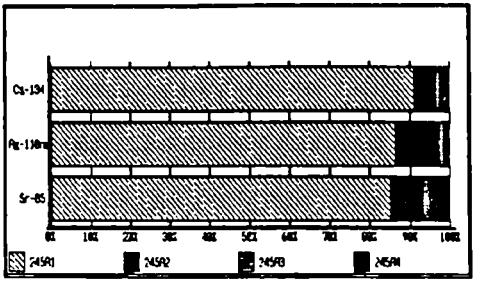


Fig. 2

Figure 1. Radionuclide distribution in soil layers before (240Al) and after (245An) irrigation treatment (Bq/Kg).
Figure 2. Radionuclide percentages in soil layers after irrigation treatment (Bq/cm^2).

4) Soil speciation.

Taking into account different chemical characteristics of studied radionuclides, two sequential extraction schemes have been applied to sandy soil to study the soil phases where radionuclides may be bound.

The first scheme (Rauret et al.) points out organic matter role in radionuclide retention. It is formed by four steps, with the following extractant reagents: $MgCl_2$, $Na_4P_2O_7$, NaOH and H_2O_2-HNO_3-NH_4AcO. The second procedure has been used in B.C.R.'s working group in solid speciation of heavy metals. It has three steps, with HAcO, $NH_2OH \cdot HCl$ and $H2O_2$-NH_4AcO as extractant reagents. Besides, to study the influence of kinetics in possible equilibriums, both schemes have been carried out on column and in batch.

Fig. 3 shows percentages of each extracted radionuclide in the different fractions. It can be observed: 1st) Sr is totally soluble in first fraction, 2nd) Ag and Cs have rather similar behaviour, 3rd) batch and column procedures lead to different Ag and Cs distributions in the same fractions. 4th) the two studied sequential extraction schemes also lead to different Ag and Cs distributions in the extractant solutions.

From these first results it can be concluded that obtained distributions are highly dependent on used methodology which, as a first step, must be improved. Besides, taking into account soil-plant transfer results that will be obtained in this project, from both soils and both crops, as well as those obtained from soil speciation, a methodology may be established to have a starting point to achieve, by means of comparative studies with other laboratories working in soil speciation, an informative, useful, widely accepted methodology to know the phase soils in which radionuclides occur.

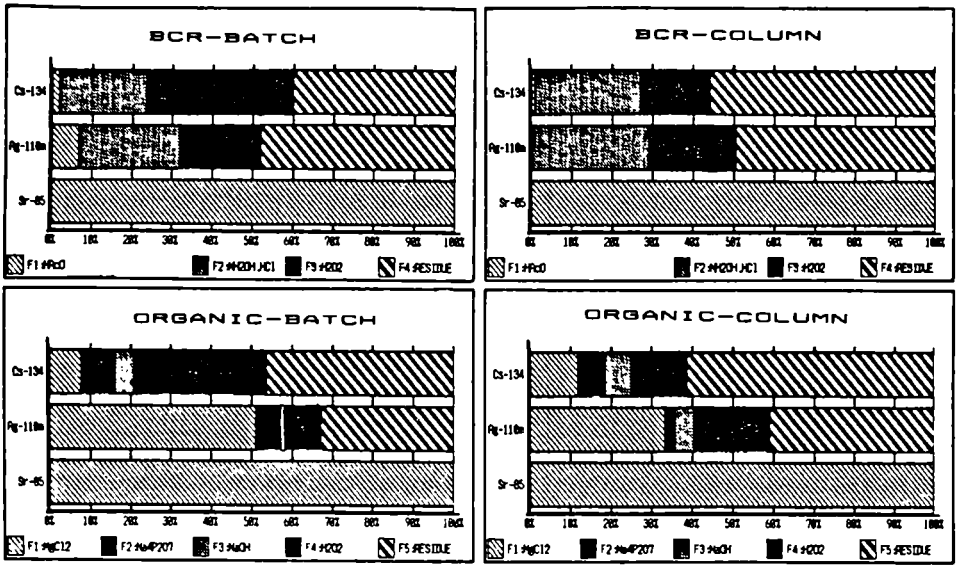


Figure. 3. Percentages of each extracted radionuclide in the different fractions.

5) Deposition on leaf surface: successive extractions.

To study the deposition on leaves surface an experiment based on successive extraction procedure has been designed. The lettuce is divided in two parts. The first one is dried and its activity is measured (24n-E). The other part is extracted with distilled water in order to wash not adhered aerosol. The extract is filtered to distinguish between soluble (24nR-A) and particulate fractions (24nF-A) and both activities are measured. Residual leaves are treated with $CHCl_3$ in order to solubilize the waxes of lettuce cuticle and to release adhered aerosol. The chloroform extract is filtered to separate soluble (24nR-C) and particulate fractions (24nF-C), and activities of both fractions as well as of residue are measured. This experiment was carried out before (n=0) and after irrigation (n=5).

In Fig.4 and 5 it has been represented percentages of each fraction before and after irrigation, respectively.

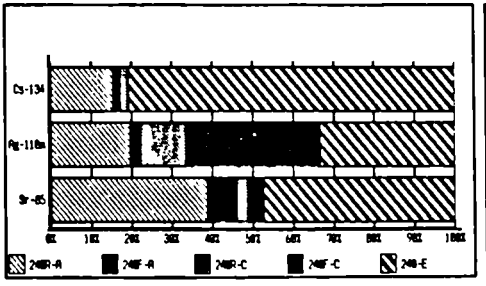


Fig. 4

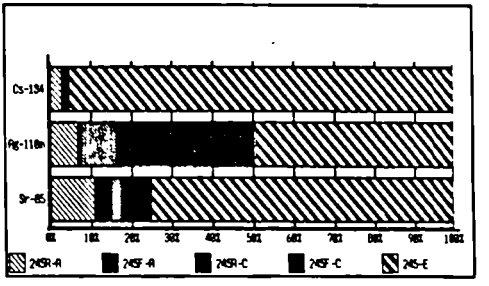


Fig. 5

Figures 4 and 5. Percentages of each extracted radionuclide in the different fractions before and after irrigation, respectively.

It can be noticed that:
- Solubility in water decreases in the following order: Sr > Ag ≈ Cs.
- Ag is mainly released by chloroform extraction, being mainly present as particulate phase, associated with the cuticle.
- A high percentage of cesium remains in the plant even when successive extraction is carried out immediately after pollution of the plant.
- Water extractable fractions in plants decreases after the period of irrigation.

Finally it has been calculated the mean isotopic Cs/Sr and Ag/Sr ratios present in the lettuce before and after irrigation and before and after applying the sequential extraction procedure (table 2). It must be pointed out a notable variation of all the isotopic ratios before and after successive extraction for the recently polluted lettuces whereas for similar samples after irrigation it is not observed any variation.

The increase of the Cs/Sr and Cs/Ag ratios after successive extractions before irrigation would show that Cs is selectively absorbed by the plant. However after irrigation isotopic ratios after successive extraction do not change significatively, probably due to a possible selective incorporation of Sr in the leaves and/or a selective leaching of Cs from the leaves.

Table 2. Isotopic ratios of radionuclides in leaves after and before successive extraction and after and before irrigation.

		before successive extraction	after successive extraction
Before irrigation	$^{134}Cs/^{85}Sr$	4.96±0.08	7.86±0.88
	$^{134}Cs/^{110m}Ag$	2.02±0.04	5.18±2.18
	$^{110m}Ag/^{85}Sr$	2.45±0.01	1.67±0.50
After irrigation	$^{134}Cs/^{85}Sr$	5.55±0.29	5.51±1.02
	$^{134}Cs/^{110m}Ag$	3.35±1.52	3.19±1.44
	$^{110m}Ag/^{85}Sr$	1.92±0.83	1.90±0.50

G. Rauret, M. Llauradó, M. Vidal and V.R. Vallejo. "Solid Speciation of Radiocaesium in Soils", in G.M. Desmet, P. Nassimbeni and M. Belli (Eds), Proc. Workshop on The Transfer of Radionuclides in Natural and Semi-natural Environments,Elsevier Applied Science, London, 1990, pp 538-545.

1 Head of Project. 4: Dr. Colle

2 Objectives for the reporting period

The aim of this project is to quantify the transfer of some elements (Cs, Sr, K, Co, Ru), during plants food-processing. This study includes collaboration with manufacturers, so it involves stable isotopes measurements. For the elapsed year, three objectives have been carried out:

1) experimental comparison of radioactive and stable isotopes behaviour during a same agro-food process,
2) trying to contact manufacturers,
3) measurements of industrial products: canned fruits, rice, vine

3 Objective for next period

1) keeping on with the comparison of stable and radioactive elements behaviour,
2) studying some processes for the following products:
- bean or peas: canning,
- potatoes: dehydrating,
- tomatoes: making juces or sauces,
- olive: oil,
- beetroot: sugar,
- wheat: flour and bread.

4 Progress achieved including publications

1) Experimental comparison of radioactive and stable isotopes behaviour during a same agro-food process

In order to work under realistic conditions, the study of transfers during food-processing uses samples taken from factories, thorought the different steps from the agricultural raw product to the proceessed food.

Environmental radioactive levels are near or under detection limits, it is therefore necessary to work on stable isotopes. This implies an experimental verification of the assumption that, for a same process, stable and radioactive isotopes of a given element behave in the same way.

Preliminary tests were made on two batches of canned green beans cultivated on two substrats: one was a soil doped whith stable element, the other was contaminated nutritive solution. Analysis data had shown that the transfer factors (concentration of processed food divided by concentration of raw plant) were identical for stable or radioactive isotopes. The transfer factors were 0,2 for cesium and 0,6 for strontium.

Theses investigations will be carried on during 1991, with different vegetables (beans, peas, spinachs).

2) Preliminary contacts whith manufacturers.

It appeared very difficult and unsuccessful to come directly in contact with manufacturers. Then, for a better efficiency, it was necessary to call in organizations which are in business connection whith the profession: CTCPA (Centre Technique de la Conservation des Produits Agricoles), LIRF (Laboratoire Interrégional des Fraudes)...
Presently, some factories, making the main foodstuffs planned in this project are ready to join this work.

3) Measurements of industrial products

- canned pears

Measurements preliminary data (ICP/MS, gamma spectrometry) on samples of canned pears taken at different steps in a production line are listed in table 1.

Cannig includes the following operations: trimming, tinning (metallic cans), adding sirup, crimmping and pasteurization at 100 °C; 1 kg of raw pear, leads to an average of 670 g of drained canned pear.

Table 1 Concentrations (µg/kg) and transfer factors (Ft: µg/kg proceeded food per µg/kg initial food) of different elements in fruits (on a fresh weight basis).

	Cs	Co	Pb	Cr	Ni	Sr	K
whole raw pear	(2,1)	6,9	25,7	123	516	1507	2,5E6
trimmed pear	(1,5)	4,3	40,4	126	393	1829	2,3E6
canned pear	(3,6)	2,8	61,7	217	810	2074	1,5E6
Ft pear whole -> canned	**(1,7)**	**0,4**	**2,4**	**1,7**	**1,6**	-	**0,6**

Data of cesium analysis seem poorly reliable because the measured levels were near detection limits. Concerning strontium, the value found for canned pears is aberrant and must be set aside. This anomaly might come either from a pollution of the sample, or from a measurement error (verifications are in progress). For lead, chromium and nickel, the global balance shows a concentration increase, which probably comes from the tin metal.

Concerning cobalt and potassium, it should be noted that canning leads to an appreciable concentration decrease in the proceeded food related to the raw item.

- rice processings

Rice processing technologies are physical treatments involving two steps: the first one changes the raw (paddy) rice in husked (complete) rice by eliminating the external teguments; the second one gives the whittened rice by removing pericarp and sprout.

In average, 1 kg of paddy rice leads to 800 g of husked rice, which gives 700 g of milled rice.

Measurements were made on round grain rice, sampled after these three steps (see table 2). Data show an important decrease of elements massic concentrations in whittened rice comparing whith paddy rice.

Table 2 Concentrations (µg/kg or Bq/kg) and transfer factors (Ft: µg/kg proceeded food per µg/kg initial food) of different elements in rice (on a fresh weight basis).

	^{40}K	Co	Sr	Cs
paddy rice	90	11,6	1335	1,8
husked rice	83	7,5	705	1,4
whittened rice	33	4,2	203	0,4
Ft rice: paddy -> husked	**0,9**	**0,6**	**0,5**	**0,8**
Ft rice: husked -> whittened	**0,4**	**0,6**	**0,3**	**0,3**
Ft rice: paddy -> whittened	**0,4**	**0,4**	**0,2**	**0,2**

- wine production and distillation

Wine was made by three processes.

a) rosé wine making: must, which is separated immediatly after grapes pressing, ferments alone.

b) red wine making: grape (without stalk) is pressed and is followed by must fermentation whith fruits pulp and husk.

c) red wine making: the whole bunch of grapes (grape and stalk) is pressed and followed by must fermentation with pulp, husk and stalk.

Analysis results (table 3) show that rosé wine making is the process leading to the lowest concentrations in wine, for measured elements. In case of red wine making, must maceration whith the whole constituants results in higher concentrations in wine wine and this fact is emphasized by pressing.

Table 3 Yield (l of wine/kg grapes) and concentrations (µg/l or Bq/l) of some elements in wine.

		Yield	^{40}K	Co	Sr	Cs
a) Rosé wine		0,46	51	1,2	301	0,1
b) Red wine:	racked wine	0,32	60	2,9	790	0,1
wine made from	pressed wine	0,11	63	4,2	867	0,2
grape	total	0,43	61	3,2	810	0,1
c) Red wine:	racked wine	0,42	61	3	982	0,1
wine made from	pressed wine	0,12	82	3,9	1130	0,2
whole bunch	total	0,54	66	3,2	1013	0,1

As for canned fruits data, values for cesium are too close to detection limits for reliability. Analysis by neutronic activation is in progress in order to complete theses results.

Afterwards, all these wines were distilled. In the resulting alcools, there was no significant amount of potassium, cobalt, strontium or cesium.

Head of Project 5: Dr P A Cawse

II Objectives for the reporting period

The objectives in 1990/91 were to (i) cultivate and/or obtain food products from a region having relatively high concentrations of radionuclides in soil, for processing in the pilot plant facilities at Reading University Department of Food Science and Technology (ii) prepare raw and processed foods for analysis of radionuclides and stable elements, and (iii) analyse the samples for K-40, Co-60, Sr-90, Ru-106 and Cs-137 together with their stable isotopes. In addition, analysis of factory processed vegetables would be started. Discussion of the measurements programme and results was required with CEN Cadarache who are studying crops specific to France.

III Objectives for next period

The objectives in 1991/92 are to maintain an equal balance between measurements of radionuclides and stable elements (as stated above) in foods that are processed by the pilot plant facilities and by factories. Products to be examined are potatoes, carrots, peas, beetroot, fruit juices (apple and blackcurrant), mushrooms and rape seed oil. Ruthenium-103 tracer will be applied to leafy vegetables that are close to maturity to assess the removal by normal culinary practice. Further discussions of results will be made with CEN Cadarache (Dr C Colle) and with CIEMAT Madrid (Dr D Cancio).

IV Progress achieved including publications

1 Crops for Food Processing

The Sellafield region of Cumbria, North West England, was chosen for cultivation by Harwell of peas, potatoes, brussels sprouts, carrots, wheat and for collection of field mushrooms. This region has a relatively high concentration of Cs-137 in soil owing to fallout from the Chernobyl accident in 1986 and historical releases of radionuclides from Sellafield Works. Concentrations of Sr-90 are ~10Bq/kg dry soil, and ~60Bq/kg for Cs-137.

A list of crops and varieties grown in 1990 is given in Table 1, together with the type of processing carried out. Varieties were selected according to their use by the food processing industry in Great Britain. In addition, milk from the Sellafield area was obtained to provide dairy products.

2 Sample Preparation for Analysis

The processed samples were oven-dried and ashed for analysis of radionuclides. A sub-sample of 50g of dry material was ground in an agate ball-mill for measurement of stable elements: 0.5g of the ground sample was then wet-ashed in nitric/perchloric acid and diluted to 10ml for analysis by ICP-MS.

3 Results from Analysis of Raw and Processed Food

Results have been discussed at a co-ordination meeting with other Contractors on 4/5 April 1991 at CIEMAT, Madrid.

3.1 Radionuclides

Results are now available for Cs-137, K-40 and stable elements in 26 samples of raw and processed vegetables. Strontium-90 has been analysed in 13 samples and further results are awaited. Cs-137 was present in the range 0.3-2.1Bq/kg dry weight in raw vegetables, except for a value of 44Bq/kg in

Table 1

Crops Used for Food Processing (1990/91)

PILOT PLANT PROCESSED CROPS		
Crop	Variety	Use
Pea	Puget	Freezing
	Maro	Canning and dehydration
	Bunting	Canning
	Princess	Canning
Potato	Record	Crisps
	Maris Peer	Canning
	Pentland Squire	Domestic (boiled)
	Pentland Ivory	Domestic (baked)
	Romano	Dehydration
	Estima	Domestic (boiled)
Brussels Sprouts		Domestic
		Freezing
Mushroom		Dehydration
Wheat	Tonic	Flour
		Bran
Milk		Skimmed milk
		Cream
		Cheese
		Whey powder
FACTORY PROCESSED CROPS		
Potato	Record	Crisps
Wheat	Mixed	Flour
	(Mercia -60%)	Bran
	(Avalon -40%)	Wheatgerm

mushroom. For Sr-90: 1.8-9.7Bq/kg and for K-40: 178-1710Bq/kg were recorded. Levels of Co-60 and Ru-106 were below 1Bq/kg in all raw crop samples. In raw mushroom, Ag-110 was found at a concentration of 28Bq/kg dry weight.

Food processing retention factors for radionuclides have been derived from data in Table 1 and are defined as activity/kg dry weight in processed food relative to the raw material. For comparison, the factors are also derived on a fresh weight basis, as in the food prepared for human consumption. A wide range of retention factors was recorded for Cs-137, K-40 (and stable Co), from 0.1 to 3 according to the processing treatment: thus an increased concentration of radionuclides in the fresh foods was sometimes evident. For Sr-90, retention factors did not exceed 0.9. These retention factors for Sr-90 and Cs-137 are shown in Figures 1 and 2 respectively.

3.2 Stable Elements

Analysis was made for Co, Cs, K, Ru and Sr. In the course of ICP-MS it was noticed that raw and processed mushroom contained relatively large amounts of Ag and Hg. Further, it was observed that canning increased the concentrations of Sn in peas and potato by an order of magnitude and also that Pb occasionally showed some increase (although smaller than for Sn) after processing, for example in canned potato and dehydrated mushroom.

In the absence of measurable amounts of Co-60 and Ru-106, the data for stable Co permitted retention factors to be derived. However, stable Ru was <0.02mg/g dry weight in all raw and processed samples except raw carrot (0.065mg/g). The soil to plant transfer factors for Ru-106 and the stable isotope are known to be low, in the region 0.05-0.2.

Figure 1

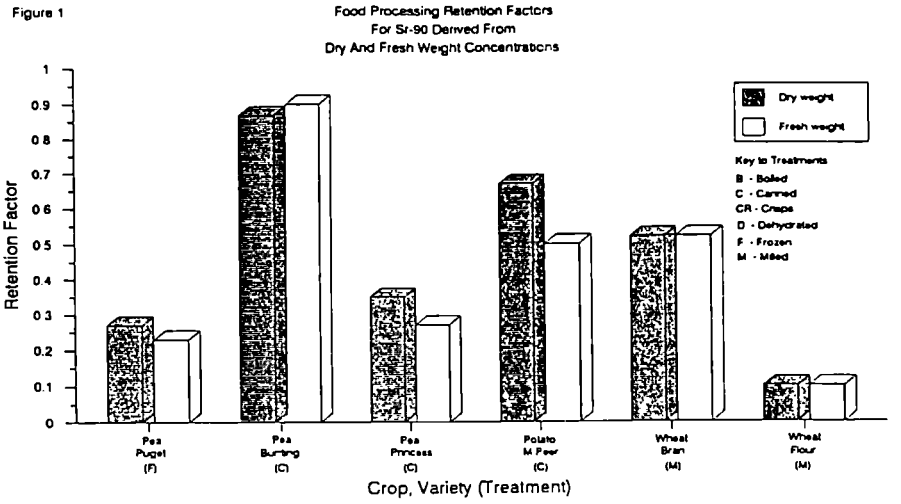
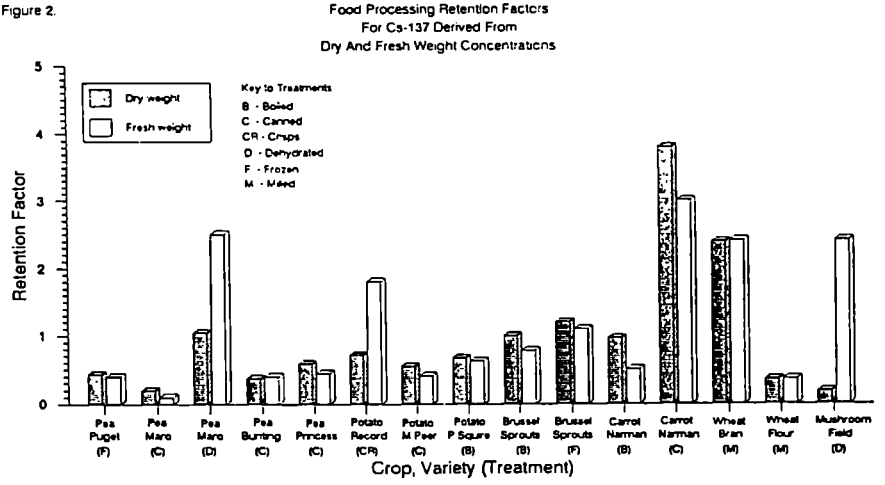


Figure 2.



3.3 Dry Matter Recovery from Prepared Foods

Losses of dry matter during food processing were recorded and retention factors were derived. In some canned crops considerable amounts of dry matter were lost, and retention factors were in the region 0.5-0.6. However, factors of 0.8-0.9 were evident for many of the boiled and dehydrated crops.

3.4 Soil to Plant Transfer Factors: Additional Data

Soil to plant transfer factors have been derived from the soil concentrations of Sr-90 and Cs-137 at locations where crops were cultivated; this provides additional information on the specific soil to plant transfer pathway. Thus the counter-effect of food processing on removal of radionuclides taken up by crops following an accidental release may also be assessed.

Head of Project 6: Dr. Grandison

II Objectives for the reporting period

To determine the extent of radionuclide transfer through the food chain to man as modified by food processing. The study includes assessment of radionuclides Sr-90, Cs-137, Co-60 and Ru-106 during pilot plant processing of raw materials produced in the Sellafield region of Cumbria, and assessment of stable isotopes of Cs, Sr, Ru and Co in raw materials and products from U.K. factories. Natural radioactivity due to K-40 will also be measured.

In the first year effort will be concentrated on measurements using the pilot plant facilities to identify the most important processes that modify the concentrations of radionuclides in food. The work includes establishment of representative processing methods and varieties of raw materials used for specific processes.

III Objectives for next period

Further studies will be carried out on production of cream, skim milk, hard and soft cheeses and whey. In addition, pilot plant and culinary preparation studies will be carried out to supplement the existing data.

The factory studies will be extended to include frozen and canned vegetables, fruit juices, rapeseed oil and dairy products.

IV Progress achieved including publications

The first year has concentrated on pilot plant studies. Processes were established to simulate commercial production of a range of products :- canned, frozen and dried peas; chipped, canned, dehydrated and boiled potatoes and potato crisps; dried mushrooms; frozen Brussels sprouts; frozen and canned carrots; flour and bran.

Samples of the appropriate varieties of raw materials with elevated radionuclide content, provided by AEA Harwell, were subjected to these pilot plant processes and to domestic culinary preparation. Products were sent to Harwell for radionuclide analysis and processing wastes for stable potassium analysis. Total dry matter recovery from prepared foods after processing was recorded.

Samples of raw materials and products have been obtained from commercial producers of potato crisps, mushrooms and wheat flour for analysis of stable elements by ICP-MS at AEA Harwell laboratories.

Results are presented in project 5 (Dr P.A.Cawse).

Food processing methods and results were discussed at a co-ordination meeting with other contractors on 4/5 April 1991 at CIEMAT, Madrid.

A paper entitled Influence of processing on the radionuclide content of food by S.Patel, A.S.Grandison, P.A.Cawse and M.J.Lewis has been accepted for presentation at the 8th World Congress of Food Science and Technology to be held in Toronto from 29th September to 4th of October 1991.

Head of Project 7: Dr. Gutiérrez

II Objectives for the reporting period.

According to the project calendar, objectives for the reporting period mainly deal with: (i) to establish the main characteristics of spanish mediterranean diet, identifying the major agricultural cultures produced and consumed, (ii) to analyze differences with other EC areas and (iii) to obtain information on soil-plant transfer factors for selected crops from relevant literature (Sr-90 and Cs-137 radionuclides); additionally some experimental work (sampling and analyses) to obtain these transfer factors would be started.

III Objectives for the next period.

Until the end of the project (June 1992) activities will be carried out to cover the following objectives: (i) to determine experimental soil-plant transfer factors, (ii) to investigate differences between the obtained parameters for the spanish mediterranean area and the "generic" parameters used in the dose models, and (iii) to establish the potential influence in dose estimation by the ingestion way.

IV Progress achieved including publications.

Characterization of agricultural productions and food comsumptions

Statistical information on agricultural productions, cultivated surfaces, yields, food comsumption habits, etc. has been collected and analysed from relevant national official sources in order to identify specific characteristics of mediterranean areas in Spain. Five geographical areas (Cataluña, Baleares, Valencia, Murcia and Andalucía) including its seventeen provinces and nine generic groups of cultures containing about 50 products have been considered. For comparison purposes, information of the whole country and global data of EC countries have also been taken into account.

Agricultural productions

From the data analysed and in addition to the general comment about the important role of mediterranean countries in the global EC agricultural production, some specific considerations can be outlined and are summarized in table 1.

TABLE 1.- PERCENTAGES OF MEDITERRANEAN CONTRIBUTION TO SOME REPRESENTATIVE AGRICULTURAL PRODUCTIONS

	RICE	TOMATOES	ONIONS	CITRUS	OTHER FRUIT	OLIVE	VINE
SP.MEDIT/TOTAL SP	88	55	53	100	55-95	84	29
EC MEDIT/TOTAL EC	100	86	66	100	--	100	90
TOTAL SP/TOTAL EC	26	19	38	50	--	42	18

- Rice is almost exclusive from mediterranean areas in Spain (88% of national production) and in EC (26% from Spain and 57% from Italy).
- For vegetables, on average, about 70% of spanish production is from mediterranean areas (ranging between 50%-85% for the analysed products). Among them tomatoes and onions are the highest production (25% and 10% of total). These two products are mainly obtained in the mediterranean countries of EC (86% and 66% respectively), Spain being a major contributor (19% and 38%) of global EC production.

- Fruit production in Spain from mediterranean areas range between 55-95% of total (excepting banana that is exclusive fron Canary Islands). Mediterranean countries are the major contributors (even exclusives in some cases) to the global EC fruit production.
- Citrus and olive are the most specific mediterranean products and practically exclusive from these areas in Spain and in global EC. The contribution of Spain to EC production is 50% and 42% respectively. In the case of olives about 95% of production is processed to oil and the remaining 5% for direct comsumption.
- Vine use distribution is 93% for wine fabrication and 7% for grape fresh comsumption. Althought the wine production is from mediterranean countries (close to 90% of global EC), it is very spread into the different areas of the countries. As an example only 29% of spanish production is from mediterranean areas.

Food comsumption

In addition to agricultural products other relevant groups of foods have been studied to determine the typical spanish mediterranean and global diets. Published data from cooperative international studies and international organizations have been consulted to assess the existing harmonization. The discrepancies between sources, that can be observed in table 2, are mainly due to inherent difficulties in handling statical data, differences in the methodologies used and interpretation and classification criteria.

TABLE 2.- FOOD CONSUMPTION IN SPAIN (Kg.Pers-1.yr-1) FROM DIFFERENT SOURCES

	SP. MEDIT		TOTAL SP			
FOOD	INE	MAPA	INE	MAPA	OCDE	CEE
A-CEREALS	123.1	91.6	114.1	86.9	83.0	75.0
B-MILK & PRODUCTS	128.6	123.0	154.4	137.2	111.7	115.1
C- EGGS	16.6	15.8	19.1	17.0	16.5	17.0
D-OIL(l)	32.8	25.6	32.3	25.2	24.3	
E-PATATOES	85.6	49.4	88.0	56.5	111.1	107.0
F-VEGETABLES	104.3	100.0	96.4	92.0	140.2	125.7
G-FRUIT	118.6	128.2	118.4	124.4	150.0	133.0
H-MEAT	75.8	69.0	76.3	67.8	74.6	83.0
I-FISH	25.8	28.0	30.7	30.1	25.4	26.0
J-WINE(l)	70.2	35.3	79.0	40.6	59.8	49.0
K-BEER(l)	103.6	87.0	83.5	64.9	70.0	70.0

In general, published data for EC countries are based on food balance sheets and usually overestimate the real comsumption. Spanish data in the tables are mainly based on house hold budget surveys carried out by national official organizations. Furthermore, data have been corrected, when necessary, to take into account comsumption outside home, so it is though to give a more reliable estimate of real comsumption.

In order to give a summary analysis of the obtained information, table 3 shows ratios between mediterranean and no mediterranean diets. From this table some preliminary remarks can be made:
- Spanish mediterranean and global diets are very close (differences are less than 20% excepting rice comsumption).
-When camparing diets from different EC areas some significant variation appear clearly mainly related to comsumption of oil, vegetables, fruits and wine.

- In spite of some differences, Spanish mediterranean diet could be representative of global EC mediterranean diet.
As a general comment mediterranean foods seem to have a progressive influence in the EC countries probably due to the growing commercial exchanges. It ressults in a higher and higher importance of the ingestion of typical mediterranean foods for dose estimations.

TABLE 3.- FOOD CONSUMPTION RATIOS (SPANISH SOURCE:INE)

FOOD	(1)	(2)	(3)	(4)	(5)
A-CEREALS	1.07	1.40	1.30	1.66	1.27
B-MILK & PRODUCT	0.83	1.04	1.15	0.96	0.83
C-EGGS	0.86	1.19	1.17	1.21	1.04
D-OIL(l)	1.01	2.43	1.75	3.73	2.12
E-PATATOES	0.97	1.06	1.25	0.93	0.75
F-VEGET & D.PULSES	1.10	0.95	0.79	1.20	1.51
G-FRUITS	1.00	1.12	0.94	1.38	1.47
H- MEAT	0.99	0.95	0.83	0.88	1.06
I-FISH	0.84	1.98	1.86	2.15	1.16
J-WINE(l)	0.88	2.03	1.11	3.64	3.28
K-BEER(l)	1.24	1.32	2.62	0.89	0.34

(1) RATIO SPANISH MEDITERRANEAN TO TOTAL SPANISH DIET
(2) RATIO SPANISH MEDITERRANEAN TO CE DIET
(3) RATIO SPANISH MEDITERRANEAN TO CE MEDITERRANEAN COUNTRIES DIET
(4) RATIO SPANISH MEDITERRANEAN TO CE NO MEDITERRANEAN COUNTRIES DIET
(5) RATIO CE MEDITERRANEAN COUNTRIES TO CE NO MEDITERRANEAN COUNTRIES DIET

Soil-Plant transfer factors

A bibliographic search on soil-plant transfer fectors for selected crops has been carried out (Sr-90 and Cs-137 radionuclides). The available information is generally referred to generic types of foods. Only a few data concern specific products as patatoes, rice and some green vegetables. Variations between data can be observed (table 4) mainly depending on the soil type and the units used for expression of activity. A lack of information concerning the most typical mediterranean products and foods is evident. In order to obtain specific information for these products, experimental work on soil and relevant vegetables sampling and Sr, Cs analyses from five spanish mediterranean areas is ongoing. Radiochemical analyses and measurements are in progress.

TABLE 4.- SOIL-PLANT TRANSFER FACTOR.DATA REVIEW SUMMARY

		Sr-90	Cs-137
CEREALS	*	0.03-0.32	0.07-0.034
	**	0.02-1.4	0.006-0.045
PATATOES	*	0.07-0.47	0.032-0.17
	**	0.014-0.06	0.003-0.02
ROOTS	*	0.44-1.8	0.027-0.17
	**	0.06-0.15	0.005
LEAFY VEGETABL	*	1.2-5.9	0.022-0.095
	**	0.06-0.7	0.004-0.02
FRUIT VEGETABL	*	0.24	0.026
	**	0.024	0.007
FRUIT	*	0.24	0.026
	**	0.01-0.03	0.02

(*) REFERRED TO DRY WEIGHT OF PLANT (**) REFERRED TO WET WEIGHT OF PLANT

III B

FOLGEN DER STRAHLENEXPOSITION DES MENSCHEN; IHRE ABSCHÄTZUNG, VERHÜNTUNG UND BEHANDLUNG

CONSEQUENCES OF RADIATION EXPOSURE TO MAN; THEIR ASSESSMENT, PREVENTION AND TREATMENT.

CONSEQUENCES POUR L'HOMME DE L'EXPOSITION AUX RAYONNEMENTS, EVALUATION, PREVENTION ET TRAITEMENT

Progress Report

Contract: Bi6-099 **Sector: B**
Title: Late somatic effects of ionizing radiation on the mammalian organism.

1 Maisin Univ. Cathol. Louvain à Woluwe

I. Summary of Project and Global Objectives

The objective of the European Late Effects Project Group (EULEP) is to improve the understanding of late biological effects of exposure to ionising radiation. Its work consists of the standardization and development of methodology in the member institutions, the co-ordination and promotion of co-operative research by means of task groups, and the organisation of training activities, workshops and symposia. Twenty-three laboratories are currently participating in th work.

(a) Standardisation and Development of Methodology

This aspect of the work is carried out by four committees:

- Committee of External Radiation Dosimetry and Techniques
- Committee of Internal Radiation Dosimetry and Techniques
- Committee of Pathology
- Committee of Cell and Molecular Biology

In addition there is an Expert Group on Physiological Methodology.

(b) Co-ordination and Promotion of Co-operative Research

The co-ordination of collaborative research work between the member institutions is organised by means of a number of problem-orientated task groups.

(c) Training Activities

EULEP is taking steps to promote the training of young radiobiologists. High priority continues to be attached to the support of scientific exchange visits between laboratories for the purpose of acquiring technical expertise. EULEP also organises special training courses, in order to promote the introduction of new methodologies into member laboratories.

Head of Project 1: Dr. Maisin

II Objectives for the reporting period

(a) to continue to develop the programme of standardisation and development of methodology through the committees;

(b) to promote the co-ordination of research by the task groups including the review of existing work and the establishment of new task groups where opportunities present themselves;

(c) to plan training activities as outlined in (I) above and to review the teaching of radiobiology in the different countries of the EC.

III Objectives for the next period

The programme of the committees and task groups will be continued as outlined below (IV). A new task group on the Effects of Radon and Radon Daughters *in vitro* and *in vivo* has been established. A training course on polymerase chain reaction methodology will be held in 1991. Efforts to promote the teaching of radiobiology will be continued. EULEP will also jointly organise two workshops at the International Congress of Radiation Research.

IV Progress achieved including publications

(a) STANDARDISATION AND DEVELOPMENT OF METHODOLOGY

Committee of External Radiation Dosimetry and Techniques

High dose total body irradiation (TBI) in combination with intensive chemotherapy followed by bone marrow transplantation (BMT) has shown to be of increasing benefit for the treatment of acute leukaemia and some other disseminated diseases. The long-term surviving patients constitute a very interesting group in which stochastic and deterministic effects of total body irradiation can be studied. Under the auspices of EULEP, the European Society for Therapeutic Radiology and Oncology (ESTRO) and the European Bone Marrow Transplant Group (EMBT), a meeting was organised on the physical, biological and clinical aspects of total body irradiation. In the proceedings (Broerse *et al.*, 1990) a proposal is made for reporting the technical and dosimetric parameters applied in total body irradiation in an unambiguous manner.

The results of the sixth EULEP X-ray dosimetry intercomparison have been analysed by the Standardisation Laboratory of the Dutch National Institute of Public Health and Environmental Hygiene. Mouse phantoms with thermoluminescent dosimeters (TLD) were irradiated at fifteen participating institutes with a prescribed dose. The readings of the TL dosimeters indicate that four institutes differed more than 5 per cent from the reference value, and two institutes showed deviations in excess of 10 per cent. In view of the accuracy of the TL dosimetry system it is now generally accepted that deviations between 5 and 10 per cent indicate the necessity of checking the absolute dosimetry of the institutes involved. Deviations in excess of 10 per cent could, in the past, always be traced to systematic errors in

the techniques or the dosimetry for animal irradiations. In the near future site visits will be made to some institutes to assist in the X-ray dosimetry procedures.

Committee of Internal Radiation Dosimetry and Techniques

While there has been no request from the task groups for assistance with problems relating to the dosimetry of internally deposited radionuclides, assistance has been requested in relation to the standardisation and comparison of various cell biological methods in several task group laboratories.

The scientific activities of the committee were concerned with detailed discussions of the new Recommendations of the ICRP (Publication 60). Several members have been involved in the assessment of the impact of the reduction of the equivalent dose limit to an average of 20 mSv/yr on the Annual Limits of Intake for radionuclides, and were also involved in the review of the biokinetic models to describe the behaviour of specific elements in the body which will be needed for the complete review of ICRP Publication 30. In another area the committee discussed the role of chelation therapy in the reduction of the risks of late effects from radionuclide incorporation. It has drawn attention to areas in which the methods of chelation therapy could be used to provide information which could assist in the elucidation of some fundamental problems in the radiobiology of internal irradiation; these relate especially to changes in dose rate and to the determination of the importance of the early and late periods of irradiation in relation to the induction of cancer or other late effects.

During the coming year, following on from the establishment of the new task group on the effects of radon and radon daughter products *in vitro* and *in vivo*, the committee plans to give consideration to the need for building up an expert group on the dosimetry of radon and its daughters in animals and in isolated cell systems. A revision of the DOSELIB computer programme for the calculation of radiation doses delivered to 21 tissues from a selection of critically important radionuclides will be started, to take account of the changed dose limits and tissue weighting factors contained in the new ICRP Recommendations.

Committee of Pathology

The main goals of this committee are (1) to standardise further and update diagnostic terminology as used by pathologists of the EULEP member laboratories, and (2) to increase the expertise of members on various morphological aspects of late effect studies in laboratory animals, including the application of new techniques.

The committee organised a half-day symposium on "Molecular-Biological Methods in Pathology" at Reisensburg. The topics covered included a description of the principles, practice and applications of PCR, the principles and applications of *in situ* hybridization, and immunocytochemical methods for the location of DNA adducts in sections.

The annual slide seminar was on "Exocrine Pancreas and Salivary Glands". Three lectures on lesions in human tissue provided background information on current classification practice, special stains and immunohistochemistry. Experimental reports included detailed descriptions of preneoplastic and neoplastic lesions in the pancreas of rats, mice and hamsters, illustrations of retrovirus and radiation-induced lesions in experimental animals, and presentation of some controversial results derived from a rat pancreatic carcinoma model.

Two new fascicles of the EULEP Pathology Atlas were published (see list of publications below). A further fascicle, "Haematopoietic neoplasms in the mouse and rat including LGL lymphoma and nonlymphoid neoplasms" by C.H. Frith, is in preparation. The committee is currently considering the possibility of having a new colour version of the Atlas published commercially. Discussions are also under way with the initiators of the Beagle Pathology Atlas, to be printed by the US DOE, on possibilities for co-operation in the form of joint symposia etc.

Committee of Cell and Molecular Biology

The committee's aim for 1990 was to intensify the efforts to provide a valuable technical and scientific background for EULEP laboratories involved in molecular and cellular projects.

As a major step in this direction a chemical synthesis project for oligonucleotide probes has been established in the Department of Molecular Biology and Plant Physiology at the University of Aarhus. The project started with the production of a stock of oligonucleotides from sequences of endogenous murine retroviruses. A database for these sequences has also been established and made accessible to EULEP laboratories. The database presently contains 1200 oligonucleotides. By 1st July 1991 an updated version containing 1500 oligonucleotides will be available. The project started with this core activity and has been expanded to offer EULEP members free synthesis of any given oligonucleotide needed. The service has been used already by some laboratories: from April 1990 to February 1991 a total of 123 oligonucleotides were prepared and distributed.

To introduce molecular methods further into the framework of EULEP activities, a practical course of PCR methods has been planned at the laboratories of the GSF for spring 1991.

Expert Group on Physiological Methodology

During the last year the committee was concerned with two projects:

(i) Late effects after total body irradiation for bone marrow transplantation. Consideration has been given to questions relating to the standardisation of functional tests in patient follow-up including dose distribution and the targets at risk in terms of expected functional disturbances. So far the functional evaluation reported by the clinicians is restricted to serum tests and the ejection fraction of the left ventricle.

(ii) Late effects after local irradiation of the heart. The group is contributing to the work of the task group on radiation effects on the heart (see below). Essentially three types of technique are in use. These are being validated and radiation-induced variations in cardiac output are being correlated.

(b) CO-ORDINATION AND PROMOTION OF CO-OPERATIVE RESEARCH

Some of the more significant aspects of progress achieved by the task groups are as follows:

Molecular Approach to the Study of Radiation-Induced Osteosarcoma

Irradiation of mice with osteosarcomagenic doses of alpha-emitting radionuclides is followed by the activation of endogenous retroviruses (proviruses). Several activated endogenous

retroviruses (murine leukaemia viruses, MuLV) have been shown to induce osteomas and osteopetrosis together with malignant lymphomas with varying incidences. The mechanisms of MuLV-induced bone lesions are still largely unknown. To facilitate further investigations on virus effects on skeletal cells, a prototype osteoma-inducing retrovirus, the RFB-virus, was cloned. Following infection as neonates, CBA mice developed multiple osteomas; in addition to these benign bone tumours NMRI mice also developed osteopetrosis and malignant lymphomas. These findings showed that the molecularly cloned RFB virus exerts the same pathogenic effects on the mouse skeleton as other molecularly cloned MuLVs isolated from radiation-induced osteosarcomas as well as from normal mouse tissues. The U3 region of the RFB LTR showed a close structural relationship with that of the lymphomagenic SL3-2 virus, suggesting that additional sequences in the viral genome are responsible for the bone-pathogenic characteristics of RFB virus.

The co-operation of activated endogenous retroviruses with the fos oncogene in the development of malignant bone tumours was studied in c-fos transgenic mice. C3H mice, carrying the transgene MT-c-fos-LTR, develop sarcomas with varying proportions of chondro-osseous differentiation. C-fos transgenic tumours show close similarities with osteogenic sarcomas induced by the FBR osteosarcoma virus (FBR MSV). Both types of tumour and cell lines derived from them expressed variable levels of the fos oncogene. Expression of endogenous retroviral sequences in transgenic tumours suggested an interaction of activated proviruses with the transgene in osteosarcomagenesis.

Cell and Molecular Studies on Radiation-Induced Haemopoietic Neoplasias

During the year the members of the task group continued to exchange DNA probes. Plans for a joint study of changes in DNA methylation patterns in a new set of radiation-induced murine acute myeloid leukaemias (AMLs) were finalised. These studies will focus on the interleukin 1 gene region which lies close to one of the most frequently observed chromosome 2 breakpoints in AMLs; evidence for changes in this region have already been obtained in 3/6 chromosome 2 - rearranged AMLs.

A total of 23 AMLs induced in CBA, SLJ and NFS mice were screened for all ras gene mutations known to confer transforming activity. Only one AML was found to carry such a mutation and it was concluded that, whereas ras mutations are a common feature of human AML, this is not the case for the equivalent murine neoplasm.

Detailed statistical analyses of chromosome 2 breakpoints in radiation-induced CBA AMLs and those seen in X-irradiated and repopulating normal marrow have been completed. These analyses further support the contention that specific chromosome 2 deletions are initiating events for AML. In addition evidence was obtained that chromosome 2 breakage is non-random and that breaks cluster strongly at specific radiation-sensitive sites on the chromosome. Molecular studies were initiated to explore a possible structural relationship between these sites and telomere-like DNA repeat sequences.

A new population of thymocyte precursors has been detected in irradiated C57Bl mice; this population was characterised by the acquisition of a capacity for self-renewal. In addition, studies have continued on the mechanism through which grafted marrow inhibits the development of lymphoma. These have provided further evidence that the production of tumour necrosis factor-alpha in the thymus may be involved in the inhibitory process.

Cell Biology of Haemopoietic Tissues

A collaborative project is under way, dealing with the induction and characterisation of radiation-induced myeloid leukaemia in NFS mice. The NFS strain has been selected because it lacks endogenous ecotropic retrovirus. Thus, recombinant retroviruses that contain an ecotropic-derived fragment cannot be generated. It has been confirmed that such mice do develop thymic lymphomas after 4 times 1.75 Gy.

The question then arose whether such mice could develop lymphomas with the indirect protocol (thymectomy - irradiation - thymic graft). Leukaemia was observed but not of the lymphoid type, indicating that endogenous ecotropic proviruses are not required to develop thymic lymphomas when the classical, direct irradiation protocol is used. In contrast, endogenous ecotropic proviruses may well play a role in the indirect model. The leukaemias obtained using the indirect protocol are presently under detailed investigation. It has been possible to adapt them to culture, without exogenous growth factors, and to establish clonal cell lines. These lines have no T-cell markers, they react with monoclonal antibodies characteristic of pre-B lymphoblasts and have a granulocytic appearance. They may possibly be good models for the human chronic myeloid leukaemia.

A related project is the characterisation in such cell lines of membrane receptors for neurotransmitters coupled to adenylate cyclase. Most remarkable is the presence of receptors for the calcitonin-gene-related peptide (CGRP). Studies are in progress, on the one hand to monitor the effects of CGRP on such cells, and on the other hand to study the effects of differentiation-inducing agents such as retinoic acid on the expression of receptors.

Cellular Basis of Late Vascular Changes in the Areas at Risk in the Irradiated Brain

The present programme consists of two complementary lines of research. The first one is the continuing investigation concerning the identification of the dose-limiting constituent in the irradiated rat brain by means of a variety of morphological and physiological methods. The second line is aimed at preventing this kind of radiation-induced change.

Further meticulous analysis of the morphological changes emphasized that minor changes in the astrocytes surrounding the blood vessels in the white matter should, with the existing techniques, be considered as the first detectable sign of damage in the parenchyma of the brain. This precedes further morphological changes. In addition, it was noted that some types of rat strain may be more sensitive to this kind of change than others. No obvious explanation could be found for this unexpected finding, which will be investigated further. In the physiological domain it was reported that no recruitment of vessels took place after irradiation, and no enlargement of the capillary network.

In the research project designed to ameliorate the development of this type of late radiation damage, the first animals have been treated with irradiation, followed by long-term administration of the drug Pentoxifylline. Discussions have been held with the manufacturer (Hoechst) concerning the optimal means of administration, the pharmacokinetics and toxicity. It was decided that a moderate dose, given over very prolonged periods of time, was well within the achievable limits.

Radiation Effects on the Heart

Three techniques are now in use to assess changes in cardiac function in the rat after irradiation. These are: an *in vitro* isolated heart preparation; a non-invasive *in vivo* method using a single crystal detector for external radioactivity measurements; and a γ-camera method as used clinically which allows the evaluation of the left ventricle ejection fraction. These techniques are complementary to each other; measurement of cardiac output is being standardised between them. Dosimetry standardisation, using TLV implants, has also been planned.

The criteria for radiation-induced heart disease have been explicitly defined for use by the participating laboratories. Functional changes have been compared with histological studies in the rat heart. There is generally good agreement between experimental findings in the rat and clinical observations.

Effects of Radiation of Pre-implantation Mouse Embryos

Studies have been performed on different mouse strains and also on guinea pigs, dealing with the radiosensitivity of the female germ cells as well as the pre-implantation embryo. The radiosensitivity of the cells has been evaluated by testing the effects of radiation on various parameters.

Studies on the induction of malformations in the mouse after exposure of pre-implantation embryos have now been extended to exposure at oogenesis stages. After doses of 2 Gy or more, a statistically significant increase in the number of malformations has been observed; the effect is dose rate dependent. Parallel studies have commenced using the guinea pig, which is thought to be a useful model for the human. Chromosomal aberrations are also being studied in both species. For this purpose, a method has been developed for culturing guinea pig oocytes and obtaining preparations of their metaphase chromosomes. Relationships have been studied between malformations, the protein pattern, and chromosomal aberrations in the mouse. The results support the hypothesis that the mechanism of induction of malformations after irradiation during oogenesis or the pre-implantation stage is related to the general labilization of the genome.

Extensive studies have been performed to investigate the adaptive response in pre-implantation mouse embryos exposed to low doses. The results obtained depended on the strain of mouse used, as well as on the experimental conditions.

Effects of Radiation on the Development of the Central Nervous System

The end-points studied comprised neuronal damage, neuro-structural damage, growth retardation and neuro-functional disorders. The timing of exposure to X-rays or neutrons was focused on the stages of enhanced sensitivity for the induction of long-term effects, ie the period of advanced organogenesis, early fetogenesis and the early post-natal stage.

Naturally occurring post-natal cell death has been examined in rats after exposure to 0.5 or 1 Gy X-rays. The numbers of dead cells in the cortical sub-plate (future white matter) was reduced after 1 Gy: these dead cells are probably the remnants of transitory populations of neurons, and can therefore reflect changes in the development of the cerebal cortex.

Cell surface glycosyl residues are known to play an important role during differentiation and development. Useful studies have continued on the pattern of specific binding sites for various lectins in embryonic mouse tissues. Radiation effects have been seen following doses as low as 0.25 Gy: binding of lectins to both endothelial cells within the CNS and to ependymal cells was enhanced. Such low doses have also been shown to affect neurotransmitter uptake by rat brain neurons cultured *in vitro*.

Studies have continued on structural damage and growth retardation in the developing brain in mice and rats. Permanent brain atrophy has been induced after only 0.025 Gy of neutrons on day 15 post-conception. Growth responses to irradiation were not dependent on dose rate for X-rays or neutrons in the rat, in contrast to previous studies on the mouse. Finally, preliminary evidence has been obtained for an effect of 1 Gy X-irradiation to the mouse fetus on the subsequent performance at 60 days of age in a maze test.

Radiation-Induced Carcinogenesis in the Liver

A carcinogenesis study has been completed on the effects of X-rays alone or combined with diethylnitrosamine (DEN) or CCl_4. Irradiation before or after administration of DEN did not affect the number or time of appearance of liver tumours.

A second study is in progress on the effects of 3 MeV neutrons and DEN on induction of liver cancer in C57Bl mice. The RBE for neutrons given at 7 or 21 days of age on life-shortening and causes of death is being determined.

These results are being compared with the induction of liver tumours in Wistar rats. 1 MeV neutrons and γ-rays are being compared; tumour induction has been shown to be sensitive to dose rate.

Interspecies Comparison of Lung Clearance

Studies have continued, the aim of which is to explain important interspecies differences in the rate of translocation to blood of dissolved cobalt oxide particles. The extent of retention in lung tissue of ionic cobalt has been investigated in the rat, guinea pig, dog and baboon: all the data in this comparison have now been collected.

The rate of dissolution of cobalt oxide particles in alveolar macrophages has also been compared in short-term culture *in vitro*. Rat macrophages proved more difficult to maintain for two weeks in culture than macrophages from either dog or baboon. However, dissolution rates in rat macrophages have now been obtained. Studies have also continued on the intraphagolysosomal pH of alveolar macrophages of different species. Some evidence suggests that there may be a correlation between pH and the rate of particle dissolution in the macrophage.

Extensive consideration has been given to the possibility of another interspecies comparison *in vivo*, using particles of a contrasting material such as uranium oxide. The behaviour of uranium oxide particles in macrophages *in vitro* differs markedly from that of materials such as cobalt oxide.

Deposition and Clearance of Inhaled Particles in the Human Respiratory Tract

This new task group has been recently formed in conjunction with EURADOS. It is primarily concerned with dosimetric modelling for inhaled radioactive particles. Initial activities have included a review of progress on the new ICRP lung model, especially relevant information on respiratory tract physiology for different age groups, and also the different ways of calculating regional particle deposition. The problems remaining in connection with particle clearance centre mainly around delayed clearance from the bronchi. Studies on man have continued to underline the importance of this phenomenon, and investigations have recently been made with non-radioactive particles suggesting that most of the inhaled bolus, which is subject to delayed clearance, remained in the conducting airways. Parallel studies are being made in the dog, using radio-labelled particles and a γ-camera. Meanwhile experiments on the rat have shown that far more particles remain on the epithelial surface of the trachea after inhalation than can be accounted for by rapid mucociliary clearance.

Studies have been initiated on a sensitivity and uncertainty analysis of the new ICRP lung model, aimed at ascertaining how robust the model is with reference to some of the model parameters, and also to identify where further research is needed to improve the reliability of the model.

The Reduction of Risk of Late Effects from Incorporated Radionuclides

Further studies have been made with DFO-HOPO and DTPA-DX on the removal of ^{238}Pu from rats after its intravenous injection. The effectiveness of DFO-HOPO after prompt oral administration was about 30 times less than after subcutaneous injection of the same dosage. The results suggested that the intestinal absorption of DFO-HOPO was similar to that observed previously for DTPA, about 3%. Also the efficacy of injected DTPA-DX was essentially independent of whether it was administered as the free acid, or as the sodium, calcium or zinc salts. The efficacy of dihydroxamic derivatives of EDTA and DTPA were compared.

3,4,3-LIHOPO has been synthesised in a highly pure state. In an on-going study with rats on the efficacy of LIHOPO for enhancing the excretion of ^{238}Pu inhaled as the tributylphosphate complex, the retention of ^{238}Pu in the lungs, liver and skeleton was less than after the administration of DTPA using the same treatment regimen. Preliminary data indicated that the ligand also removed appreciable amounts of ^{241}Am from the body, in marked contrast to DFO-HOPO.

Some diphosphonate analogues of EDTA and DTPA have been tested for their ability to remove uranium from the rat. Some of the substances tested so far were moderately successful, but the retention of uranium in the body was still greater than 50% of that present in untreated animals. Studies have also been designed to test the efficacy of orally administered ZnDTPA for enhancing the excretion of ^{238}Pu and ^{241}Am inhaled as nitrate.

Stem Cell Studies after Contamination with Alpha-Emitters

In a collaborative project pregnant mice have been contaminated with ^{239}Pu at various stages of gestation. There was a wide disparity (> 100 fold) in uptake and retention of ^{239}Pu by the

offspring of mice contaminated in early or late pregnancy. Although the resultant damage to haemopoietic stem cells was the same in both cases, the deficiencies in number were the result of different mechanisms: the low level of retention following early contamination (4d) had a direct effect on the stem cell population while the higher level retained from later contamination affected the stem cells' regulatory stromal microenvironment. On the basis of these studies, a long-term leukaemogenesis study has been initiated but to date, no leukaemias have been recorded.

In a comparable study with ^{241}Am, an additional group was included in which the father was contaminated prior to conception. All groups including the latter one had reduced levels of committed haemopoietic progenitor cells and increases in at least one of the stromal components. Paternal contamination with ^{239}Pu had failed to affect haemopoiesis, but further experiments with more comparable timings have been initiated.

Two new groups from Madrid have recently joined the task group. One is measuring the functional capacity of granulocytes after radiation-induced haemopoietic damage. A greatly elevated superoxide anion production level has been observed, also a complementary production of growth factors by the haemopoietic microenvironment. The other group is studying differentiation of haemopoietic stem cells in contaminated animals by the incorporation of the neomycin-resistant gene into purified stem cell populations using the pXTI retrovirus.

Metabolism, Dosimetry and Effects of Bone-Seeking Radionuclides

A number of co-ordinated studies have continued on the spatial distribution of radionuclides in bone. The dose rate from incorporated ^{239}Pu to the endosteum of rat bone varied considerably in different parts of the skeleton. The distribution also depended on the amount of ^{239}Pu injected.

A large investigation has continued into the distribution and consequent effects of different actinides, where the dose distribution in bone varied but the average dose was approximately the same. The dose to bone marrow increased with time for ^{239}Pu but very little ^{233}U was seen in marrow.

Radionuclide distribution in bone has also been measured for certain accident cases and patients. In the case of thorotrast, the material was found principally in the cellular bone marrow and hardly at all in yellow marrow. Useful data predicting the long-term retention of radium in bone has been gained from volunteer studies with ^{133}Ba. Studies have also continued on natural levels of α-particle emitters in human tissues, including measurements on fetal tissues. The hypothesis has also been explored that environmental levels of radon can result in significant α-particle irradiation of bone marrow, as a result of radon solubility in fat droplets in the marrow.

On-going studies of late effects in mice have questioned the assumption that the effects of two radionuclides administered at the same time are the sum of the effects of each radionuclide given separately. In the CBA mouse it is now very clear that there is a level of administered ^{224}Ra where the incidence of myeloid leukaemia is considerably greater than that of osteosarcoma. Experiments are continuing with multiple injections for studying the

effect of a reduced dose rate. Finally, it now appears that patients treated with ^{224}Ra for ankylosing spondylitis have shown a significant increase in the incidence of leukaemia.

Fetal Dosimetry and Effects of Incorporated Radionuclides

Several large studies are being co-ordinated, in the light of considerable interest in fetal dosimetry eg by ICRP. It has been possible to measure α-radioactivity in human autopsy samples from 18 weeks gestation to term. The major radionuclide was ^{210}Po; the distribution of α-activity was determined autoradiographically with CR-39. In experimental animals ^{238}Pu was retained in the fetoplacental unit to a degree which increased with advancing gestation. Transfer to the embryo or fetus was however limited; at later stages it accumulated primarily in the skeleton.

The effects of radionuclides on the fetus have concentrated firstly on damage to the developing CNS by ^{125}I, comparing sodium iodide with iododeoxyuridine. In both cases, damage to the cerebral cortex was much less than observed after exposure to external irradiation. Secondly, age-dependent haemopoietic responses have been assayed in mice treated with ^{239}Pu. Depletion of pluripotent stem cells was compensated by over-production of committed progeny, resulting in a normal output from the marrow. Later in gestation, the stem cells were unaffected but direct damage to the microenvironment resulted in their poor growth.

Retention and Absorption of Ingested Radionuclides and Irradiation of the Gastro-Intestinal Tract

The aim of this task group is to provide experimental data for use in radioprotection and to define specific risks associated with radionuclide ingestion.

A human volunteer study to measure the absorption of ^{239}Np and ^{242}Cm has been completed. Administration of the citrate complexes to five subjects resulted in f1 values of about 2×10^{-4} for both nuclides.

Factors affecting the absorption of ^{210}Po are being studied using rats and guinea-pigs. Results obtained for the fractional absorption of polonium after administration as the nitrate, citrate or incorporated into liver were in the range 0.06 - 0.13. Experiments with primates have shown that ingestion of plutonium with soya-bean increased absorption by about a factor three compared with values for plutonium nitrate but no increase was observed for plutonium incorporated into winkles. The tissue distribution of plutonium after administration in biologically incorporated forms was different from that observed after ingestion of the nitrate or the citrate: most plutonium was retained in the skeleton with less than 20% in the liver.

In neonatal baboons, absorption of plutonium administered as the citrate was as high at 2% in some individuals. Increased absorption appeared to be due to uptake in distal epithelial cells which contained an apical canalicular system connected to transport vacuoles. The number of these cells, characteristic of fetal intestinal epithelium, varied considerably between individual animals and was related to the observed differences in absorption of about an order of magnitude.

(c) SYMPOSIA AND TRAINING ACTIVITIES

The external EULEP symposium in 1990 was held in Oxford on "The Role of the Alveolar Macrophage in the Clearance of Inhaled Particles". This attracted some 80 participants. In addition, a symposium was organised at Reisensburg on "Molecular-Biological Methods in Pathology". The 1991 symposium at Reisensburg was on "Molecular Markers for Differentiation".

Training activities in 1990 included numerous inter-laboratory exchange visits for communicating expertise from one laboratory to another; other activities with a training component organised by the standardisation committees are outlined above.

Finally, EULEP has commenced an investigation into the possibility of providing course material for the teaching of radiation biology. Current practice in nine EULEP institutes is being reviewed. It is now aimed to produce material for four hours of lectures which could be made available for teachers of medical students including radiologists.

(d) PUBLICATIONS

EULEP Newsletter: Six issues were published during 1990 (Nos. 55-60).

EULEP Pathology Atlas: 2 new chapters were published:

(i) Preneoplastic and neoplastic lesions of the kidney of the rat, by P. Bannasch and H. Zerban;
(ii) Neoplastic lesions of the mouse lymphoid system, by P.K. Pattengale.

Broerse, J.J., Dutreix, A., and Noordijk, E.M., Proceedings of international meeting on Physical, Biological and Clinical Aspects of Total Body Irradiation, Radiotherapy and Oncology 18, supp.1, 1-162, 1990.

Proceedings of EULEP symposium on Skin - Its Relevance in Radiation Accidents and Radiological Protection, organised by J.W. Hopewell, International Journal of Radiation Biology 57, 737-896, 1990.

Contract: Bi7-032 Sector: B11
Title: Biophysical models for the effectiveness of different radiations.

1	Paretzke	GSF Neuherberg
2	Goodhead	MRC Radiobiological Unit
3	Terrissol	ADPA
4	Leenhouts	RIVM

I. Summary of Project and Global Objectives

This project carries out experimental and theoretical research with the aim of achieving a better understanding of the biological effects of different radiation fields with particular emphasis on low doses and low dose rates. It is designed to arrive at an improvement in our present knowledge of somatic and genetic radiation risks in man, and to help develop radiation protection instrumentation which measures the characteristic properties with regard to these endpoints in mixed radiation fields. In addition, the combined action of radiation and chemicals (also of those prevalent in the environment) will be investigated on a mechanistic level. This goal shall be reached by the development of new models based on;

- the improvement of biophysical track structure calculations for relevant radiation fields (photons, neutrons, electrons, ions) in particular by introducing structured cell geometry, condensed state cross sections, time dependency, and chemical and biological reactions; various codes of other authors will be compared in critical bench mark calculations;
- the analysis of such physical-chemical-biological track structures will be improved using new cluster algorithms and by testing biophysical models developed by participants 3 and 4.
- selective radiation biological experiments with soft X-rays and UV-photons will be performed, as well as with alpha-particles and gamma-rays; the biological systems will include appropriate transformational and inactivation assays, etc.

The usefulness of a better understanding of radiation effects on members of the public has often been emphasised as being highly desirable in the radiation protection literature. This understanding is necessary also to improve the protection of workers and the public in the ALARA-sense of the ICRP, where over-estimates of radiation risks might lead e.g. to a not optimum allocation of large resources.

Collaboration is foreseen with other projects working on the improvements of dosimeters and on biological radiation effects.

Partner 1 (GSF) will improve the physical track structure codes for fast electrons, photons, neutrons, protons, alpha particles and HZE ions encountered in space, he will introduce complex geometry describing structured targets and condensed state cross sections for the DNA and other relevant biological molecules.

Partner 2 (CPA) will improve the cross sections for slow electrons and apply a different approach to condensed state cross sections (to permit a sensitivity study), he will be the leading partner in the intercomparison of results from various other track structure codes in a benchmark and validation study, and he is responsible for the introduction of the chemical reactions in the common code.

Partner 3 (MRC) will analyse dedicated track structure calculations to test and improve his biophysical model for radiaiton actions of different fields, he will accumulate for the comparison of theory and experiment selected radiobiological data from the literature and will perform own experiments with soft X-rays, alpha particles, etc. using appropriate combinations of genetic, chromosomal, transformational and inactivation assays.

Partner 4 (RIVM) will analyse results from dedicated track structure calculations in the framework of his DNA damage model, will try to make a sensitivity analysis of model parameters to understand their significance and the influence of different irradiation conditions. He will investigate the interaction of radiation with other DNA damaging agents to better understand the influence of such agents on the effects of low radiation doses. He will experimentally study effects of UV radiation of different wavelengths and the interaction with damage from gamma irradiation. The predictions of models of partners 3 and 4 will be compared.

All partners finally will try to derive conclusions regarding the quantification of stochastic risks at low doses and dose rates.

Head of Project 1: Dr. Paretzke

II Objectives for the reporting period

(a) Calculation of secondary electrons produced in a water molecule and in a water cluster by proton and electron impact to investigate the influence of physical state on double differential ionization cross sections.
(b) Generalization of results to obtain an empirical analytical set of cross sections for water and other biological targets (e.g. DNA).
(c) Testing of the complex geometry routines simulating a lymphocyte.
(d) Calculation with PARTRAC of SSB, DSB, and fields of dicentric chromosomes taking chemical reactions and diffusion into account and using simple models of DNA interaction.

III Objectives for next period

(a) It is intended to extend the cross section calculations for electrons and protons to DNS and other molecules of biological interest. For this purpose it is intended to use density functional theory to calculate the electronic properties and electron density of regions of these large molecules, i.e. the DNA bases, because this theory can handle at least 1000 atoms. It is also intended to calculate cross sections for α-particles, light and heavy ions which introduces the problem of including in the calculation the effect of projectile electrons.
(b) In order to understand the experimental data on induction of dicentric chromosomes, detailed simulation of the movement of chromosomes inside the cell nucleus is needed, during and after irradiation. An attempt will be made to include this process in PARTRAC.

IV Progress achieved including publications

Progress has been made in two areas

(a) Calculations of inelastic cross sections for Monte Carlo codes.
A comprehensive set of calculations of the double and single differential cross sections for secondary electron emission as a function of angle and secondary electron energy have been completed for the case of proton impact on a water molecule and a clustser of water molecules[1] using methods developed for electron impact [2]. These cross sections have been integrated to yield the total cross section as a function of incident energy and the energy loss per unit path length. The calculations have been carried out over an extended incident energy range of protons from 10 keV to 10 MeV. The central molecule of the cluster can be compared to a molecule in liquid water. In this manner the change in the cross sections due to the change in phase has been investigated. Results for the double and single cross sections are shown in Figs. 1 and 2. The energy loss is shown in Fig. 3. The theoretical results have been compared to experimental results and to an empirical model developed by Rudd.

A comprehensive empirical model for electron cross sections for secondary electron emission from water has been developed and has been extended into the relativistic energy range. The necessary analytic formulae for this region have been derived. This model written as a subroutine will provide the necessary input of cross sections for our PARTRAC Monte Carlo

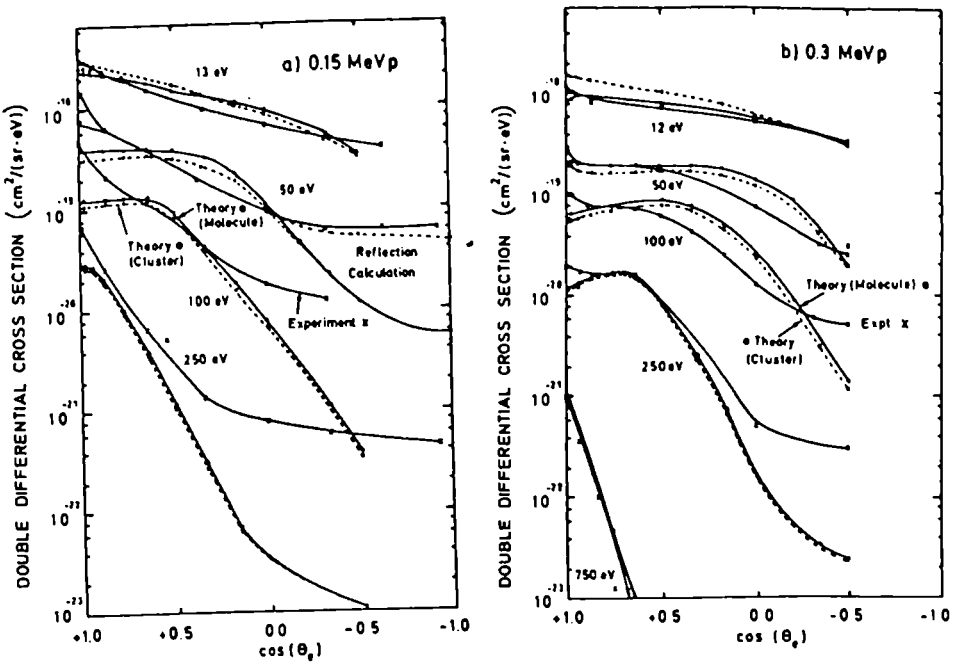
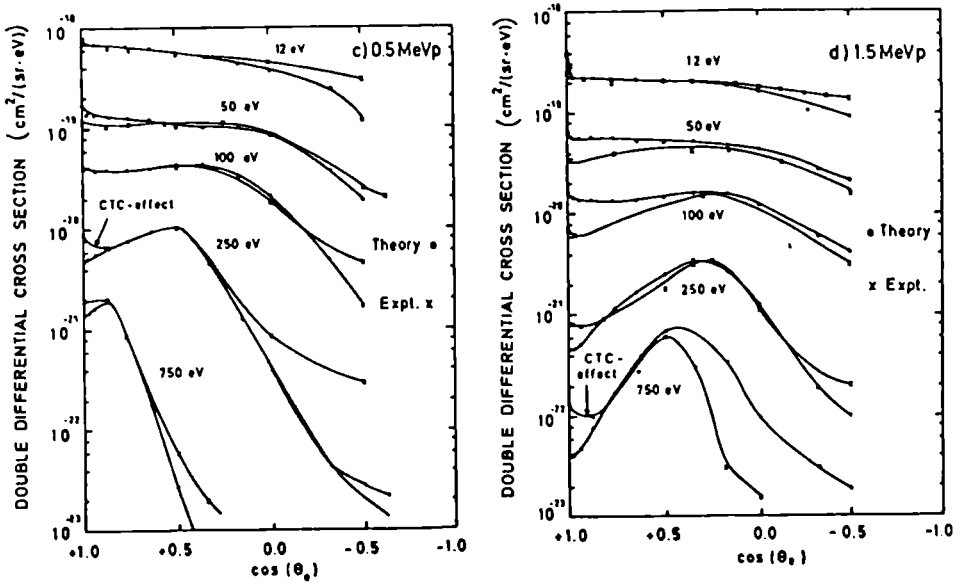
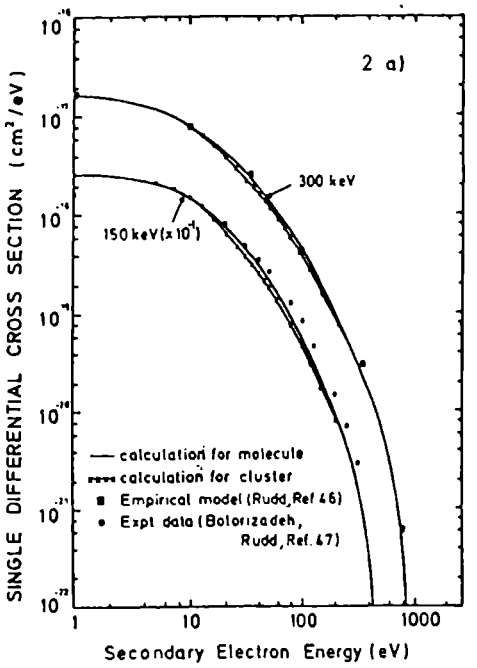
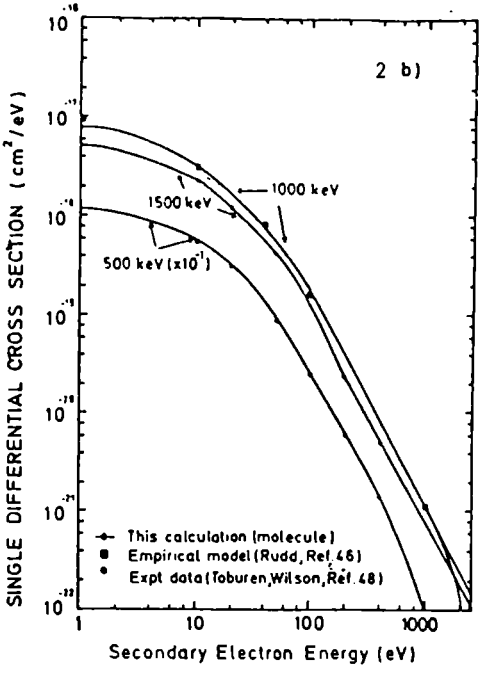

a) 0.15 MeVp
b) 0.3 MeVp
DOUBLE DIFFERENTIAL CROSS SECTION (cm²/(sr·eV))
cos (θe)
13 eV
50 eV
100 eV
250 eV
750 eV
12 eV
Theory e (Molecule)
Theory e (Cluster)
Reflection Calculation
Experiment x
Theory (Molecule) e
Expt x
e Theory (Cluster)

Fig. 1


c) 0.5 MeVp
d) 1.5 MeVp
DOUBLE DIFFERENTIAL CROSS SECTION (cm²/(sr·eV))
cos (θe)
12 eV
50 eV
100 eV
250 eV
750 eV
CTC-effect
Theory e
Expt. x
e Theory
x Expt.


2 a)
300 keV
150 keV(x10⁻¹)
calculation for molecule
calculation for cluster
Empirical model (Rudd, Ref 46)
Expt data (Bolorizadeh, Rudd, Ref. 47)
SINGLE DIFFERENTIAL CROSS SECTION (cm²/eV)
Secondary Electron Energy (eV)


2 b)
1000 keV
1500 keV
500 keV (x10⁻¹)
This calculation (molecule)
Empirical model (Rudd, Ref. 46)
Expt data (Toburen, Wilson, Ref. 48)
SINGLE DIFFERENTIAL CROSS SECTION (cm²/eV)
Secondary Electron Energy (eV)

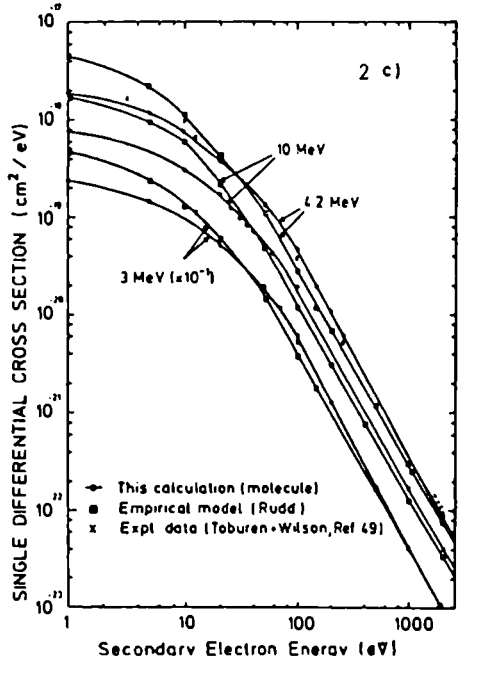
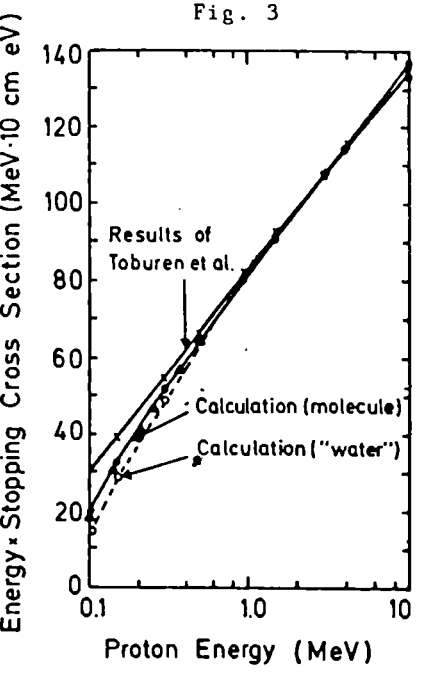

2 c)
10 MeV
4.2 MeV
3 MeV (x10⁻¹)
This calculation (molecule)
Empirical model (Rudd)
Expt data (Toburen+Wilson, Ref 49)
SINGLE DIFFERENTIAL CROSS SECTION (cm²/eV)
Secondary Electron Energy (eV)

Fig. 3


Results of Toburen et al.
Calculation (molecule)
Calculation ("water")
Energy x Stopping Cross Section (MeV·10⁻¹⁵ cm² eV)
Proton Energy (MeV)

code for electrons from 10 eV up to very high energies (100 MeV) and beyond. A relativistic extension to the model of Rudd for protons is also being prepared.

(b) PARTRAC-Development

During the reporting period, the computer code PARTRAC (PARticle TRACks) was developed to include modelling of induction of primary and secondary lesions (i.e. the formation of dicentric chromosomes) and was applied to simulate radiation effects on human lymphocytes induced by different photon radiation qualities.

For this purpose, a human T-lymphocyte has been simulated Fig. 4) including its chemical and geometric structure (cytoplasm, nucleus,

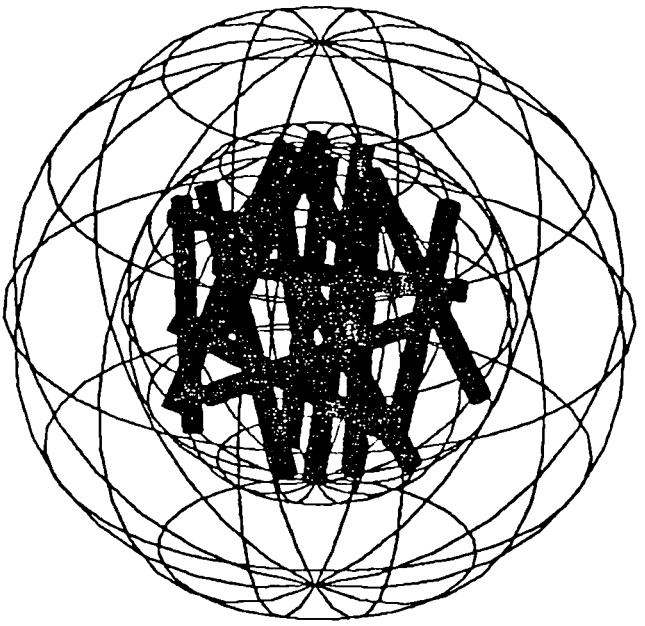


Fig. 4 Simulation of Chromosomes in a human lymphocyte

chromatin, DNA). For various X-ray sources (C-K (0.28 keV), Al-K (1.49 keV), 30-kV, 150-kV and 220-kV) track-structure calculations within a lymphocyte and its environment were performed to study the radiation damage to DNA. For that reason, direct energy deposition events within DNA and indirect effects by OH-Radicals produced in the surrounding water were taken into account (Fig. 5).

$$OH^{\bullet} + H^{\bullet} \rightarrow H_2O$$
$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2$$
$$OH^{\bullet} + e^-_{aq} \rightarrow H_2 + H_2O_2$$
$$e^-_{aq} + e^-_{aq} \rightarrow H_2 + 2OH^-$$

$$H^{\bullet} + H^{\bullet} \rightarrow H_2$$
$$e^-_{aq} + H^{\bullet} \rightarrow H_2 + OH^-$$
$$e^-_{aq} + H_3O^+ \rightarrow H^{\bullet} + H_2O$$

$$OH^{\bullet} + \text{Desoxy.} \rightarrow \text{ESB}$$
$$OH^{\bullet} + \text{Base} \rightarrow \text{PMT}$$

Species	PARTRAC	Zaider	Chatterjee	Turner
H_3O^+	4.7	4.7	5.0	6.3
$\dot{O}H$•	5.3	6.0	5.9	8.4
H•	1.7	0.8	0.9	2.1
e^-_{aq}	4.8	4.7	5.0	6.3

Fig. 5 Table for comparison of chemical yields in water as calculated by different models

For the different radiation qualities, dose effect curves were calculated for DNA single- and double strand breaks.

The spatial distribution of the DNA double strand breaks which are the primary lesion for chromome aberrations was analysed. Assuming a decreasing probability for production of dicentric chromosomes with increasing distance of the primary lesions, the efficiency of the different radiation qualities in producing dicentric chromosomes via intratrack- and intertrack effect was simulated and compared to experimental linear- and quadratic coefficients of the dose-effect curve (Fig. 6). For Al-K, for example, a pronounced decrease in the linear coefficient was observed.

To study dose rate effects, a dose fractioning experiment with 150 kVp X-rays was simulated to compare the effects on the linear and quadratic component with experimental data. In consistency with the experimental data, the linear component was not affected by the fractionation.

Publications

[1] K.A. Long and H.G. Paretzke. "Comparison of Calculated Cross Sections for secondary Electron Emission from a Water Molecule and Clusters of Water Molecules by Protons". J. Chem. Phys., in press, July, (1991)

[2] K.A. Long, H.G. Paretzke, F. Mueller-Plathe and G.H.F. Diercksen. "Calculation of Double Differential Cross Sections for the Interaction of Electrons with a Water Molecule, Clusters of Water Molecules, and Liquid Water". J. Chem. Phys. 91, 1569 (1989)

[3] H. Nikjoo, D.T: Goodhead, D.E. Charlton and H.G. Paretzke. "Energy in Small Cylindrical Targets by Monoenergetic Electrons". International Journal of Radiation Biology (in press).

[4] H.G. Paretzke. "Biophysical Models of Radiation Action-Development of Simulation Codes". In: Early Effects of Radiation on DNA, M. Fielden, P. O'Neill, Eds. Springer Verlag, Heidelberg, 1991

[5] H.G. Paretzke. "Advances in Radiation Track Structure Calculations". In: Physical and Chemical Mechanisms in Molecular Radiation Biology, W.A. Glass, R. Wood, M. Varma, Eds. Dept. of Energy, USA, in print

[6] H.G. Paretzke, J.E. Turner, R.N. Hamm, H.A. Wright. "Spatial Distributions of Inelastic Events Produced by Electrons in Gaseous and Liquid Water". Radiat. Res., in print

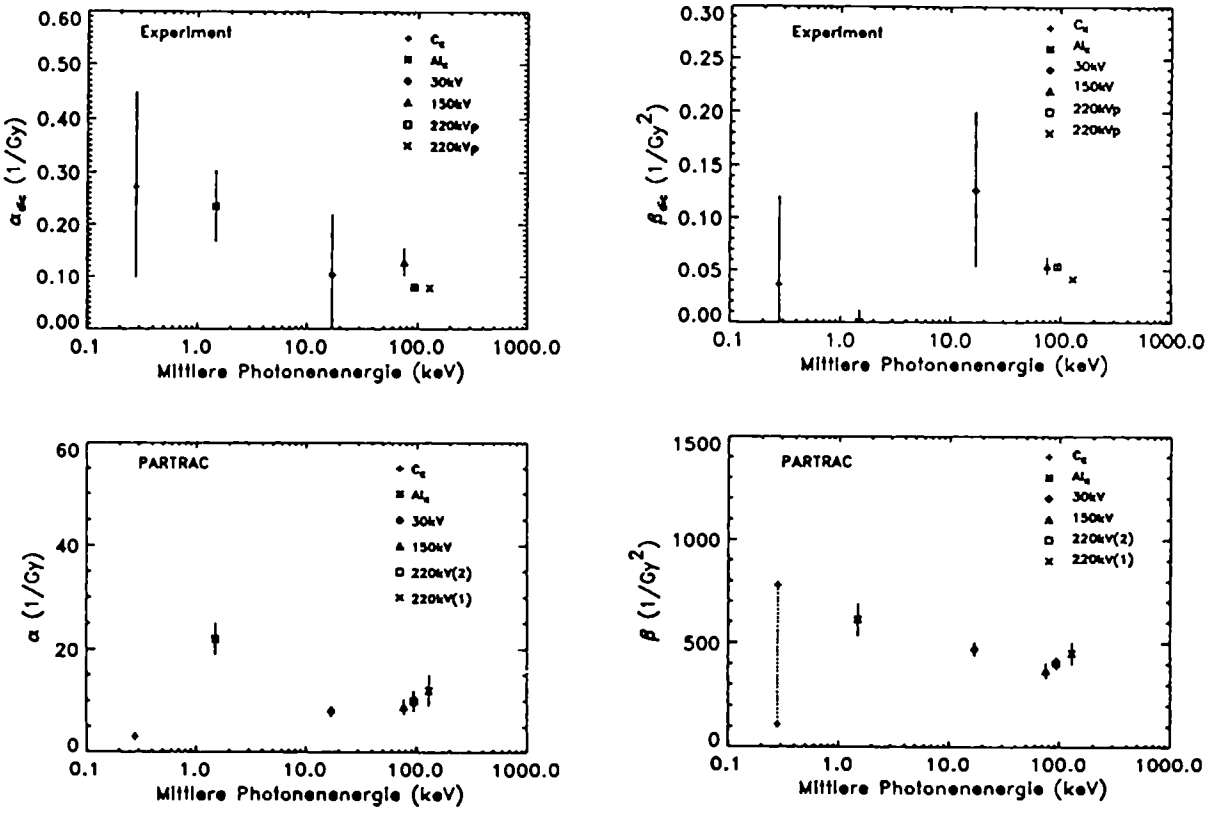


Fig. 6 Experimental and calculated linear and quadratic dose coefficients for the induction of dicentric chromosomes by different photon fields.

Figure Captions

Fig. 1 Double differential cross sections for proton impact on a water molecule calculated (•) at a proton energies of 0.15 (a), 0.3 (b), 0.5 (c), and 1.5 MeV (d), and on 'water' at 0.15 (a) and 0.3 MeV (6) at a selection of secondary electron energies as a function of the cosine of the secondary electron emission angle compared to the experimental results (only for the molecule) of Bolorizadeh and Rudd at 0.15 MeV and of Toburen and Wilson at 0.3, 0.5 and 1.5 MeV(χ).

Fig. 2 Single differential cross sections for proton impact on a water molecule calculated (•) at a proton energy of 0.15 to 10 MeV, and on 'water' (χ) at 0.15 and 0.3 MeV as a function of the secondary electron energy. The calculated results (•) are compared to the experimental results (•,χ), and to the empirical model of Rudd (■).

Fig. 3 Energy loss per unit path length multiplied by the proton energy plotted against the proton energy as compared to the experimental data (triangles) and calculations of Toburen and Wilson (crosses). The calculations for the molecule are represented by dots and those for 'water' by empty circles.

Fig. 4 Simulation of chromosomes in a human lymphocyte.

Fig. 5 Table for comparison of chemical yields in water as calculated by different models.

Fig. 6 Experimental and calculated linear and quadratic dose coefficients for the induction of dicentric chromosomes by different photon fields.

Head of Project 2: Dr. Goodhead

II Objectives for the reporting period

1. Simulation and scoring of monoenergetic electron tracks to generate frequency distributions of energy deposition in microscopic volumes.

2. Develop methods and obtain experimental data on biological effects of radiations with well-defined track structures.

3. Compare theoretical and experiment data to seek track properties of relevance.

III Objectives for next period

1. Compile and publish monograph of electron-scoring tabulations; extend to additional radiations of special interest; score and compare with tracks from other simulation codes and media.

2. Investigate induction by α-particles of $HPRT^-$ mutations in haemopoietic cells and V79 cells including the effect of reducing dose rate.

3. Evaluate the implications of track properties especially for low-level effects.

IV Progress achieved including publications

Progress has been made in three related parts of our component of the contract as follows:

(a) Track structure analysis

We have used the Monte-Carlo track structure code MOCA8b of Paretzke to compute distributions of the absolute frequencies of energy deposition by electrons in small cylindrical targets in water. For this purpose statistically representative numbers of full slowing down tracks, in water, of initially monoenergetic electrons were generated for initial electron energies at intervals from 0.1 to 100 keV. These were randomly sampled with cylindrical volumes (diameters 1 to 100 nm; lengths 0.5 to 64 times diameter) to obtain statistically stable frequency distributions of energy deposited within the cylinders, per Gy of absorbed dose to the macroscopic medium. A summary and discussion of these extensive data was published [1], including some comparisons with high-LET α-particles (4 MeV). The complete set of results are being prepared in tabular form for publication as a monograph, to accompany our previously published monographs on energy deposition in similar volumes by protons and α-particles of diverse energies and ultrasoft X-rays of selected characteristic energies. Together these monographs provide an extensive and consistent data base for comparison of the energy-deposition properties of different radiations in cylindrical target volumes including those of dimensions similar to subcellular biological structures such as DNA, nucleosomes and chromatin fibre.

The track structure code has also been used to compare the abilities of different low-LET radiations to deposit their energy in the form of low-energy secondary electrons. The results indicate that low-energy electrons of 0.1-5 keV account for about 30-50% of the total dose imparted to a medium irradiated by any conventional low-LET radiation [2] and hence that these electrons may play a dominant role in the biological consequences.

Our existing data base on frequencies of energy deposition by different radiations has been derived exclusively from the codes MOCA8b and MOCA14 of Paretzke and Wilson, which are based on water vapour cross sections with adjustment to the density of liquid water. We have initiated studies to test the generality of our data, and conclusions drawn from them, by carrying out identical scoring computations on tracks from the few alternative codes currently in existence or under advanced development. A selection of monoenergetic electron tracks generated by the liquid water code of Terrisol has now been obtained and prepared for this purpose.

The track structure codes are also being used to develop models of the initial stages of damage to DNA [3] with a view to incorporating chemical processes.

(b) Biological experiments on radiation-quality

To complement our theoretical studies of radiation track structure we are investigating their biological consequences in a number of ways. Alpha particles of about 3 MeV have LET in the region of maximum relative biological effectiveness for most effects. We have previously constructed, and now extensively characterised and described, a versatile irradiator for radiobiological studies with α-particles of about this energy [4]. It is based on a disc of plutonium-238 and allows irradiation, of thin biological samples, with an approximately parallel beam of monoenergetic α-particles in the track-segment mode. We have developed methods for irradiation of attached monolayers of cells or thin volumes of cells in suspension. Dose rates can be varied over wide limits, with the sample dishes being maintained under gassed and incubated conditions.

This irradiation is currently being applied to a variety of cell types including rodent fibroblasts and haemopoietic progenitor cells and human peripheral lymphocytes. Studies include the induction of $HPRT^-$ mutations, including the consequences of reducing dose rate. Preliminary indications are that this may cause an increase in the frequency of induced mutations, in contrast to the effect on inactivation or chromosome aberration frequencies.

Mechanistic interpretation of the effects of ultrasoft X-rays or α-particles, in terms of their individual tracks, requires accurate measurement of the dimensions of the cells, or their nuclei, under the exact conditions of irradiation. For this purpose we have developed methods of confocal microscopy to make measurements of thicknesses [5] and areas [6] on living individual cells in the monolayer cultures as irradiated. The thicknesses were compared with measurements on the same cell populations obtained by the more conventional methods of electron microscopy after fixation and sectioning [5].

(c) Comparative analysis of track properties with biological effectiveness

In the publications quoted above we have drawn a variety of inferences on the biologically critical features of radiation tracks from low- and high-LET radiations. The implications have been considered further in relation to potential classes of initial DNA damage and quantitative models of radiation action. We have suggested that a particularly important feature of tracks may be the very locally clustered ionisations/excitations that can produce complex damage to DNA and associated structures, even for low-LET radiations, and especially for high-LET radiations. We have also discussed the repairability of different types of measurable DNA damage and their possible relationship to cluster size and explored the consequences for low level radiation effects that are due to single tracks alone [9,10].

Publications

[1] H. Nikjoo, D.T. Goodhead, D.E. Charlton and H.G. Paretzke. "Energy in small cylindrical targets by monoenergetic electrons." International Journal of Radiation Biology (in press).

[2] H. Nikjoo and D.T. Goodhead. "Track structure analysis illustrating the prominent role of low-energy electrons in radiobiological effects of low-LET radiations." Phys. Med. Biol., 36, 229-238 (1991).

[3] H. Nikjoo. "What basis for the development of radiation-induced DNA damage." In: The Early Effects of Radiation on DNA. Eds. E.M. Fielden and P. O'Neill (Springer-Verlag, Heidelberg) (in press).

[4] D.T. Goodhead, D.A. Bance, A. Stretch and R.E. Wilkinson. "A versatile plutonium-238 irradiator for radiobiological studies with α-particles." International Journal of Radiation Biology, 59, 195-210 (1991).

[5] K.M.S. Townsend, A. Stretch, D.L. Stevens and D.T. Goodhead. "Thickness measurements on V79-4 cells. A comparison between laser scanning confocal microscopy and electron microscopy." International Journal of Radiation Biology, 58, 499-508 (1990).

[6] S. Marsden. "Non-invasive nuclear area measurements using confocal laser scanning fluorescence microscopy for the assessment of alpha particle inactivation of plateau phase V79-4 Chinese hamster cells." M.Sc. Thesis, University of St. Andrews (1990).

[7] D.T. Goodhead. "Models to link DNA damage to RBEs for final cellular effects." In: The Early Effects of Radiation on DNA. Eds. E.M. Fielden and P. O'Neill. (Springer-Verlag, Heidelberg) (in press).

[8] D.T. Goodhead. "Summary comments from a physicist." ibid.

[9] D.T. Goodhead. "Biophysical features of radiations at low doses and low dose rates." In: New Developments in Fundamental and Applied Radiobiology. Eds. C.B. Seymour and C. Mothersill (Taylor and Francis, London), pp.4-11 (1991).

[10] D.T. Goodhead. "Radiation tracks in biological materials: initial damage in cells, DNA and associated structures." In: Genes, Cancer and Radiation Protection. (NCRP, Bethesda) (in press).

Head of Project 3: Dr. Terrissol

II Objectives for the reporting period

During last years we have set up computer codes using stochastic models to obtain the complete transport and the chemical evolution of species created by incident radiation between 10^{-15} s. and seconds. This Monte Carlo code gives excellent results but, rather diverges (computer memory space and execution time), and it is more efficient to use concentrations to study the kinetics following the radiolysis. We have begun to set up a deterministic model to solve the set of coupled differential equations describing the temporal and spatial evolution of the reactive species. The written codes are now tested and methods and results will be presented in the next report.

A main objective of this first year was an intercomparison study of track structure codes. Since water media and low energy electrons play an important role in the analysis of radiobiological data, we have concentrated the comparison on low energy electron track simulation on liquid water and we present here some topics. The comparison has also be done for water vapor.

III Objectives for next period

We plan to achieve the intercomparison of track structure codes and try to extract some rules for the use of cross-section or input data in such or such situation.

The complete set up of the deterministic method for modelling the diffusion and reaction of radicals and ions produced by incident particle will be done, in order to check and help the stochastic code in special cases.

Basic input data and cross-sections used in the set up of codes will be searched for biological condensed media, rather than liquid water, and peculiarly for low energy electron.

IV Progress achieved including publications

To do the intercomparison of track structure codes, we have, during 1990, collected informations from research groups represented by : M. BERGER from NBS, Gaithesburg(USA); B. GROSSWENDT from PTB, Braunschweig(Germany); A. ITO from IMS, Tokyo(Japan); I. KAPLAN from KIPC, Moscow(USSR); H. PARETZKE from GSF, Munich(Germany); M. TERRISSOL from CPA, Toulouse(France); J. TURNER from ORNL, Oak-Ridge(USA)

As a matter of fact, these codes tend to obtain same kind of results, giving the data available to the radiobiologists, but are they giving same accuracy? Sure, mainly of the output of a code depends of the input data, but also of the hypothesis done to establish calculation algorithms. Different codes may be written as a function of the expected results with increasing accuracy (and complexity !) as far as one goes close to the atomic or molecular structures or tries to join the Physico-Chemistry and Biology. To obtain gross parameters or data useful for

tissues, bones or muscles studies, it is suffucient to use codes based on stopping power or continuous slowing down approximation, while an extensive set of differential cross-sections for all events must be involved for the atomic or DNA levels and radiobiological or radiochemical studies.

Six Institutes, GSF, NBS, KIPC, PTB, IMS and CPA, have written codes for water vapor; three of them apply the same code -same input data with density correction- to the liquid phase. Three Institutes : KIPC, ORNL and CPA, have written codes specially for liquid water, taking into account the physics and chemistry of the track.

There is no room here to present all the intercomparison -it will be soon published- we only present some topics on the comparison of real liquid water codes : their input data and some interesting results on species yields. In this energy domain and for condensed media, all is not well known and a set of uncontested cross-sections does not exist. So, depending of the age of the code, the model of computer used and the results expected, the analysts are doing assumptions and nobody do exactly the same. It is also possible that, since several months, a few points in the large cross-section sets and different assumptions made have been improved and inserted in the codes, but results presented have been obtained with the described set of cross-sections.

Cross-sections used for liquid water

Institute	Energy range	Cross-sections used
KIPC	< 200 eV	experimental data of Danjo-Nishimura assuming : liquid = vapor
	> 200 eV	Thomas - Fermi
ORNL	0 - 10 eV	experimental data of Itikawa assuming liquid = 0.6*vapor
	< 1000 eV	phase-shift Mott-Dirac formula
	> 1000 eV	Thomas - Fermi
CPA	0 - 8.4 eV	experimental data of Sanche-Michaud assuming liquid = ice
	up to 30 keV	phase-shift Mott-Dirac formula

Table 1 - Elastic cross-sections used in liquid water Monte Carlo codes.

Institute	Excitation state	Energy	Cross-sections used
KIPC ORNL CPA	A^1B_1	8.4 eV	Integration of energy loss function within the limits of each peak width, using differential oscillator strengths determined with dielectric response function derived from experiments
	B^1A_1	10.1 eV	
	Rydberg(A+B)	11.26 eV	
	Rydberg(C+D)	11.93 eV	
	Diffuse bands	14.1 eV	
	Collective	21.4 eV	
ORNL	Subexcitation electrons	< 8.4 eV	Stopping power calcultated with optical data and Fermi age theory
CPA	Subexcitation electrons	< 8.4 eV	Exper. cross sections of Sanche-Michaud, assuming liquid = ice

Table 2 - Excitation cross-sections used in liquid water Monte Carlo codes.

Institute	Ionization level	Cross-sections used
KIPC	Oxygen K shell	Asymptotic Bethe cross-section
	For outer shells :	
	8.76 - 25 eV	Use of dielectric response function and Jain-Khare semi-empirical cross-sections
	> 25 eV	
ORNL	Oxygen K and $1b_1$, $1b_2$, $2a_1$, $3a_1$ shells	Partitionning of the imaginary part of the dielectric function between the five levels with sum rules
CPA	Oxygen K-shell	Gryzinski cross-section
	For outer shells : $1b_1$, $1b_2$, $2a_1$, $3a_1$	Partitionning of the dielectric response function between the four levels

Table 3 - Ionization cross-sections used in liquid water Monte Carlo codes.

Results comparison for liquid water

The Institutes KIPC, ORNL and CPA have developped Monte Carlo codes, simulating water radiolysis taking into account the physical, physico-chemical and the chemical stages. As one can see in Tables 1-3 the basic input data are similar but nevertheless different. Such large codes make assumptions and use algorithms certainly close together but "self-specific" for many motives, so that comparisons cannot be done in a large scale.

Table 4 present initial ionization and excitation yields (about 10^{-15} second), resulting from the physical step. Differences may be due to the including or not the desexcitation scheme and then must disappear in Table 5, obtained after the physico-chemical step.

Institute	g_{ion}	g_{ex}
KIPC	6.04	1.43
ORNL	4.36	3.09
CPA	4.02	2.05

Table 4 - Initial radiation yields, for 5 keV electron in liquid water.

Institute	H_3O^+	OH	e^-_{aq}	H	H_2	H_2O_2	OH^-
KIPC	4.8	6.2	4.8	0.61	0.39		
ORNL	6.3	8.4	6.3	2.1	0.3		
CPA	4.51	4.8	4.5	1.5	0.4	0.98	0.01

Table 5 - Yields at 10^{-12} sec. for 5 keV electron in liquid water.

Table 6 is presented at 10^{-7} second, wihich is the beginning of a stationary phase. And for greater times it is then possible to calculate the so-called Fricke G value with :

$$G_{Fricke} = 2G_{H2O2} + G_{OH} + 3(G_H + G_{e\text{-}aq})$$

Table 7 shows Fricke G values calculated for 1 and 5 keV. The KIPC value is calculated at 10^{-7} second, with data of table 6.

Institute	H_3O^+	OH	e^-_{aq}	H	H_2	H_2O_2	OH^-
KIPC	3.78	4.65	3.32	0.85	0.62	0.80	0.46
ORNL	2.62	2.2	1.63	0.92	0.76	0.92	
CPA	2.6	1.5	2.05	0.95	0.9	1.45	0.55

Table 6 - Yields at 10^{-7} sec. for 5 keV electron in liquid water.

Institute	1 keV	5 keV
KIPC		18.7
ORNL	12.1	12.9
CPA	13.8	13.4

Table 7 - Calculated Fricke G values at 0.28 10^{-6} sec. in liquid water.

Head of Project 4: Dr. Leenhouts

II Objectives for the reporting period

- Interaction of radiation types: modelling of selected data sets of interaction of different types of ionizing radiation using the linear-quadratic dose-effect-relationship, as part of the use of the model to define the influence of other types of DNA damaging agents on the dose-effect relationship.
- Comparison of UV effects: use of other wavelengths to study the comparative effect and interaction of UV with ionizing radiation.
- Application of models to low-dose risk assessment: investigating the combination of the linear-quadratic dose relationship with the Knudson-Mollgavkar two-mutation carcinogenesis model in order to make the link between cellular radiation effects and the occurrence of malignancy.

III Objectives for next period

- Track structure: application of the track structure model to the analysis of the initial slope of the dose-effect relationship of chromosomal aberrations in human lymphpocytes for different radiations; sensitivity analysis to investigate the influence of the various components of the model.
- Comparison of UV effects: interactions of UV of different wavelengths with gamma rays; influence on dose relationships.
- Fractionation effects: continue the work on the effect of fractionated irradiation of gamma rays with different time intervals.
- Application of models to low-dose risk assessment; continue the investigation of the application of the Knudson-Moolgavkar model for radiation to study the implications for the absolute and relative risk models.

IV Progress achieved including publications

Interaction of different radiation types:

Application of the linear-quadratic-dose-effect relationship for cell survival, as derived e.g. in the molecular model for radiation biology, leads to a straightforward description of the effect of the combination of two types of radiation. If the first radiation type is characterised by α_1, β_1, and a dose D_1 and the second by α_2, β_2, and a dose D_2, then survival S_{in} in case of interaction is given by:

$$S_{in} = \exp[-(\alpha_1 D_1 + \alpha_2 D_2) - (\beta_1 D^2 + \beta_2 D^2) \cdot 2\ \mathrm{sqrt}(\beta_1 \beta_2) D_1 D_2].$$

The interaction in fact is given by the term containing the square-root function.
Interaction only occurs, if the irradiations are given simultaneously or within a very short time interval (in the order of minutes). When the time interval is longer, interaction is reduced and ultimately disappears because of repair.
Further, the dependence on radiation type is given by a higher α-value for more densely ionising radiation, the β-value being constant for sparsely and medium-densely ionising radiation and decreasing at higher densely ionising radiation types. This implies that for sparsely and medium-densely ionising radiation only three parameters per data set characterise the fit.

Within these constraints several sets of data (data of Bird, McNally, Ngo et al., Railton, and Raju) were analysed successfully. A goodness of fit was given to indicate the difference between analysis with and without interaction. The results will be presented at the workshop at Padua on Biophysical modelling. If no interaction was found, arguments will be discussed why not.

Comparison with UV.

Continuing the experiments started in the previous contract with UV radiation of 254 nm wavelength, cell survival was studied in stationary CHO cells using UV-radiation of wavelengths around 300 nm. At this wavelength still the type of lesion causing cell death can be expected to be similar to that for 254 nm.
The results indicate a mixed cell population of cells in G_0 and G_2, as might also be expected from the reports on these cells of Nelson et el. Repair was found to be effective for this type of UV, but the lesions in the two phases of the cell cycle behave differently. Further experiments are designed to study this difference.

Application of the models to low-dose risk assessment.

The two-mutation carcinogenesis model of Knudson and Moolgavkar provides a framework for the application of the model developed in previous contracts for the description of radiation induced malignancy. In the model it is assumed that malignancy behaves as a recessive genetic character, which can be suppressed by a normal chromosome. Radiation is assumed to react by eliminating the normal suppressive gene in a carreer cell. Using the Knudson-Moolkavkar model these processes are logically explained and the role of radiation in malignancy can be examined.

Radiation is assumed to influence three parameters in the carcinogenesis model, the two mutational steps and the cell division process in the expansion of the intermediate stage of a cell developing malignancy. The combined model is used to analyse lung tumors in mice and rats after acute and chronic irradiation with different types of ionising radiation (X-rays, gamma rays, neutrons and "radon"). The results show good fits of the model with experimental tumor incidence. The model explains the age dependence of the sensitivity of the animals to radiation, dose-effect relationships for different radiation types, time-incidence relationships and age dependence of radiation sensitivity. An example is given in fig. 1.

Publications

[1] H.P. Leenhouts and K.H. Chadwick. "The molecular basis of stochastic and nonstochastic effects". Health Physics 57, 343-348 (1989).
[2] H.P. Leenhouts and K.H. Chadwick. "The influence of dose rate on the dose-effect relationship". J. Radiol. Prot., 10-2, 95-102 (1990).
[3] H.J.M. Pruppers, H.P. Leenhouts and K.H. Chadwick. "A track structure model for the spatial energy deposition of ionising radiation". Rad. Prot. Dos. 31, 351 (1990).
[4] H.P. Leenhouts, M.J.M. Pruppers and K.H. Chadwick. "Track structure, target structure and radiation effectivness". Rad. Prot. Dos. 31, 351 (1990).
[5] H.P. Leenhouts and K.H. Chadwick. "The cellular basis of stochastic and non-stochastic radiation effects and the implications for low-dose effects". Z. Phys. Med. Baln. Med. Klim. (Sonderheft 2) 19, 54-58 (1990).

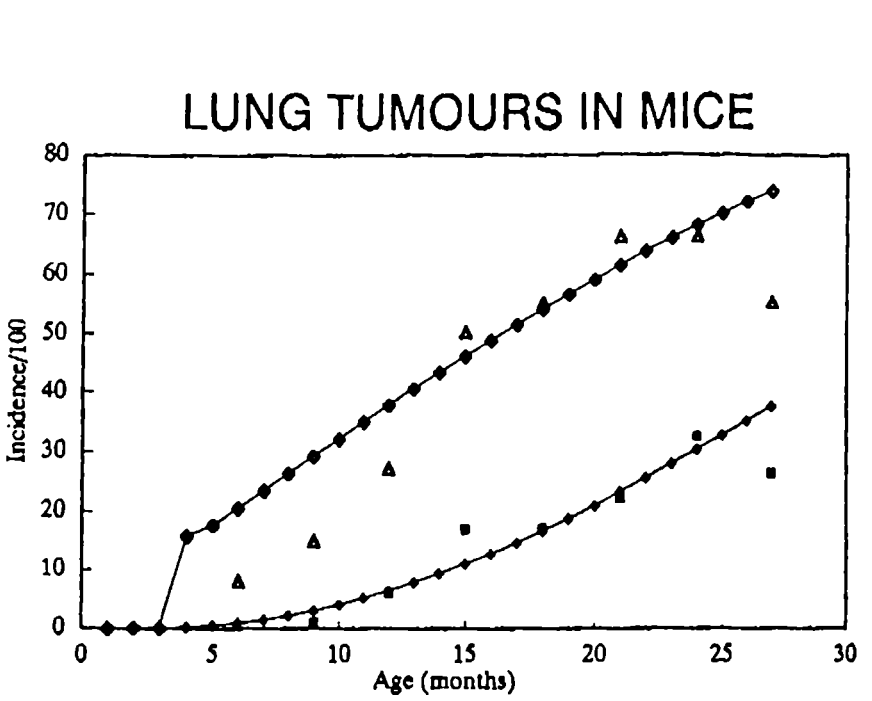


Figure 1: Experimental (squares and triangles) and calculated (black and open diamonds) incidence of lung tumors in male mice as a function of age for, respectively, control animals and animals exposed to 5 Gy acute X-rays at 3 months age. Experimental data of Coggle (1988).

Progress report

Contract: Bi7-040 **Sector: B11**

Title: Specification of radiation quality at the nanometre level.

1	Colautti	INFN - Legnaro
2	Watt	Univ. St.Andrews
3	Harder	Georg-August Univ. - Göttingen
4	Leuthold	GSF Neuherberg
5	Izzo	Univ. degli Studi di Roma

I. Summary of Project and Global Objectives

Research concerning the best way of specifying radiation quality for radiobiology and radiation protection by physical parameter expressing the track structure-target structure relation remains a central task of microdosimetry. Since both molecular biology and radiobiological analysis (track segment method) suggest for cellular radiation effects, critical target sizes of few nanometre, the imperfection of present microdosimetric simulation of volumes in the micrometer range have become evident. For all ionizing particles or particle configuration (e.g. Auger cascade) which produce a high concentration of deposited energy on the nanometre scale, but having ranges much smaller than the micrometer dimensions, the spatial resolution of present microdosimetric detectors is inadequate. The project has four main aims.

1. Track structure studies for nanometre targets

AIM

To establish the approximated constancy of the delta-ray contribution to the energy deposition fluctuation in a nanometre target. To establish the constancy of the ratio of restricted LET to linear primary ionisation.

Track structure studies, based on computer simulation, recent cross section updates and adequate statistical concepts such as distribution parameters, pattern recognition and target modelling, will provide the physical basis for the validation of the proposed quantities linear primary ionisation or restricted LET. The phenomenon of d-ray cutoff at nanometre target boundaries will need further study and the proposed close correlation of these quantities with lineal energy in simulated nanometre volumes will have to be substantiated. The work will include updated cross-sections and genomic target structure.

2. Biological validation of the best suited parameter

AIM

To select bench-mark sets of survival, chromosome aberration and molecular lesion data to test and confirm the ability of linear primary ionisation and restricted LET to determine their variation with radiation quality.

The ultimate decision concerning the suitability of the new radiation quality parameters must be provided by their ability to predict the dependence of radiobiological yields on radiation quality. This work, already started by the cooperating groups in promoting linear primary

ionisation or restricted LET, needs further effort in broadening the biological data base and stepping forward from retrospective analysis to predictive approach.

3. Experimental studies of associate detector systems

AIM

Measurements of the ionisation pattern around charged particles tracks and study of a portable device able to simulate T.E. volumes of few tens of manometres in size.

The actual experimental studies, which aim to determine the lowest simulation limit of a TEPC, will continue with the use of slow ions as probes to explore the avalanche characteristics of single-wire and field-grid TEPC. A tissue-equivalent multistep parallel plate avalanche chamber will be manufactured to measure single ionisations in order to study the correlation between primary ionisation and restricted LET. The possibility to manufacture a small cylindrical avalanche chamber will be studied. In parallel with the gas-filled detectors, a feasibility study will be carried out with the object of simulating the biological response to radiations in nanometer dimensions in condensed phase detectors. The optimum method will be selected, guided by the biological analysis, and work will begin on a device.

4. Quantification of indirect action from single tracks.

AIM

To conduct an experimental study of the yield and spatial distribution of paramagnetic free radicals formed in the wake of individual tracks by measurement of relaxation time and using ESR technique. To compare the experimental results with the predictions of a semplified theoretical model of biological effectiveness.

ESR measurements will be used to explore the spatial distribution, mean life times and reaction rates of free radicals generated by charged particle tracks in nucleic acids, proteins, aminoacids from cell cultures and possibly whole tissues. Measurement of radical density is based upon the dependence of the saturation value of microwave magnetic fields upon the spin-spin relaxation time. The possibility of adapting simplified theoretical methods, developed for enzyme inactivation by indirect action, will be explored in an attempt to obtain a more meaningful model of radiation action for radiation protection purposes.

Head of Project 1: Dr.Colautti

II Objectives for the reporting period

To investigate the working characteristics of a cylindrical TEPC at very low pressures.
To develop a gas counter able to measure the ionisation pattern near the track of a charged particle at nanometre level.

III Objectives for next period

To measure the ionisation distributions near the track of a charged particle in the range from few nanometres to few tens of nanometres from the particle track.

IV Progress achieved including publications

Our coordinated experiment foresees to measure the ionisation distributions as close as 20 nanometres, or less, to the track with enough spatial resolution to test the Monte Carlo calculations which point out that the ionisation distributions very close to the particle path are independent on the particle type and energy.

Gas detectors working in pulse mode can measure ionisation distributions. The experimental problems to solve are mainly two:

- to measure one single electron;
- to obtain the information on the initial position of the electron within a few nanometres uncertainty.

The diffusion of electrons in gas is related to the gas pressure (P) and the travelling path (X) through the relation : $\sigma \propto \sqrt{\frac{1}{E/P}} \cdot \sqrt{\frac{X}{P}}$ where E/P is the reduced electrical field. However in microdosimetry we are interested to the simulated lengths rather than to the real lengths in the gas, then the diffusion is meaningful when expressed in equivalent terms:

$$\sigma \propto E_q \cdot \sqrt{X \cdot P}$$

where X is the real travelling length in the gas of the electron (e.g. millimetres) and σ is in equivalent length units (e.g. nanometres of soft tissue at density 1). E_q is the equivalence ratio for the gas used, namely $E_q = \frac{X(nm)}{X(mm)}$ at unity of pressure. It is possible to see that decreasing the gas pressure we improve the detection precision of the single electron even if the the diffusion in the gas increases at low pressures, that is due to the fact that in this experiment the precision is related to simulated lengths.

For pure propane gas, for instance, we have then:

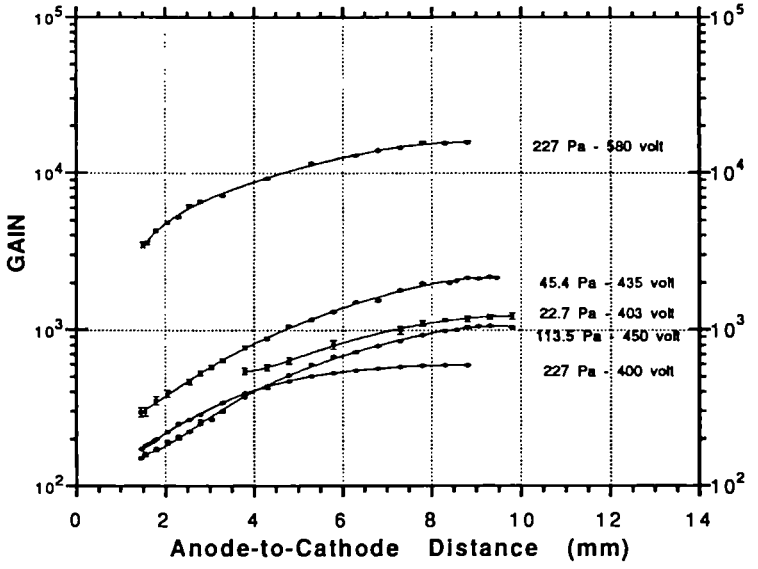
$$\sigma(nm) \approx 2 \cdot \sqrt{P(torr) \cdot X(mm)}$$

That means that in order to obtain a position uncertainty inside the nanometre range it is necessary to work at about 1 torr of pressure or less and with drift lengths of about 1 millimetre.

Our idea is to manufacture a track detector made up of two parts: the drift region and the multiplication region. The drift region is defined by two parallel plates few millimetres apart; the charge particle crosses the drift region at selected lateral distances from two parallel-to-beam slits defining the ionisation collecting region. The electrons produced in that region are made to drift into the multiplication region where they are detected.

The first track detector we shall assemble will have a single wire cylindrical proportional counter as multiplication region.

The multiplication characteristics of the cylindrical proportional counter equipped with a 10 μm anode have been measured in the propane based T.E. mixture at different pressures down to 22.7 Pa (0.17 torr) by using the experimental set-up described elsewhere[1], modified in order to use low velocity ions as probe[2]. The results (see figure) show that a drift region (the plateau in the figure) exists even at very low pressures This finding assures the possibility to separate properly the drift and the avalanche region even if the grid, which will separate the two regions, were not able to confine completely the electrical field.



Gain curves towards the ion probe position for different pressures and applied voltages. 0 is the anode position, the cylindrical cathode is at 10.2 mm.

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Parallel-to-Anode Ion Probe in a Cylindrical TEPC at Simulated Lengths Less Than 1 μm
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2. P.Colautti, G.Talpo, G.Tornielli
Stocastic Variables in the Energy Deposit and their Meaning in the Hazard of Neutrons
Laboratori Nazionali di Legnaro, Annual Report 1989, LNL - INFN (REP) - 030/90

Head of Project 2: Dr.Watt

Objectives: (i) to pursue detailed analyses of the validity of the proposed new quality parameters, dose restricted LET and linear primary ionisation, for correlation of radiobiological data in a manner which is independent of radiation type. This involves development of a model for radiation action and its comparison with other proposed models with the intention of obtaining improved methods for extrapolation of effects to low doses near environmental levels.

(ii) On the basis of (i), to define the desired response function for design of a new generation of detectors which will be capable of providing an absolute measure of radiation effectiveness.

(iii) to appraise the feasibility of making suitable detectors.

1. Interpretation of radiation effects and model development.

1. (a) Calculation of basic quantities for interpretation of biological effect.

To facilitate interpretation of radiation effects in terms of physical parameters of special interest to those in our collaborative group, and to provide formal information to other interested parties, a publication has been prepared which contains calculations performed in the csda approximation for a wide range of electron and photon energies and for some commonly used radioisotopes. Quantities calculated are: track and dose average LET, restricted LET (=100eV), relative variances, the mean linear primary ionisation and the corresponding mean free path, csda ranges, the mean energies required to produce a *primary* ion pair and kerma factors for electrons and photon radiations interacting in water. The minimum cut-off energy is 30eV. Results are tabulated for monoenergetic electrons (50eV to 30 MeV), characteristic K_α X-ray line spectra (carbon to uranium), some commonly used bremsstrahlung (50kV to 300kV) and gamma-spectra (^{241}Am, ^{137}Cs, ^{60}Co, ^{125}I and ^{131}I) and for some typical radionuclides that decay by ß-emission or electron capture accompanied by Auger electron cascades (^{3}H, ^{14}C, ^{32}P, ^{125}I and ^{131}I). Each set of tables is divided into four parts, viz. data for the instantaneous electron energies; averages over the whole primary electron tracks (track averages); averages for the secondary charged particle equilibrium spectrum; information on the build-up factors, spatial concentration of primary and secondary source electrons, fluence of primary and secondary electrons and a quality modified fluence. For photon irradiation there is additional information on the weighted mean free paths for electron production, the weighted mass energy transfer coefficients, the mean photoelectron and compton electron energies and their net mean range. Wherever relevant, graphical displays are given to facilitate interpolation.
Plans to produce part 2 on neutrons and Part 3 on protons and accelerated ions from protons to uranium ions are progressing well.

continued /

1 (b) Radiobiological Data for Analysis.

An extensive data base is being compiled of published results for a wide range of relevant biological endpoints and radiation types. Endpoints included are chromosome aberrations, inactivation, mutations, transformations, DNA strand breaks and inactivation of mammalian cells. Initial results show that all of these end-points have a common causal mechanism attributed to double-strand breaks in the DNA. The radiosensitive target size is demonstrated unambiguously to have a mean chord thickness of about 2 nm and not larger as is sometimes claimed by others. The maximum effect cross-section of about 45 μm^2 for heavy particles, observed and reported by many in the past, is interpreted as the product of the projected cross-sectional area of the DNA in the cell nucleus ($3\mu m^2$) and the number of DNA segments penetrated on average by a particle traversal of the mean chord through the cell ($\sim$15 segments). Electrons in equilibrium, at their most damaging energies of about 100eV, cannot penetrate more than one segment and hence for these the maximum effect cross-section is about 3 μm^2. The ratio of the maxima cross-sections for high and low LET radiations, about 15, gives a bio-physical explanation for the magnitude of the allocated quality factors for these radiations. These results have led to a simple model of direct radiation action which has led to novel interpretations of the (inappropriately named) inverse dose-rate effect for neutrons and heavy particles and the conclusion that the linear-quadratic model is meaningless for extrapolation of effects to low doses. Irradiation time rather than dose-rate emerges as a significant parameter. In the past year studies of the component of damage due to indirect action have been pursued with the object of improving the model for application to electron and photon radiations.

1(c) Indirect action by radicals.

New work has been started on the analysis of indirect effects. The philosophy applied is based on the assumption that since DNA strand breaks are the dominant lesion then if inactivation of single hit targets (e.g. enzymes) can be adequately well quantified for model purposes, it will be a simple process to apply statistical arguments to determine the probability for induction of double strand breaks by indirect action in mammalian cells.

Towards this objective experimental measurements have been carried out in this laboratory to investigate the biophysical mechanisms of direct and indirect radiation action using the metallo-enzyme Dihydro-orotic dehydrogenase (Di-Dnase). By measuring the inactivation of the enzyme in solution and in the dry state, with Cu K_α X-rays at different dose-rates, it is possible to isolate the respective contributions to damage from direct and indirect (radical) action. Also, the diffusion length can be estimated for the radicals that cause the major damage. The results are consistent with this being the $OH^\cdot$ radical. A simplified model has been constructed to take account of direct and indirect action at different concentrations and dose-rates. From this the probability of damage to the DNA in cellular material can be deduced, assuming Poisson statistics apply, and an appropriate model formed. It remains to explore the reliability of the model for a wider range of photon energies. Experimental data (unpublished) for inactivation of ribonuclease in solution is available from earlier studies in this laboratory and is currently being analysed with the object of testing the ability of the model to correlate the data in a unified way.

As the model, in effect, predicts the number of double-strand breaks in cellular material, comparison with data on dsb production in the DNA in cells is seen as a crucial test. Such information is now appearing in the literature and is being compiled on a data base. The model is reasonably sophisticated in the sense that it allows for direct and indirect action, repair, rate effects, irradiation times, varied concentration etc.

2. Progress in novel detectors for absolute dosimetry.

2(a) MOSFET - type devices.

If it is accepted that a double strand break in the DNA of mammalian cells constitutes the fundamental lesion responsible for biological effectiveness then, in principle, a physical detector having a response made to simulate that of the DNA will provide a reading which is directly proportional to biological effectiveness. Exploratory studies are being made on various novel types of detector which it is thought offer good prospects of simulating the response of the cellular DNA. No knowledge of the type, intensity or quality of the radiation field is required. Research is being carried out in solid phase materials and is intended to complement the studies in gaseous and liquid phase detectors being pursued by our collaborative partners at INFN, Legnaro.

Initial experiments have been performed with MOSFET devices which are being operated in a new configuration. In the first instance measurements have been made of the five characteristic modes of operation of various semiconductor devices having different configurations and doping materials (p- and n- type), obtained from commercial suppliers. It was found that the n-type MOSFET responded encouragingly well to alpha, X- and gamma- radiation exposures. An exciting feature of the new system used is that it has the ability to distinguish between different radiation types! The source-drain current was found to be a linear function with respect to the drain voltage and corresponded to the dose-rate delivered. A method has been developed to ensure that the signal can be accurately reproduced in successive measurements. In these exploratory experiments, the intrinsic efficiency of the commercially obtained devices is close to 100% . So far experiments have been performed only in the integrated mode. Single particle, and single electron, counting have yet to be investigated. There are no theoretical reasons preventing single particle detection, and indeed, single *charge* sensitivity, if appropriate design modifications are made and if the device is coupled to apparatus with a higher specification. These detectors are expected to offer a variety of possbilities: detector arrays; position sensitive counters with high spatial resolution; particle type identification (they are already capable of distiguishing between high and low LET radiations), in addition to the main objectives of applying the devices to measurement of biological effectiveness. Future progress will depend on gaining access to specialist National nano-electronic facilities, and appropriate funding, so that custom designed devices may be fabricated.

2(b) Thin film scintillators.

Although commercially available plastic scintillators can be prepared in nanometre layers they are unsuitable for use for the present dosimetry purposes because of their limiting threshold energy of 1 keV for a useable light output. Nevertheless it is quite possible that more efficient scintillants can be developed. Meantime some preliminary research has been started on the design of the required response for an absolute dosimeter. Towards this objective small spheres of scintillant of micron dimensions were suspended in a matrix of non-scintillating plastic at a concentration selected to give the desired mean spacing between spheres. Studies of light output were analysed in terms of proximity function theory and mean chord distributions to try to relate the light pulse to the mean number of spheres penetrated at the appropriate spacing. In this way it is hoped to be able to scale to nanometre dimensions and to simulate the number of double strand breaks produced simultaneously by individual tracks. Then the signal can be related to biological effectiveness. Much further work remains to be done in this area. Present theory in this area is unsatisfactory. However, ideas on a new theory of the scintillation yield for particles of

different LET are being developed and will be tested as a guide to improving scintillation yield.

2(c) Langmuir Blodgett films and other devices.

Contact has been made with specialists in the application of LB organic films with a view to applying these to detection of single electrons. Initial discussions have been arranged and will take place soon to explore the feasibility of their application to dosimetry at the nanometre level. There is the possibility of construction of a solid phase proportional counter and the feasibility is being investigated. Other approaches to be investigated are : superconducting particle detectors; low-dimensional quantum well structures and molecular electronics. It is expected to complete the survey in the next year.

3. Reference:

Track structure data for ionizing radiations in liquid water. Part 1: electrons and photons. D.E.Watt University of St. Andrews Report BIOPHYS/10/89 (90).

Head of Project 3: Dr Harder

Biological Validation of the Best Suited Parameters

An essential task of microdosimetry is to identify a physical parameter of ionizing particle track structure which is able to predict the yield variations of chemical and biological radiation effects with radiation quality. Unrestricted linear energy transfer L_∞, which has been used for many years as an approximation to this ideal, has been dismantled when it became clear that:

a] particles with the same unrestricted LET but with different partition of the energy deposition between the track core and the long-range δ-rays have different cross sections for cellular radiation effects;

b] unrestricted LET - comprising in its definition the integral over the spectrum of energy losses in atomic collisions - is inherently unsuitable to determine uniquely the stochastic fluctuation of the energy deposition associated with a particle traversal through a target of cellular or subcellular size. The width of this fluctuation is decisive for the yield of second-or-under reactions between sequential molecular products on the chemical or biochemical level.

A new concept for the solution of the above-mentioned task, now carried on by the reporting group, can be deduced from the topologic relation between target size and δ-ray ranges: although it is a universal regularity that the fluctuation of energy deposition per particle traversal has essentially two components - the fluctuating number of the primary ionizations and the fluctuating energy deposition per δ-ray - the resulting compound stochastic distribution has fundamentally different properties for targets with micrometer and nanometer dimensions. In micrometer regions, δ-rays frequently remain *insiders*, i.e. they are generated and reabsorbed within the region. Therefore, their initial spectrum is reflected in the stochastic fluctuation of energy deposition per particle traversal, forming one of the two fluctuation components which depend upon primary particle type and energy, the other component is the fluctuation in the number of primary ionizations. However, in nanometer regions, a large fraction of the δ-rays behave as *starters*, i.e. they escape from their region of origin, so that the exterior parts of their trajectories do not contribute to energy deposition within the region.

Although this topologic cut-off effect bears an obvious tendency to reduce the dependence of the δ-ray component of energy deposition fluctuation upon the initial spectrum of the δ-rays, it was a surprise when the reporting group discovered that the δ-ray fluctuation component becomes practically *invariable* with the primary's type and energy for targets in the nanometer range. According to this invariability, only the number of primary ionizations remains as the fluctuation component varying with the primary particle's type and energy. This in turn means that merely a *single parameter*, namely mean linear primary ionization density, is left as a primary-particle dependent variable of energy deposition fluctuation in a nanometer target, since the number of primary ionizations on a given track segment is Poisson distributed.

Thus, the topologic study of the δ-ray escape from targets small in comparison with δ-ray ranges has brought back a unique chance for microdosimetry to characterize radiation quality with a single radiophysical parameter when targets of nanometer dimensions are concerned. The further efforts of the reporting group were and are focused upon the biological establishment and physical generalization of this new and far-reaching observation. Monte Carlo histories of particle tracks projected upon cylindrical targets of various lengths and diameters in the nanometer range have been studied for electron and photon radiations as well as for protons and alpha particles, and the mean primary ionization density respectively the restricted linear energy transfer l_Δ, which is proportional to it for small values of Δ, was thereby further qualified as the single determinant of energy deposition fluctuation varying with the primary particle. Restricted LET is defined to exclude kinetic energies of δ-rays larger than Δ. The low cutoff energy Δ = 100 eV was chosen to secure this proportionality.

We are now in the process of proving this as a general regularity, utilizing the fact that the invariability of the δ-ray fluctuation contribution for nanometer targets rests on the invariability of the low-energy portion of the δ-ray spectrum, which is due to the physical regularities of "glancing collisions". Another task envisaged as urgent is the calculation of the spectra of restricted LET, which have to comprise the contribution by long-range δ-rays. Some spectral mean values of $\bar{L}_{100,T}$ and $\bar{L}_{100,D}$ have already been calculated and more will be produced.

On the biological side, the correlation of various cellular effects with restricted LET is being investigated. A convincing linear dependence of yield coefficient α for dicentric chromosomes in human lymphocytes upon $\bar{L}_{100,D}$ has been observed and is more and more confirmed by new experimental results. We are now investigating the restricted LET dependence of other cellular and molecular radiation effects, which may lead to combined applications of $\bar{L}_{100,D}$ and $\bar{L}_{100,T}$.

Cooperation with the associated groups was discussed at the contractors' meeting in April 1991. Dr Leuthold intends to apply his Monte Carlo code for further proofs of the constancy of the δ-ray fluctuation contribution, Dr Watt cooperates in discussing the relation to his plots of biological cross-sections versus mean linear primary ionization density, and Dr Colautti is performing direct microdosimetric measurements of the δ-ray component of energy deposition fluctuation in nanometer regions. Instruments for the measurement of restricted LET have been developed by other groups, using the saturation characteristics of ionization chambers. We have proposed to ICRU that restricted LET be included in their new report on radiation quantities and units.

Head of Project 4: Dr. Leuthold

II Objectives for the reporting period

1. Extension of track simulation calculations and energy deposition analysis to lower proton energies (Ep > 10 keV).

2. Analysis of the dose mean lineal energy in in spherical nanometer targets.

III Objectives for next period

1. Calculation of distribution functions of ionizing events during the random passage of protons through small spherical volumes in order to study the "invariant straggling contribution " of secondary electrons postulated by the Goettingen group.
2. Calculation of proximity functions and dose mean lineal energy for protons below 100 keV.

IV Progress achieved including publications

1. The cross section data set for the Monte-Carlo simulation program has been extended to proton energies below 100 keV using analytical functions given by M.E. Rudd (Rad. Protec. Dos. Vol.31, p.17-22, (1990)) for the ejection of secondary electrons from different shells in water vapour. By subtraction of the kinetic energy of the secondary electrons and the ionization potential energy from stopping power cross sections the excitation cross section was derived under the assumption of a mean excitation energy of 12.6 eV. The energy deposition by simulated proton tracks traversing spherical targets of 1 to 100 nm diameter was calculated for 10 to 100 keV. Below 10 nm straggling effects lead to asymmetric distributions.

2. The invariance of secondary electron track component with regard to proton energy for nanometer targets was analysed. Fig. 1 show the contribution of single secondary electron tracks to the dose mean lineal energy for nanometer targets over the proton energy range from 0.2 MeV up to 15 MeV. It was derived from the corresponding proximity function component. For 1 and 2 nm targets only a small variation can be seen. This indicates the postulated invariance.

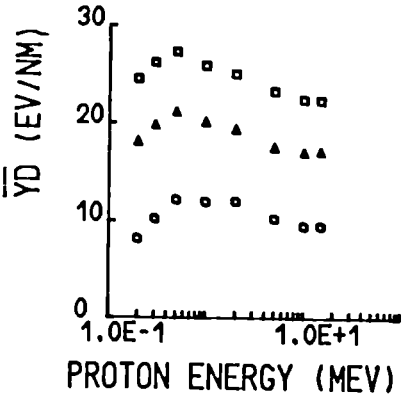


Fig. 1: Dose mean lineal energy of single secondary electrons as function of proton energy for spheres of 1 nm (squares), 2 nm (triangles) and 10 nm (circles) diameter.

Head of Project 6: Dr. Izzo

II Objectives for the reporting period

1. Adaptation of existing ESR spectrometer to precise measurements of the spin-spin relaxation time by means of the determination of microwave field in the cavity.
2. Modification of the ESR spectrometer to the measurements of liquid and semiliquid (tissues) samples frozen down to the temperature of liquid nitrogen.
3. Determination of local radical densities in gamma ray irradiated samples of solid biochemicals as a function of imparted dose.
4. Development of software for absolute measurement of radical density.

III Objectives for next period

1. Installation of refrigeration system for the ESR measurements on tissue and cell samples (transferred from the first year of project).
2. Measurements of local radical densities in the irradiated frozen tissue and cell samples. Both low and high LET radiations will be tried.
3. Study of the effect of dose rate on the local radical density.
4. Interpretation of results.

IV Progress achieved including publications

ad 1. Determination of the spin-spin relaxation time by the method of saturation requires knowledge of the intensity of the field inside the cavity. The Slater method, originally developed for accelerators considers changes of the frequency of cavity $\Delta\omega$ in consequence of an introduction of a very small conducting sphere to the point where the sample is placed during ESR measurements. The metal sphere was mounted on a micropositioner and the measuring system, corresponding to Fig. 1 was used to determine the necessary quantities. The intensity of the magnetic field H was found from :

$$H = \tfrac{1}{2}\left[(\omega^2 - \omega_0^2)/(\omega\omega_0^2)\right] \times \left[(1 - |\Gamma_\omega|^2) \times P \times Q\right) / (2\pi r^2 \omega_\omega)\right]$$

where Q is a Q-factor for a resonator without a sample.
ω_0 is the frequency of cavity without probe
$\omega = \omega_0 + \Delta\omega$
P is the forward power
Γ_ω is the reflection coefficient.

Once the output attenuator of the ESR spectrometer was absolutely calibrated by the above method, the calibration needs to be repeated only rarely, owing to aging of the klystron source.

The degree of saturation of the ESR line and the values of relaxation times were found from the method of Katsumura (Y. Katsumura et al. Rad. Phys. Chem 16. 255. 1980).

ad 2. The low temperature facilities were not installed in the period covered in this report owing to administrative problems outside our group and entirely outside any control or influence of ours. For this reason the studies were limited to solids which could be studied at room temepartures.
It is hoped that this situation will be remedied in the second part of the project.

ad. 3. The average concentrations of radicals in the bulk of the solid (or more generally: condensed) matrix are found easily from total absorbed dose and the intensity of ESR line. The procedure is lengthy, involving comparison of the unknown sample with the known amount of DPPH. However, DPPH spin concentration is known to no better than about 5 % and after taking all known uncertainty factors the absolute values of yield were found with an uncertainty of at least of ± 8 % .

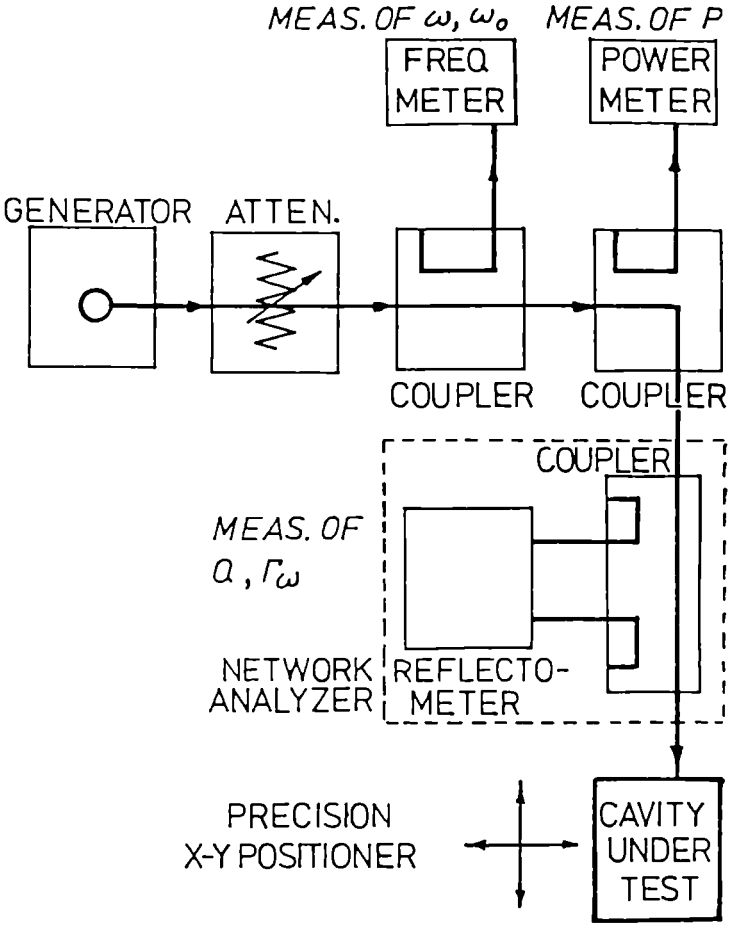
In condensed media the electrons, released in consequence of interaction of photons with medium, lose energy in the ionization regions : spurs, blobs, short tracks and principal tracks. The local radical density in spurs and blobs is the object of our interest. The blobs are having dimensions of an order of nanometers. The local radical concentration inside an ionization region does not increase with dose as our measurements have confirmed for amino acids. We have been using radiation doses of gamma rays from ^{60}Co in the region of linear response because appearance of dose saturation signifies an overlap of spurs. The typical concentrations of radicals in amino acids are between 5 x 10^{17} to 2 x 10^{20} radicals per cm^3. For

two- and three-component polyaminoacids the concentrations are about 50 % smaller. The compounds in which the local radical densities were measured include some carbohydrates as well as DNA and some nucleosides.

In order to arrive at the local spacing between radicals we need to know the volume of the trapping region within the spur. This, can be estimated from the knowledge of energy deposited in a spur. E.g. for a spur with E_{max} = 100 eV the trapping region is a sphere of a radius of about 1.7 nm .

4. To assist in the measurements utilizing the Katsumura method of determining the degree of signal saturation, two computer programs were written in FORTRAN. One of the programs calibrates the readout, eliminates the skew and smoothes the lineshape, the second one obtains the product of spin-spin and spin-lattice times.

Paper : K.V.Ettinger, J.Holowacz and C. Franconi : Dosimetric Information From The Shape Analysis Of ESR Spectral Lines. Accepted for the Third International Symposium ESR Dosimetry And Applications. National Institute of Standards and Technology, Washington DC USA (October14 - 18 , 1991 .



MEASUREMENT OF MAGNETIC FIELD INSIDE RESONATOR

Progress Report

Contract : Bi7-0022-C (CD) Sector : B12
Title : Individual radiosensitivity and its relation to colo-rectal cancer.

I. Summary of Project and Global Objectives

Aim of the Study

In human population, heterozygote carriers for the gene of familial adenomatosis polyposis coli (APC), mapped on chromosome 5, are highly predisposed to form polyps and subsequently adenocarcinomas of the colon.

This process of carcinogenesis is not simple. Several other genomic alterations are usually involved, and a susceptibility of these individuals to various mutagenes is suspected. A study is developed to look for an eventual chromosomal instability of the patients, in lymphocytes and epithelial cells from polyps and adenocarcinomas. In addition, studies on radiation sensitivity of the lymphocytes are conducted by comparing, in the same families, affected and non affected relatives, using cytogenetic and molecular techniques.

Material and methods

1) Ascertainement of the cases and characterization

Patients are first ascertained on clinical criterial, in various specialized consultations and clinics of Paris region. When an APC syndrome is suspected, a familial analysis is performed, and a pedigree established. Then, blood samples of members of the family are obtained. Aliquots of these samples are used for DNA extraction. DNA is cut by different restriction enzymes permiting the detection of polymorphisms, with tested markers located on both sides of the APC gene. Then, using Southern's technique, the haplotype of the chromosomal segment 5q21-q22 is established for each individual. The 2 haplotypes of each individual are compared with those of their relatives to determine which one is associated with the disease and to localize the mutant gene. This part of the work is performed by the group of Gilles THOMAS, at Institut Curie (Paris).

2) Cytogenetic analysis

They are developed in several directions :

1) Characterization of "spontaneous" chromosomal anomalies in blood lymphocytes. After molecular confirmation of the status of the patients, heterozygote carriers are selected. Short term cultures are

developed and 100 R-banded metaphases are analysed per patient. Each suspected anomaly leads to the establishment of the karyotype. Metaphases from synchronized cultures are also analysed to detect eventual constitutional microdeletion of chromosome 5.

The same study is developed on patients ascertained for colorectal cancer, but without FAP syndrome, to obtain a control group.

2) Cytogenetic study of benign and malignant tumors. Polyps and adenocarcinomas are dissected and cell suspensions are cultured for a short term to obtain metaphases. The karyotypes of polyps and adenocarcinomas from APC and non APC patients are compared.

3) Effect of radiations on blood lymphocytes. An aliquot of the blood from APC patients and relatives is irradiated by X-rays very soon after sampling, in Paris. The blood samples are sent to Brussels, where short term cultures are developed. Cell kinetics are studied using a BrdU incorporation technique, and the chromosomal lesions of cells exposed to 0,02 and 2 Gy irradiations are scored, and compared in APC patients and relatives, and in cancer patients without APC syndrome. The clastogenic effect of radiations during G2-phase will be studied in a second step.

Points 1 and 2 are developed in Paris (URA 620 CNRS, Institut Curie), coordinating the researches. Point 3 is developed in Brussels by the TEMU Laboratory.

2) DNA breakage analysis

Blood samples from APC patients are irradiated by X-rays at doses of 0.2, 1 and 2 Gy. DNA breaks are analysed using fluorimetric analysis of DNA unwinding (FADN) method. The role of DNA repair systems, and in particular the activity of poly (ADP-ribose) polymerase is studied in relation with the production of DNA breaks and the kinetics of DNA repair.

The part played by active oxygen species, through modulation of endogenous levels of catalase activity and usage of OH scavengers is also studied. This part of the work, performed on blood samples obtained locally, is developed by the department of Genetics of UNL (Lisboa). Another study will be done by the same group on lymphoblastoid cell lines from APC patients developed at Institut Curie (Paris).

Head of Project 1 : B.Dutrillaux, Institut Curie, Paris.

II. OBJECTIVES FOR THE REPORTING PERIOD :

Ascertainement of patients affected by familial adenomatous polyposis coli (APC), establishement of pedigrees, and of haplotypes of the 5q21-q22 chromosome region and analysis of the karyotypes from lymphocytes.

III. OBJECTIVES FOR THE NEXT PERIOD :

Increase the number of patients under study, start analyses comparing control samples composed of healthy donors and patients affected by colorectal cancer without polyposis.

IV. PROGRESS ACHIEVED :

Ascertainement of cases

In the course of 1990, 161 proposinus affected by familial APC could be ascertained. Pedigrees were established for 37 families, and a demographic study was performed for all cases. A genetic analysis was performed on DNA from peripheral blood of 260 individuals belonging to 25 families. In all cases, the use of 12 probes for polymorphic locus from APC region of chromosome 5 provided a significant information on either side of the gene. This allowed us to give a risk estimate to develop APC for 70 young relatives (less than 25 year old). We could also determine the parental origin of 2 mutations.

Cytogenetics of non irradiated lymphocytes

Methods : PHA stimulated peripheral lymphocytes were cultured for 72 h. Chromosomes were R-banded, and 100 metaphases per patient were photographed. Chromosomes were analysed on photographs by two independent observers and karyotypes were established when an anomaly was suspected. BrdU incorporation technique was applied in some cases.

APC patients : the analysis of 12 cases is almost achieved. Another patient, affected by a Peutz-Jeghers syndrome (variant from APC) was also studied. This last case seem to have a higher rate of chromosome anomalies than the other APC patients, for whom we found fairly homogeneous results.

Non APC colorectal cancer patients : the study of 12 cases is also almost achieved. They seem to have similar rates of chromosome breakage and aberrations. Although statistical analysis is not yet

performed, there is apparently no difference between APC and non APC patients.

Finally, data previously obtained on healthy controls and preliminary comparison with APC and non APC patients favour the following hypotheses :

1) Lymphocytes from patients affected by a colorectal cancer have not a high rate of chromosome aberrations, by comparison to controls.

2) Lymphocytes from patients affected by a genetic constitution (APC) predisposing to colorectal cancer have not a high spontaneous rate of chromosome aberration.

3) More studies are required on Peutz-Jeghers syndrome to know if, in this genetic constitution, there is an increased rate of chromosome aberrations.

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Head of Project 2 : A.Léonard

II. OBJECTIVES FOR THE REPORTING PERIOD

The activities of the TEMU laboratory of the Catholic University of Louvain were devoted to the comparison of the cytological and cytogenetical radiosensitivity of CPA carriers and normal individuals from the same family.

III. OBJECTIVES FOR NEXT PERIOD

Additional CPA carriers and normal individuals from the same family will be examined for their cytological and cytogenetical radiosensitivity. Similar observations will be performed on carriers already displaying such cancer and on patients presenting colo-rectal cancers without polyposis.

IV. PROGRESS ACHIEVED INCLUDING PUBLICATIONS

Blood samples from 4 CPA carriers and 6 normal individuals were exposed to 0,1,2 or 4 Gy of γ-radiation within 2-3 hr after collection. The cells were set up in culture at 37°C in 5 ml Ham's F-10 medium supplemented with foetal calf serum (15 %), phytohemagglutinin and antibiotics. The cells were cultured for 48 h or 72 h and preparations were made according to the method routinely used in our laboratory. The slides coded for blind analysis were examined for structural chromosome aberrations, (200 cells per individual and per treatment), cell kinetics (100 cells per individual and per treatment) and mitotic index (on 1000 stimulated cells per individual and per treatment).

The results obtained up to now (Tables I-III) demonstrate that the interindividual variations largely exceed the differences between the groups of CPA carriers and normal individuals.

	Dose (Gy)	Cells with aberrations (per 100 cells)	Chromatid aberrations (per 100 cells)		Chromosome aberrations (per 100 cells)				
			Gaps	Fragments	Translocations	Fragments	Centric rings	Dicentrics	Tricentrics
NORMALS	0	0.5±0.2	-	0.3±O.1	-	0.3±0.1	-	-	-
	1	15.9±1.6	0.5±0.4	0.5±0.6	0.6±0.4	4.9±3.4	0.6±0.2	10±2.4	-
	2	42.9±8.6	1.0±0.5	1.6±1.1	2.5±1.1	14.4±6.6	2.6±1.9	34.9±5.9	0.3±0.1
	4	80.5±11.5	0.8±0.6	3.2±3.5	5.2±1.2	31.6±11.2	8.1±3.9	94.8±19.9	1.6±1.1
CARRIERS	0	1.5±1.0	0.7±0.5	0.3±0.1	-	0.9±0.5	-	-	-
	1	15.0±2.3	0.3±0.5	0.5±0.5	0.7±0.3	4.9±2.7	0.7±0.3	9.5±1.0	-
	2	38.9±6.8	-	1.7±1.6	1.2±1.6	12.3±6.2	3.0±2.2	31.8±0.8	0.4±0.3
	4	84.0±3.8	1.2±1.0	1.2±1.0	4.7±1.2	35.7±5.5	8.0±3.0	104.5±8.9	2.2±2.1

Table I: RESULTS OF THE CYTOGENETIC OBSERVATIONS IN NORMAL AND CARRIER PERSONS

	Dose (Gy)	Dicentrics and centric rings (per 100 cells)	Dicentric + Centric ring distribution (per 100 cells)					
			0	1	2	3	4	5
NORMALS	0	0	-	-	-	-	-	-
	1	10.7±1.4	89.9±17.0	9.9±2.1	0.4±0.5	-	-	-
	2	37.7±7.1	67.5±5.1	27.6±4.4	4.7±2.4	0.3±0.3	-	-
	4	105.9±22.4	28.3±6.9	41.6±6.2	22.6±3.9	5.3±3.0	0.7±0.5	0.1±0.2
CARRIERS	0	0	-	-	-	-	-	-
	1	10.2±1.1	90.5±0.9	8.9±0.8	0.7±0.3	-	-	
	2	35.5±1.8	70.0±1.8	25.2±1.5	5.0±0.9	-	-	-
	4	117.0±13.9	25.2±2.4	40.6±6.5	26.7±3.9	5.0±1.8	1.9±1.3	O.2±0.3

Table II: DISTRIBUTION OF DICENTRICS AND CENTRIC RINGS IN NORMAL AND CARRIER PERSONS

	Dose (Gy)	M1 %	M2 %	M3 %	MI‰
NORMAL	0	84±8.9	16±8.9	-	70.5±23.5
	1	90.8±6.3	9.3±6.3	-	46.8±19.2
	2	94±4.2	6±4.2	-	48±15.3
	4	96.3±4.9	3.8±4.9	-	26.3±12.04
CARRIER	0	87±3.5	13±3.5	-	75.3±11.6
	1	91.6±3.2	8.3±3.2	-	57±19.6
	2	96±3	4±3	-	59.3±29.7
	4	96±4.3	4±4.3	-	39.3±19.8

Tableau III: CELLULAR KINETICS AND MITOTIC INDEX IN NORMAL AND CARRIER PERSONS

Head of Project 3 : J.Rueff

II. OBJECTIVES FOR REPORTING PERIOD :

Work for the reporting period concentrated in : (i) establishing the radiation doses for normal subjects which induced DNA breakage ; (ii) studing DNA breakage in normal and Familial Adenomatous Polyposis (FAP) patients.

III. OBJECTIVES FOR NEXT PERIOD

Extending the number of patients to be studied for DNA breakage and studing the role of Poly (ADP-Ribose) polymerase.

IV. PROGRESS ACHIEVED

Material and Methods

A total of 7 patients with FAP belonging to 4 different families were studied. Fourteen controls were concurrently studied for breakage.

DNa breakage was assessed through the FADU method.

Doses of 1 Gy and 2 Gy (^{60}Co) were used throughout the study after the establishement of effectives doses.

Results

Preliminary studies for establishing the irradiation doses, showed that 2 Gy produced a statistically significant breakage $P < 0.05$) in normal individuals. Table 4 summarizes the results.

The results for the induction of DNA strand breaks in normal and FAP patients for 1 Gy and 2 Gy are presented in Figure 1. Although there seems to be a trend for greater DNA breakage in FAP patients the difference between normals and FAP patients did not attain statistically significant values.

Preliminary studies were carried out for the role of poly(ADP-Ribose) polymerase in both normals and FAP patients using 3-aminobenzamide as inhibitor of the enzyme. The results obtained in 3 FAP patients with 1 Gy and 2 Gy did not show a statistically significant difference.

Table IV : Double stranded DNA in peripheral leucocytes of controls after gamma - irradiation

Individual	Dose (Gy)		
	0	1	2
1	54.34	84.73	63.03
2	35.70	32.04	38.22
3	38.41	29.22	21.58
4	40.08	39.13	34.29
5	68.08	69.17	74.36
6	51.65	52.01	14.32
7	60.85	50.64	38.22
8	65.29	50.72	61.76
9	42.28	44.07	44.96
10	70.85	65.18	51.13
11	60.60	88.54	68.55
12	95.14	80.52	79.18
13	100.00	79.95	68.05
14	73.06	65.23	63.92
Mean	61.17	59.37	51.54
S.E.M.	5.28	5.26	5.35
P		NS	0.05

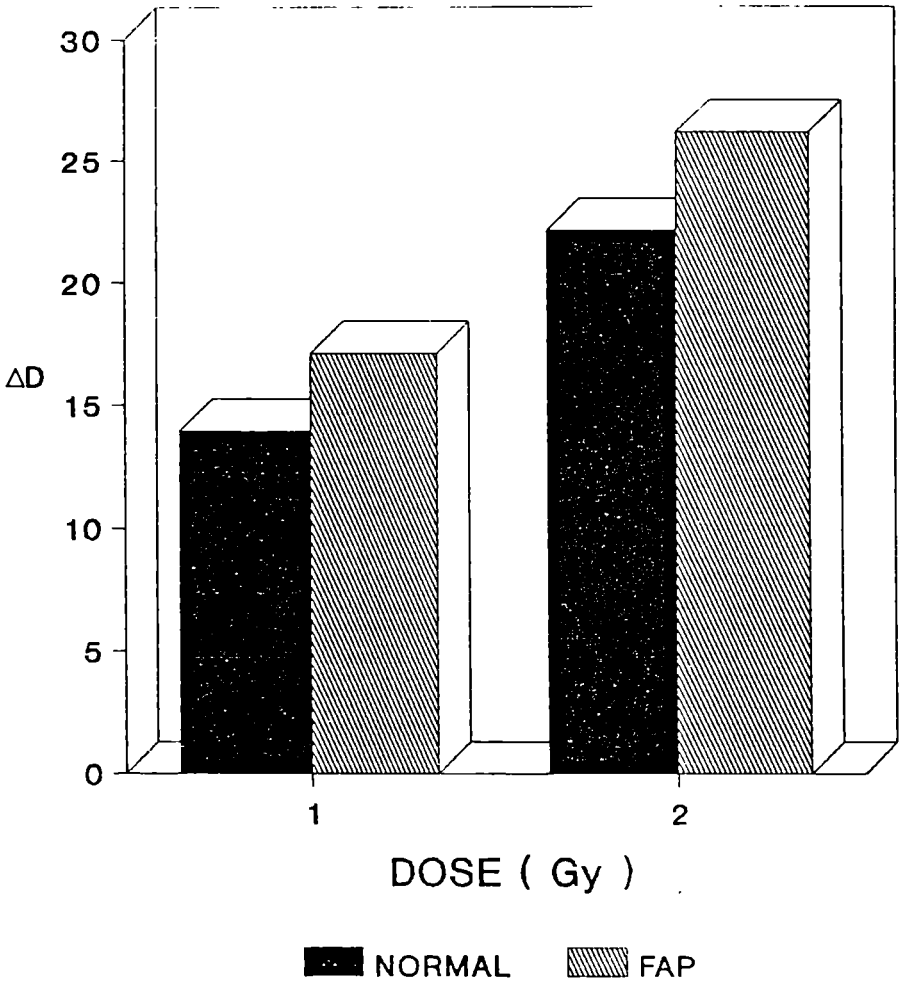

Figure 4 - The effect of gamma-irradiation on development of DNA strand breaks as determined by FADU. The F test did not reveal any statistically significant difference between normal and FAP patients.

Progress Report

Contract: Bi7-026 **Sector: B12**
Title: The genetic and biochemical basis of human DNA repair and radiosensitivity

1	Lohman	Univ. Leiden, Sylvius Labor.
2	Bridges	MRC Cell Mutation Unit
3	Bootsma	Erasmus University Rotterdam
4	Moustacchi	Institut Curie
5	Thacker	MRC Radiobiological Unit

I. Summary of Project and Global Objectives

Differences in radiosensitivity between neonatal and adult blood derived lymphocytes as well as between those from AT and normal donors have been detected by clonal assay. A different approach including measurement of micronuclei in fibroblasts revealed evidence for increased sensitivity of AT-heterozygotes. In addition a single microgel assay has now been established as a promising method to measure heterogeneity of human radiation response (MRC Cell Mutation Unit). Radiosensitive mutants isolated from rodent cell lines have been further characterized and assigned to complementation groups (Univ. of Leiden). These cell lines are fed into projects which are concerned with genes involved in repair. The ERCC-3 and ERCC-6 genes correcting UV-sensitive rodent mutant 27-1 and UV61 respectively, have been further characterized. The deducted aminoacid sequences of both genes suggest that these repair genes may code for DNA helicases (Erasmus Univ.Rotterdam). Preliminary data suggest that ERCC6 is deleted in one allele of a patient suffering from Cockayne's syndrome. Two human homologues of the yeast (S. cerevisiae) Rad 6 gene involved in postreplication repair, have been cloned by sequence homology and their chromosomal location has been assigned (Erasmus Univ.Rotterdam). Progress has been made in isolation of the ERCC1 protein by overproduction in E. coli (Erasmus Univ.Rotterdam). Cloning of repair genes in the fission yeast S. pombe appears to be rather successful: 12 genes have been cloned and they are currently characterized by sequencing and expression. Microcell mediated chromosome transfer is successfully used to correct rodent xrs mutants sensitive to ionizing radiation (MRC Cell Mutation Unit). It is shown that radiation resistance is restored by chromosome 2. DNA transfection techniques have been employed to correct the mitomycin C sensitivity of Fanconi anemia (FA) cells (Institut Curie). Cloning of c-DNA has been achieved and sequencing is in progress. The influence of DNA repair on mutagenesis has been assessed in rodent and human cells. Analysis of UV-induced HPRT mutations in repair proficient and deficient hamster cells has revealed differences in both types and strand specificity of mutation (Univ. of Leiden). The hypomutability observed in FA cells is associated with a reduction of point mutations (Institut Curie). The high frequency of deletions is likely to reflect the established chromosomal instability characteristic for FA. Deletions in the human HPRT have also been analysed for breakpoints (MRC Radiobiological Unit). The process of DNA repair is

further investigated at the level of chromatin and by in vitro assays. In human cells UV-induced cyclobutane dimers are preferentially repaired in active genes. Xeroderma pigmentosum group C cells have a defect in repair of inactive sequences, whereas Cockayne's syndrome cells are unable to perform preferential repair of active genes. Chromatin encapsulated in agarose microbeads, is used as template to study factors involved in preferential repair, in an in vitro assay (Univ. of Leiden). Nuclear extracts have been employed to analyse efficiency and fidelity of DNA-break rejoining in vitro. In AT extracts the rejoining fidelity was impaired (MRC Radiobiological Unit).

The collaborating laboratories will continue to focus on (i) heterogeneity and variability in human radiation response; (ii) isolation of new radiosensitive mutants; (iii) cloning and characterization of repair genes and gene products; (iv) analysis of effects of repair on mutagenesis and other cellular processes; (v) influence of chromatin organization on repair, and (vi) establishment and analysis of repair by in vitro assays.

Head of Project 1: Prof. Dr. Lohman

II Objectives for the reporting period

(1) To assess the role of chromatin structure in DNA repair employing repair proficient and deficient mammalian cell lines.
(2) To determine mutation spectra in repair proficient and deficient cell lines.
(3) To isolate and characterize repair deficient mutants to study the relationship between DNA repair and mutagenesis.
(4) Cloning of repair genes.

III Objectives for next period

(1) Further development of methodologies to measure radiation induced DNA damage in defined genomic regions.
(2) Further establishment and characterization of an in vitro repair system to study repair of transcriptionally active DNA.
(3) Determination of mutation spectra in repair proficient and deficient hamster and human cell lines.
(4) Isolation of repair deficient mutants and cloning of repair genes.

IV Progress achieved including publications

(1) DNA repair and chromatin structure

We have demonstrated that cyclobutane pyrimidine dimers (CPD) are preferentially removed from transcriptionally active DNA in UV-irradiated hamster and human cells. In V79 hamster cells repair of CPD in the active HPRT gene is confined to the transcribed strand only, with little repair of the nontranscribed strand. In human cells preferential repair of CPD as determined in housekeeping genes (ADA, DHFR) refers to differences in rates of CPD removal in both strands. During the first 4 hours following UV-treatment (10 J/m²) CPD repair is faster in the transcribed strand than in the nontranscribed strand, but after 24 hours repair of both strands is almost complete. Experiments with a human ADA deficient (SCID) cell line carrying a deletion in the promotor region of the ADA have shown that preferential repair of the transcribed strand is depending on transcription. Human cells exhibit a less efficient CPD repair of X-chromosomal repressed genes, compared to active housekeeping genes, even in the absence of transcription of a housekeeping gene, suggesting several hierarchies of DNA repair in mammalian cells.

Different levels of CPD repair in transcriptionally active and inactive DNA sequence are found in cells derived from UV-sensitive genetic disorders (xeroderma pigmentosum, Cockayne's syndrome). Xeroderma pigmentosum group C fibroblasts efficiently repair the transcribed strand of active genes, but not the nontranscribed strand or transcriptionally inactive DNA. In Cockayne's syndrome fibroblasts active genes are repaired with a similar rate and efficiency as inactive genes without differences between transcribed and nontranscribed strands. Therefore factors defective in Cockayne's syndrome, regulate both the rate and extent of repair of CPD in active genes.

The methodology to determine pyrimidine (6-4) pyrimidone (6-4 PP) photolesions in defined DNA sequences have been established. Isolated DNA is treated with photolyase to monomerise CPD; the sites of remaining damage predominantly 6-4 PP are cut by E. coli UVR-ABC nuclease. Preliminary experiments with hamster cells did not show differences in repair of 6-4 PP in the active HPRT and inactive c-mos gene.

Encapsulated cells have been employed to isolate intact chromatin as template for in vitro repair synthesis. These chromatin preparations are able to carry out in vitro replication without adding extracts. In exponentially growing immortalized human cells the in vitro replication is strongly inhibited by UV-irradiation. In confluent normal human fibroblasts incorporation by replication is sufficiently low to detect UV-induced repair synthesis. Repair synthesis was not detectable in confluent XP-A fibroblasts.

(2) Mutation spectra in repair proficient and deficient cells

Mutation spectra have been established in the repair proficient CHO and V79 cells and the repair deficient derivatives UV-5 and VH-1. Comparison of UV-induced HPRT mutations in repair proficient and deficient cells show two striking differences:

(i) All types of transitions and transversions are present in mutation spectra of repair proficient cells, whereas spectra in repair deficient cells are dominated by GC-AT transitions.

(ii) In repair proficient cells UV-irradiated with a dose of 2 J/m^2, mutations are generated predominantly by damage sites in the poorly repaired nontranscribed strand. The opposite result is observed in repair deficient cells: mutations are primarily generated by photolesions in the transcribed strand. These results are the first proof that strand specific repair leads to strand specificity of induced mutations.

(3) Characterization of UV-sensitive mutants

The V79 cell mutant VB-11 has previously assigned to a new complementation group 7 of UV-sensitive rodent mutants. The removal of 6-4 PP is not impaired in VB-11 consistent with a normal level of unscheduled DNA synthesis. However repair replication was 50% reduced and removal of CPD from the HPRT gene was significantly slower. Despite these defects no effect on UV-induced mutagenicity was observed.

A partial revertant (RH-26) of the UV-sensitive mutant VH-1 was isolated and characterized. The revertant shows normal level of 6-4 PP, but the absence of CPD repair from the transcribed strand of the HPRT gene. However, in spite of this defect in CPD repair the frequency, the types and strand specificity of HPRT mutations were similar to the parental repair proficient V79 cells. These data suggest, that 6-4 PP is the main mutagenic lesion in hamster cells.

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Head of Project 2: Prof. Bridges

II Objectives for the reporting period

(1) To establish reliable laboratory techniques for measuring cellular radiosensitivity with (a) clonal assays with T-lymphocytes, and (b) the single cell microgel assay.
(2) (a) To search for deletions in chromosome 11 in ataxia-telangiectasia cells, (b) to localise the human gene corresponding to the xrs mutation, (c) to clone and characterise DNA repair genes from S. pombe.

III Objectives for next period

(1) To evaluate the single cell microgel assay as a test for radiosensitivity.
(2) To apply techniques which measure the repair of clastogenic damage in the detection of radiosensitive individuals.
(3) To correct the radiation sensitivity of the xrs mutants by transfection with human DNA.
(4) To further characterise DNA repair genes from S. pombe, and to begin to clone their human homologues.

IV Progress achieved including publications

Sub-project 1.

New techniques for measuring radiosensitivity

1. We have completed an extensive study of the radiosensitivity of T-lymphocytes. These investigations have reinforced our conviction that this cell type can provide the most rapid (≈14 days from blood separation) estimate of radiosensitivity based upon a clonal assay. It may also more closely reflect the in vivo situation since no extensive period of in vitro expansion is required between biopsy and test such as is necessary when working with fibroblasts or keratinocytes.
   The results show: (i) neonatal cord blood derived T-lymphocytes (18 samples) are significantly more radiosensitive ($\bar{D}$ = 1.54 Gy) than T-lymphocytes derived from adult blood ($\bar{D}$ = 1.90 Gy, p = <0.001). (ii) Although the range of sensitives between T-lymphocytes ($\bar{D}$ = 1.26 - 2.15 Gy) and fibroblasts ($\bar{D}$ = 0.90 - 1.68 Gy) from normal donors are similar there was little evidence for a correlation in radiosensitivity between the two cell types. These results are based on a sample of 34 donors. (iii) T-lymphocytes from ataxia-telangiectasia (A-T) donors are significantly more radiosensitive than T-lymphocytes derived from normal donors.
2. We have established the single cell microgel assay of Singh et al. (Expt. Cell Res., 175 (1988) 184-191) as a reliable laboratory procedure. This has required setting up image intensification and computerised scoring facilities in addition to defining critical experimental parameters. We have trained visiting workers from other institutions in the use of this assay.
   (i) The procedure is efficient, fast (results within 1 day) and can be

used with any tissue from which a single cell suspension can be obtained, it requires a minimal number of cells (20 µl of blood provides sufficient material).
(ii) We have shown that the assay can detect breaks produced indirectly during the excision repair of UV-C damage in T-lymphocytes.
(iii) We have shown that we can detect breaks induced directly by gamma-irradiation at doses down to 0.25 Gy. There do not appear to be any differences in initial damage between cells from normal or A-T donors. It is too early to decide how effective the technique will prove to be in attempt to discriminate between the response of normal and radiation sensitive cells under conditions which maximise differences in repair of damage.

3. Procedures which permit the repair of potentially lethal damage (RPLD) have been shown by us to maximise the differences in cellular radiosensitivity between normals and between normals and radiosensitive individuals. We have attempted to couple the RPLD experimental design to the study of the repair of clastogenic damage by measuring changes in the induction of micronuclei in fibroblasts. Ataxia-telangiectasia heterozygotes, like the probands, are defective in the repair of this damage. The realization that A-T heterozygotes are cancer-prone and are believed to represent ≈20% of premenopausal breast cancer prompted us to initiate a pilot study to investigate the repair of potentially clastogenic damage in cells from such patients. The first results show that a substantial proportion are defective in repair of clastogenic damage.

Sub-project 2

Cloning of DNA repair genes

1. The genes for ataxia-telangiectasia (groups A and C) are known to map to chromosome 11q22. We have carried out a search in A-T cell strains for deletions of DNA containing markers known to be closely linked to the A-T genes. Using pulse-field gel electrophoresis we have not detected any deletions in large fragments containing the Thy-1 or pYNB3.12 markers.
2. Hamster xrs mutants are sensitive to the lethal effects of ionizing radiation, as a result of a defect in the ability to rejoin double-strand breaks. We are attempting to localise the human xrs gene using microcell-mediated chromosome transfer. As chromosome donor we use a panel of somatic cell hybrids, each containing a "tagged" human chromosome. Following fusion of xrs hamster cells with microcells from the donor, selection is applied for the tagged human chromosome. The ability of the individual human chromosomes to correct the radiation sensitivity of the xrs cells is then assessed. So far we have shown that human chromosomes 5, 6, 9, 12, 13, 15 and 21 do not correct the xrs defect, but human chromosome 2 does restore radiation resistance. We can tentatively assign the human xrs gene to chromosome 2.
3. We are using the fission yeast Schizosaccharomyces pombe to clone DNA repair genes. This organism is being used both as a model system to study eukaryotic genes, and as a stepping-stone to cloning DNA repair genes from mammalian cells. We have so far cloned 12 DNA repair genes from S. pombe, and they are currently being characterised by DNA sequencing and expression studies. Comparison with DNA repair genes from S. cerevisiae identifies important functional domains, and may provide indicators for appropriate strategies for cloning the homologous mammalian genes. In the excision repair pathway, we have shown that the S.pombe rad 16, rad13 and rad15 genes are respectively homologous to the S. cerevisiae RAD1, RAD2 and RAD3 genes. In each case the homology is different. With rad16/RAD1 there is about 35% identity along the gene,

whereas with rad13/RAD2 two highly homologous domains of 80-100 amino acids are separated by a large non-homologous region. The two conserved domains are likely to be of functional importance. Rad15/RAD3 contains 7 highly conserved helicase domains, with reasonable conservation throughout the gene.

In the recombination-repair pathway we have fully characterised two DNA repair genes, rad4 and rad9. The rad4 gene has a vital function. It has several interesting domains, including a zinc finger, a nuclear location signal and a basic tail. The latter is not essential for gene function. A segment of the gene shows significant homology to the human XRCC-1 gene which is involved in joining of strand breaks. This area of homology again points to an important functional domain, e.g. active site or area of protein-protein interaction. The rad9 gene contains 4 exons. Only the first two exons are required for correction of radiation sensitivity of rad9 mutants. No homology has been identified with other cloned genes.

The cloned genes have all been physically mapped on the S.pombe chromosomes using pulse-field gel-electrophoresis.

Publications

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Head of Project 3: Prof. Bootsma

II Objectives for the reporting period

1. Isolation of human genes involved in excision repair by DNA transfection or by sequence homology with genes of yeast (*S.cerevisiae* and *Ss.pombe*) and *Drosophila.*
2. Characterization of the cloned repair genes and their gene products at the molecular level. Emphasis was on the cloned genes *ERCC-1*, *ERCC-3* and *ERCC-6.*

III Objectives for next period

1. Continuation of cloning human DNA repair genes by DNA transfection and sequence homology.
2. Further characterization of the genes and gene products of *ERCC-1*, *ERCC-3*, *ERCC-6*, and the human homologues of the yeast *RAD6* gene.
3. Preparation of constructs required for expression *ERCC*-genes in transgenic mice.

IV Progress achieved including publications

1. Isolation of human genes involved in excision repair.

ERCC-3

Using DNA mediated gene transfer to the repair-deficient rodent mutant 27-1, the human excision repair gene *ERCC-3* was recently isolated which corrects the UV-sensitivity and unscheduled DNA synthesis (UDS) of mutants belonging to complementation group 3. The *ERCC-3* gene (chromosomal localization 2q21) is ~ 45 kb in size and consists of at least 15 exons. It specifies a protein of 782 aa. The deduced aa sequence suggests it to be a chromatin binding DNA helicase. There is no significant homology to known repair genes of yeast or *E.coli*, although the gene is strongly conserved. The yeast (*S.cerevisiae, S. pombe*) cognates have recently been cloned using human *ERCC-3* probes. The sequence of the predicted *ERCC-3* homologs in these species is consistent with the functional domains postulated for the human protein. Genetic disruption in *S.cerevisiae* carried out by S. and L. Prakash and coworkers (Rochester) suggest that like the RAD3 gene *ERCC-3*^{sc} encodes an essential function.
Striking parallels exist between *ERCC-2* and *ERCC-3*: mutation in the two genes induce similar cellular phenotypes, i.e. similar sensitivity to UV and mutagens that form bulky DNA adducts, no pronounced cross-sensitivity toward X-rays and DNA cross-linking agents. A similar level of UV-induced mutagenesis, and a defect in early (pre) incision steps of the excision repair pathway. Furthermore, both genes have yeast homologs that exhibit 50% sequence identity to their human cognates and encode an essential function. Finally, the two genes encode (presumed) DNA helicases of similar size. These data suggest that they play an equivalent not overlapping role in excision repair. Finally, the *ERCC-3* gene is found to be

responsible for XP complementation group B; a very rare form of XP that is simultaneously associated with Cockayne's syndrome (CS). Using the gene, 2 new patients have been assigned to this group. In spite of the severe excision repair deficiency in these two patients, they have not developed skin cancer even at an advanced age. This suggest that additional genetic factors are required for cancer proneness.

ERCC-6

The UV-sensitive, nucleotide excision repair-deficient, Chinese hamster mutant cell line UV61 was used to identify and clone a correcting human gene, *ERCC-6*. UV61, belonging to rodent complementation group 6, harbours a deficiency in the repair of UV induced cyclobutane pyrimidine dimers but permits apparently normal repair of (6-4) photoproducts. This suggests that the defective protein is involved in damage recognition. Genomic (HeLa) DNA transfections of UV61 resulted, with a very low efficiency, in several primary and secondary, UV-resistant transformants. One of the secondary transformants was used to clone the entire 115 kb human insert. The *ERCC-6* gene appears to cover more than 80 kb, which makes it one of the largest genes cloned by genomic DNA transfection.

Two lowly expressed mRNA transcripts of 6.5 and 8.5 kb are detected with *ERCC-6* probes. Human cDNA clones have been isolated and sequenced. The two mRNA species appear to be due to alternative polyadenylation site selection. Although it is uncertain whether the entire N-terminus is present on the partial cDNA clones isolated, the ORF seems to encode a predicted protein of minimally 1500 aa with a putative chromatin binding domain and a serine phosphorylation site. In addition it shows an almost perfect match with 7 consecutive domains conserved between two superfamilies of helicases, which makes *ERCC-6* the third putative helicase involved in DNA excision repair: the others being *ERCC-2* and *-3*.

Preliminary data suggest that the *ERCC-6* gene, which is located on human chromosome 10q11-21.1, is deleted in one of the alleles of a patient suffering from the excision repair disorder Cockayne's syndrome (CS). This finding opens the possibility that *ERCC-6* is implicated in CS.

RAD6 human homologs

The *Saccharomyces cerevisiae RAD6* gene plays a central role in postreplication repair, damage induced mutagenesis and sporulation. The 172 amino acids RAD6 protein is a ubiquitin conjugating enzyme (E2) that *in vitro* (poly)ubiquitinates histones. By evolutionary walking based on nucleotide sequence homology we have cloned two human *RAD6* homologs (designated *HHR6A* and *HHR6B*) using the *Schizosaccharomyces pombe* and *Drosophila melanogaster* genes as "intermediates". The two 152 amino acids human proteins, one of which identical to a recently cloned human E2(M_r=17000) enzyme (Schneider *et al.* (1990) *EMBO J.*, 9, 1431-1435), share 95% sequence identity with eachother and approximately 70% and 85% overall identity with the yeasts (*S.cerevisiae* and *S.pombe*) and *D.melanogaster* homologs respectively. Intriguingly, none of the RAD6 homologs possesses the acidic C-terminal sequence present in the *S.cerevisiae* RAD6 protein, which is required for sporulation. The central part, harbouring the cysteine residue involved in thiolester formation with ubiquitin, displays significant homology with other ubiquitin conjugating and activating enzymes. Genetic complementation experiments reveal that HHR6A as well as HHR6B can carry out the repair and mutagenesis functions of RAD6 in *S.cerevisiae*

rad6Δ mutants but not its role in sporulation. The striking conservation of RAD6 structure and function throughout eukaryotic evolution suggests that the essential components of the repair- and mutagenesis-machinery with which RAD6 interacts have also been conserved. Using biotinylated probes the *HHR6A* gene was localised on the human X chromosome, band q24-25, where it could form part of a cluster of genes involved in ubiquitin conjugation. The *HHR6B* gene was assigned to human chromosome 5q23-31.(work in collaboration with E.Smit, A.Hagemeijer and B.Oostra, Rotterdam). The X-chromosomal localisation makes it unlikely that the *HHR6A* gene is implicated in the postreplication repair deficient variant complementation group of xeroderma pigmentosum. The HHR6B gene, however, is still a possible candidate gene.

2. Characterization of human repair genes and proteins

Functional analysis of the ERCC-1 gene and protein

The human excision repair cross-complementing gene *ERCC-1* specifically corrects UV and mitomycin C-sensitive CHO mutants from complementation group 1 (CG1). Its encoded protein is 297 aa long and has a central part with strong homology to the yeat repair protein *RAD10*. The presence of a characteristic helix-turn-helic domain in this area suggests that the protein has DNA binding activity. Towards the C-terminus areas are present with significant homology to parts of uvrA and uvrC from *E.coli*.

In order to study the significance of these areas and putative domains, cDNAs with specific mutations were constructed and assayed for functionality by transfection to CG1 mutants. Deletion mutants showed that the N-terminal 92 aminoacids of the protein are not essential for correction of the CG1 phenotype. In contrast, not more that 6 aa can be missed from the strongly conserved C-terminus. Several mutations in the putative DNA-binding domain resulted in loss of correcting ability. Studies of other aminoacid changes and of hybrid constructs containing substituted *uvr*C-sequences from *E.coli* are underway.

When overexpressed in *E.coli* on its own, the full-length *ERCC-1* protein is quickly degraded. Therefore, rabbit antisera were raised against a fragment of the protein in fusion with the Ig-binding part of staphylococcal protein A. These antibodies specifically recognize the protein synthesized *in vitro* from an *ERCC-1* mRNA, which has an apparent MW of 39 kD on SDS-PAGE. After extensive affinity-purification of the antiserum, using an *ERCC-1* fragment liberated from the fused protein A moiety by specific digestion, only very low levels of *ERCC-1* protein are detected in immunoblots of human cell extracts. While 5-10 times higher levels of protein were found in CHO cells with amplified genomic *ERCC-1* sequences, this increase contrasts with the mRNA concentrations which were elevated ≥500x. The data suggest, that excess *ERCC-1* protein is rapidly degraded and are compatible with the idea that it is part of a complex.

Recently, we have succeeded in establishing, in *E.coli*, a large overproduction of an ubiquitin polypeptide C-terminally extended with full-length *ERCC-1*. The ubiquitin moiety which protects *ERCC-1* from degradation by the host, can be specifically removed by ubiquitin lyase, an enzyme which is present in extracts from yeast as well as mammalian cells. Currently, the *ERCC-1* protein is being isolated and purified from this source in large quantities, for further enzymological studies.

Thesis
Weeda G.
Molecular characterization of a DNA repair defect in xeroderma pigmentosum and

Cockayne's syndrome.

Roza L.
Processing of UV-induced DNA damage in human skin cells.

Publications

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linkage between CKMM and the ERCC-1 and ERCC2-genes.
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Head of Project 4: Dr. Moustacchi

II Objectives for the reporting period

Fanconi anemia (FA) has in common with the other human inherited diseases studied in the contracting group a high spontaneous and induced chromosomal instability associated to an abnormal processing of specific DNA lesions and to cancer predisposition. Two main topics during the reporting period received particular attention. The first one concerns the characterization of DNA sequences implicated in the correction of FA cells hypersensitivity. The second deals with the quantitative and molecular analysis of mutations induced at a specific locus in normal and FA cells.

III Objectives for next period

The molecular rescue of the DNA sequences implicated in the FA correction allows us to attempt the chromosomal location by *in situ* hybridization of the probe. The sequencing of the cDNA (partial gene) actually available will tell us if it corresponds or not to a known gene product. The mutagenesis studies in FA show that this disease may constitute a good model for analyzing the mechanism of deletions. This will be pursued. Steps of DNA repair in individual chromosomes as analysed by pulse field electrophoresis will be also continued. Following studies on the correction of the FA defect by cocultivation with normal human or mouse cells, we discovered a novel characteristic of this disease, that is anomalies in the regulation of production of at least two cytokines (IL-6 and TNFα). How these anomalies are related to the DNA repair defect and to the failure in differenciation of the hematopoïetic system will be the object of our future research.

IV Progress achieved including publications

We have demonstrated this year that FA cells in culture are hypomutable at the *HPRT* and at the Na^+/k^+ *ATPase* loci compared to normal. This is true for the two genetic complementation groups A and B whether the mutation frequencies are expressed as a function of dose or of survival levels *(3)*. Using Southern blot hybridization, the molecular analysis of the spontaneous and induced mutants was performed. In FA cells, the deletion mutants predominate where in normal cells the vast majority of mutants were due to point mutations *(4)*. The hypomutability in FA cells associated with a reduction in the frequency of point mutations suggests that FA cells are defective in a mutagenic process operating in normal cells. The high frequency of deletions at a specific locus is likely to reflect the well established chromosomal instability characteristic of the disease. How this relates to cancer predisposition remains to be understood.

The strategy of cloning the gene(s) involved in this disease is based on phenotypic complementation of FA cells *in vitro* by DNA transfection. Correction of transfection of either genomic normal human (Proc. Natl. Acad. Sci. USA, 1986, 83, 7034-7038) or mouse DNA *(9)* has been achieved in our group. In some instances, the clastogenic and cellular responses to DNA cross-linking agents could be phenotypically dissociated in transfectants, possibly indicating the presence of two domains in the correcting protein(s). By conventional rescue techniques, a λ phage library has been constructed from FA group B transfectants demonstrating an increased resistance to MMC. The library has been screened using mouse repetitive DNA and the biological activity of positive phage recombinants has been tested by transfection of FA group B fibroblasts. From the three out of 20 transfected phage recombinant DNA capable to correct the sensitivity of FA cells to mitomycin C, only one led to a substantial level of correction (75% of normal response). With single copy phage insert DNA probes a 4 kb transcript in normal polyadenylated mRNA can be detected by Northern blot hybridization. Cloning of the cDNA has been achieved and sequencing is in progress.

Publications

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10. Fanconi anemia : Genetic and molecular aspects of the defect. E. MOUSTACCHI, C. GUILLOUF, D. FRASER, F. ROSSELLI, C. DIATLOFF-ZITO & D. PAPADOPOULO. *Nouv. Rev. Fr. Hematol.*, **32**, 387-389 (1991).

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14. Formation of cyclobutane thymine dimers photosensitized by pyridopsoralens : Quantitative and qualitative distribution within DNA. A. MOYSAN, A. VIARI, P. VIGNY, L. VOITURIEZ, J. CADET, E. MOUSTACCHI & E. SAGE. *Biochemistry* (1991) (in press).

Head of Project 5: Dr. Thacker

II Objectives for the reporting period

(i) To assess the efficiency and fidelity of rejoining of site-specific DNA double-strand breaks in a novel cell-free system, using extracts from individuals with ataxia-telangiectasia and normal persons to see if these differ. (ii) To examine recently-isolated radiosensitive mutants (*irs*) for complementation by fusion to A-T cells and normal human lymphocytes. To assess the ability of the *irs* mutants to recover from damage under low-dose-rate irradiation conditions. (iii) Development of methods to analyse the molecular nature of large deletions in a defined mammalian gene (*hprt*) in early-passage human cells.

III Objectives for next period

(i) To assess whether lack of fidelity of rejoining of DNA breaks by human nuclear extracts is specific for A-T cells, and to identify the enzymes active in rejoining and mis-rejoining of breaks. (ii) To map the human homologue of the *irs2* mutant, and to establish the recovery potential of the *irs1* and *irs3* radiosensitive mutants using low dose-rate irradiations. (iii) To isolate breakpoints of deletions in the *hprt* gene of early-passage human cells and to sequence these; to pursue high resolution chromosome banding, pulsed-field gel electrophoresis, and use of X-linked probes to size radiation-induced deletions.

IV Progress achieved including publications

(i) The introduction of defined and localized sites of damage into purified DNA molecules, followed by exposure of the DNA to a cellular environment, is an important technique in the molecular analysis of DNA repair and mutagenesis in mammalian cells. A major type of DNA damage, induced by agents such as ionising radiation, is the double-strand break (dsb). A number of studies implicate the dsb as a lethal lesion in cells, and some radiation-sensitive mutants of mammalian cells appear to have a reduced ability to rejoin this type of damage. Individuals with the disorder ataxia-telangiectasia (A-T), who suffer both radiosensitivity and cancer-proneness, may also be unable to repair DNA breaks adequately in comparison to normal human cells.

In the present study we are attempting to refine the measurement and analysis of DNA-break rejoining with the use of nuclear extracts from human cells applied to defined molecules carrying specific enzymatically-induced dsb. We have compared the activities of extracts from an A-T cell line (AT5BIVA) with those from a lines showing normal radiation-sensitivity. The simple recombinant plasmid pUC18 was used as a substrate because it has a number of different enzyme break sites closely spaced at the same location (the multicloning site) on the molecule. This site is within the *lacZ* gene of pUC18, allowing the fidelity of rejoining to be assessed by expression of normal gene activity after extract treatment.

Physical rejoining of DNA was monitored by Southern analysis after gel separation, and the fidelity of rejoining by expression of the *lacZ* gene after bacterial transformation with the treated plasmid.

Breaks at the *Sal*I and *Eco*RI sites of pUC18 were rejoined by

extracts to form circular monomers, but the efficiency of rejoining was much higher at the *SalI* site. However, measurement of rejoining at several adjacent sites having different types of termini, generally showed a range of efficiencies with 5' 4-base > 3' 4-base overhangs and 4-base > 2-base > no overhang. Similar efficiencies were found for nuclear extracts from transformed cell lines, both 'normal' and A-T derived, and from non-transformed normal cell cultures. Little rejoining to give circular monomers was found for blunt-cut sites, or for mismatched ends (e.g., a 5' overhang paired with a 3' overhang).

While the efficiency of rejoining did not vary with the cell type used for extract preparation, the fidelity of rejoining was poor at some sites and with some extracts. In particular, at sites with a low rejoin efficiency, the fidelity of rejoining was very much lower for the A-T extracts than for normal cell extracts. Mis-rejoining was, however, unrelated to rejoin efficiency at other sites, suggesting that factors such as the exact sequence at the break site on the molecule may also influence the fidelity of rejoining.

Molecular analysis of mis-rejoined sites revealed that the molecules had suffered deletions of <100 base pairs around the original break-site. To investigate the mechanism of deletion formation, more than 30 misrepaired molecules were sequenced. All deleted molecules showed similar features: the deleted DNA was situated between short direct repeats (mostly 4 bases, but also 2 or 3 bases) and one repeat was retained in the mutant (misrejoined) molecule. Certain sequences appear to predominate in these repeats but more work is required to establish the exact specificity.

(ii) In an attempt to map the human gene complementing the radiosensitive mutant *irs2*, which has a very similar phenotype to A-T cells, *irs2* cells were fused to human lymphocytes. The fusion products were put through a stringent selection for resistance to X-rays or to the topoisomerase I-inhibitor camptothecin, since this gives an increased selective potential to distinguish hybrids from parental cells. However, after checking a number of clones surviving this treatment, it was found that none represented true hybrids, presumably because either the selective potential and/or the fusion efficiency
was too low. Attempts to improve these parameters did not result in success. The use of microcell-mediated transfer of human chromosomes is now being undertaken to achieve this aim, in collaboration with Dr. P.A. Jeggo (MRC Cell Mutation Unit, Brighton).

An important cellular indication of repair deficiency is inability to recover from the lethal effects of radiation when the dose is given at a low rate. Normal cells show a large recovery factor when irradiation is protracted in comparison to acute irradiation. However, for example, cells from A-T patients do not show this recovery. Some difficulties have been encountered in getting the *irs* mutants into the plateau-phase state necessary for protracted irradiation, to avoid complications of cell-cycle effects and population renewal. However, satisfactory experiments have been completed for the

irs2 mutant, showing that like A-T cells they recover little under protracted irradiation. This result further indicates that *irs2* belongs to a similar class of radiosensitive mutant to the major human type, exemplified by A-T.

(iii) It has been established that the predominant type of mutation induced by ionising radiations in mammalian cells is the large deletion or rearrangement. Much of this work was carried out with established lines of hamster cells, and the analysis has not proceeded beyond crude molecular mapping of the breakpoints involved in deletions. To understand the mechanisms of deletion formation in relevant cells, we have reinitiated mutation experiments with primary human fibroblasts, using X-rays and the *hprt* gene for analysis. The sequence of the complete human *hprt* gene and some of its flanking region is now available (56 kb), allowing the development of refined methods for the analysis of sites of radiation-induced mutation. In addition, several anonymous probes which map to the chromosomal region of *hprt*, Xq26, are available for checking the extent of deletions around the gene. We have established a mutation system with early-passage male human cells (HF12), and selected a number of spontaneous and radiation-induced mutants. A certain level of loss of mutants had to be accepted through poor growth potential or senescence. However, deletions in *hprt* were detected in several mutants using Southern blot analysis with a full-length human cDNA probe. Breakpoints are being assessed using the polymerase chain reaction (PCR), where primer sets can be created along the genomic region within and around the *hprt* gene. As yet none of these mutants has been found to co-delete anonymous sequences at the 3' end of the gene.

Publications:

THACKER ,J., and A.N. GANESH DNA break repair, radioresistance of DNA synthesis, and camptothecin sensitivity in the radiation-sensitive *irs* mutants: comparisons to ataxia-telangiectasia cells. Mutation Research 235 (1990) 49-58.

THACKER, J., E.W. FLECK, T. MORRIS, B.J.F. ROSSITER and T.L. MORGAN Localization of deletion breakpoints in radiation-induced mutants of the *hprt* gene in hamster cells. Mutation Research, 232 (1990) 163-170.

THACKER, J. Molecular nature of ionising radiation-induced mutations of native and introduced genes in mammalian cells. In Ionising Radiation Damage to DNA: Molecular Aspects, (Eds. S. Wallace & R.B. Painter), Wiley/Liss: New York. 1990, pp.221-229.

THACKER, J., and R.E. WILKINSON The genetic basis of resistance to ionising radiation damage in cultured mammalian cells. Mutation Research, 254 (1991) 135-142.

NORTH, P., A. GANESH and J. THACKER The rejoining of double-strand breaks in DNA by human cell extracts. Nucleic Acids Research, 18, (1990) 6205-6210.

Progress Report

Contract: Bi6-225 Sector: B13

Title: Evaluation of the frequencies of chromosomal aberrations induced in human blood lymphocytes by low doses of neutrons.

1	Lloyd	NRPB
2	Natarajan	Univ. Leiden Sylvius Lab.
3	Obe	Univ. Essen
4	Verschaeve	CEN - SCK
5	Palitti	Univ. degli Studi della Tuscia

I. Summary of Project and Global Objectives

The project forms an extension of work that was funded by CEC to investigate the lov dose response for chromosomal aberrations in human lymphocytes exposed in vitro to x-rays. That work showed, over the dose range 0-50 mGy, that the data fitted well to a linear regression. However, below 20 mGy, despite 200,000 cells being scored from 24 donors, the uncertainties on the data were such that one could not discount the possibility of a threshold. Therefore below 20 mGy the data were unable to support or reject the Pohl-Rüling hypothesis that such low doses to G_o lymphocytes could induce repair and indeed result in aberration yields below those for zero dose controls.

The purpose of the present project is essentially to repeat the work but to use low doses of fission spectrum neutrons. Five collaborating laboratories, listed above, are supported by the CEC and this progress report has been written to cover them jointly. In addition Dr. E.J. Tawn of BNF plc Sellafield, UK, is also collaborating in the work.

Head of Project 1: Dr. Lloyd

II Objectives for the reporting period

a) To irradiate blood to 8 doses; 0, 0.25, 0.5, 0.8, 1.25, 2.5, 12.5 and 62.5 mGy of fission spectrum neutrons of incident mean energy, 1.0 MeV. This is to be replicated with blood from 4 donors.

b) To culture the lymphocytes, make coded metaphase preparations and to distribute them to the collaborating laboratories.

c) To commence the microscope analysis.

III Objectives for next period

a) To complete the microscope analysis.

b) To collate and decode the data.

c) To analyse the results for inter donor and inter laboratory variation and to infer the dose response relationship.

IV Progress achieved including publications

All the irradiations have been completed. They were done at the Reactor Centre, Petten, The Netherlands. The lymphocytes were successfully cultured and chromosome preparations were made at the University of Leiden. The slides have been distributed to the participants and the microscope work is approximately 25% completed. As it is being scored "blind" it is not possible to comment on the results so far.

Publications

1. Lloyd et al. 1988, Frequencies of chromosomal aberrations induced in human blood lymphocytes by low doses of x-rays. Int. J. Radiat. Biol. 53 49-55.

2. Lloyd et al. ms in preparation. Chromosomal aberrations in human lymphocytes induced in vitro by very low doses of x-rays.

FINAL REPORT

Title of the project no.: BI6-E-312-D

Formation of micronuclei in human lymphocytes
after partial and whole body irradiation

Heads of project:

Prof. Dr. C. Streffer, Privatdozent Dr. W.-U. Müller

Scientific staff:

H.-W. Gantenberg, K. Wuttke

I. Objectives of the project:

Development of reliable methods to determine radiation dose in accidentally exposed persons using the micronucleus technique.

II. Objectives of the reporting period:

1. Establishing a micro-culture technique for human lymphocytes.

2. Introducing the cytochalasin B method and fixation of cells with preservation of cytoplasm.

3. Proliferation kinetics of stimulated lymphocytes.

4. Comparison of spontaneous micronucleus frequency in unexposed people.

5. Radiation induced micronuclei in lymphocytes of healthy donors exposed in vitro and in lymphocytes exposed either in vivo or in vitro

III. Progress achieved:

METHODOLOGY

1. Culture conditions: An aliquot of 15.000 cells obtained from heparinized blood was transferred to 1 ml RPMI 1640 medium supplemented with 15% fetal calf serum, L-glutamine, antibiotics, and phytohemagglutinin. The cultures were incubated in small tubes in a water bath at 37° C.

2. Fixation of cells: In the case of proliferation studies, cells were fixed on a glass slide using a standard protocol (hypotonic treatment with 75 mM KCl, fixation in ethanol/acetic acid 3:1). In the case of application of cytochalasin B, this inhibitor of cytokinesis was added to the medium, when the simultaneously performed proliferation studies signalled the successful stimulation of lymphocytes (= when the sum of the S- and G_2-cells exceeded 10%). Cells were fixed much more cautiously in order to preserve the cytoplasm (hypotonic treatment with 100 mM KCl, fixation with ethanol/acetic acid 5:1).

3. Staining of fixed lymphocytes: For the proliferation studies, the nuclei of the lymphocytes were stained with ethidium or propidium iodide and the relative DNA content was determined by quantitative fluorescence measurements with a microscope photometer. For counting micronuclei in binucleated cells, fixed lymphocytes were stained with Giemsa.

RESULTS

1. Methodological improvements

Usually, lymphocyte culturing is carried out in macrocultures (about 8 drops of blood in 8 ml of medium). In order to save material, microcultures (1 drop of blood in 1 ml of medium)

were established in our lab, and it could be shown that stimulation and growth of lymphocytes was the same in both culture systems. Due to the now lower numbers of lymphocytes available, we could no longer follow cell proliferation by using flow cytometry (as it was announced in the proposal). Thus, we switched over to microscope photometry which allows control of cell proliferation at much lower cell numbers than flow cytometry.

A second very important improvement was introduced when a publication of Fenech and Morley came to our attention, in which they described a method for identification of those lymphocytes that divided just once after stimulation by PHA. An exact knowledge of the fraction of lymphocytes that has divided is crucial, because micronucleus expression is strongly dependent on the number of mitoses carried out after radiation exposure. We therefore adopted the method of Fenech and Morley, in which cytochalasin B is used to prevent cytokinesis, whereas karyokinesis takes place unimpaired. Those lymphocytes that have completed a mitosis therefore show two cell nuclei per cell.

2. Proliferation of lymphocytes in vitro

Study of proliferation of lymphocytes in vitro after stimulation with phytohemagglutinin revealed, that even under exactly identical conditions proliferation varies considerably from individual to individual. This was true for the fraction of proliferating cells (between 15 and 50% of all lymphocytes), as well as for the start of proliferation (between 24 and 40 hours after stimulation). This means, that it is very dangerous to use a standard protocol in a way, that cytochalasin B is added and the cells are harvested at constant time points. Thus, continuous monitoring of proliferation is required in order to add cytochalasin B and to harvest the cells at the optimal time points.

3. Spontaneous frequency of micronuclei in healthy donors and patients

One of the prerequisites of the use of micronuclei as biological dosemeter is a spontaneous micronucleus frequency with low variability. Table 1 shows that indeed variability is rather small. In the healthy donor group the mean value amounts to 0.020 micronuclei per binucleated cell and in the patient group the mean is 0.014 micronuclei per binucleated cell. The difference between both groups is statistically not significant.

4. Radiation induced micronuclei in lymphocytes of healthy donors exposed in vitro and in lymphocytes of patients exposed either in vitro or in vivo

Evaluation of 48,028 binucleated cells of healthy donors after radiation exposure in vitro (doses: 1.25, 2.5, 3.75, or 5 Gy) and of 25,586 binucleated cells of patients exposed under the conditions mentioned above resulted in the following dose response equations:

a) Healthy donors: $y = 0.008 + 0.120\ D + 0.023\ D^2$
b) Leukemic donors: $y = -0.007 + 0.170\ D + 0.005\ D^2$

The quadratic coefficient is somewhat higher for healthy donors; this, however, is entirely due to a slightly lower response of lymphocytes of patients after 5 Gy. No differences can be detected between healthy and leukemic donors for doses lower than 5 Gy.

It was not possible to obtain binucleated lymphocytes after radiation exposure exceeding 2.5 Gy in vivo. This is probably due to the heavy medication that accompanies whole body irradiation of the patients. No differences were observed between in vitro and in vivo exposure after 1.25 Gy; after 2.5 Gy a slightly lower number of micronuclei was found in vivo

(0.316 micronuclei/cell) than in vitro (0.452 micronuclei/cell). However, evaluation of the in vivo exposed lymphocytes was very difficult after 2.5 Gy (and the heavy medication), so that the difference perhaps can be attributed to this reason.

TABLE I:
MICRONUCLEUS FREQUENCY AND DISTRIBUTION IN UNEXPOSED LYMPHOCYTES OF HEALTHY OR LEUKEMIC DONORS

Donor	Number of cells	Micronucleus distribution/cell 0	1	2	3	Micronuclei per cell
a) Healthy donors:						
1	721	704	15	1	1	0.028
2	563	561	2			0.004
3	1389	1378	8	2	1	0.011
4	1852	1835	17			0.009
5	787	776	10	1		0.015
6	3987	3913	69	4	1	0.020
7	234	233	1			0.004
8	1209	1172	33	4		0.034
9	3400	3299	96	5		0.031
10	999	969	27	3		0.033
11	1000	993	7			0.007
12	1000	995	5			0.005
sum:	17141	16828	290	20	3	0.020
b) Leukemic donors:						
1	1100	1087	10	1	2	0.016
2	608	603	5			0.008
3	595	577	18			0.030
4	2008	1994	13		1	0.008
5	903	883	16	4		0.027
6	450	447	3			0.007
7	261	252	9			0.034
8	1400	1391	8	1		0.007
9	823	808	15			0.018
sum:	8148	8042	97	6	3	0.014

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-E-206-GR

Greek Atomic Energy Commission
Nuclear Research Center
"Demokritos"
Aghia Paraskevi Attikis
GR-153 10 Athens

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 01-651.1360

Title of the research contract:

A new analysis of radiation-induced cytogenetic damage in human lymphocytes using the PCC technique, and its implications for biological dosimetry and the understanding of cell-cycle-dependent radiosensitivity fluctuations

List of projects:

1. A new analysis of radiation-induced cytogenetic damage in human lymphocytes using the PCC technique, and its implications for biological dosimetry and the understanding of cell-cycle-dependent radiosensitivity fluctuations.

Title of the research contract NO. BI6-E-206-GR:

A new analysis of radiation-induced cytogenetic damage in human lymphocytes using the PCC technique, and its implications for biological dosimetry and the understanding of cell-cycle-dependent radiosensitivity fluctuations.

Head of the project:

Dr. G.E.Pantelias

Scientific staff:

G.Politis, M.D.
K.Sambani, Ph.D.

I. Objectives of the project:

1. To develop a sensitive biological dosimeter, based on the analysis of C-banded peripheral blood lymphocyte prematurely condensed chromosomes (PCCs), for the early assessment of radiation injury and the establishment of absorbed dose estimates in accidental overexposures.

2. To elucidate the mechanisms of radiation action at the molecular, chromosomal and cellular levels by the study of the:
   a) effects of DNA repair inhibitors on the repair of radiation damage,
   b) effects of BrdUrd incorporation on radiation damage,
   c) effects of hyperthermia on the induction and repair of radiation-induced damage, and
   d) induction and repair of radiation damage in an X-ray sensitive CHO mutant cell line.

II. Objectives of the reporting period 1/1/89 to 30/9/90

MECHANISMS OF RADIATION ACTION

To study the effects of hyperthermia on the induction and repair of radiation-induced chromosome damage as visualized by the PCC technique.

To study induction and repair of chromosome damage in an X-ray sensitive CHO mutant cell line (xrs-5).

To study the effect of BrdU incorporation on cell radiosensitization as well as on the induction and repair of chromosome damage in plateau phase xrs-5 cells.

III. Progress achieved

1. BIOLOGICAL DOSIMETRY

Methodology

Cytogenetic dosimetry following ionizing radiation usually involves the analysis of metaphase chromosomes from mitogen stimulated peripheral blood lymphocytes. This project aimed at the development of a new cytogenetic approach for biological dosimetry which would allow the early assessment of radiation injury and the establishment of absorbed dose estimates in accidental overexposures. This new approach to biological dosimetry is based on the analysis of radiation-induced chromosomal aberrations scored in C-banded peripheral blood lymphocyte prematurely condensed chromosomes (PCCs). Lymphocytes are separated from irradiated whole blood by Ficoll-Paque sedimentation and fused with mitotic Chinese Hamster Ovary (CHO) cells for PCC induction[1]. Radiation-induced chromosomal fragments increase the number of PCCs beyond the diploid chromosome number of 46. The yield of fragments, however, decreases with time after irradation due to repair processes and formation of exchanges such as dicentrics and rings. Since conventional Giemsa staining of PCCs does not allow visualization of chromosome centromeric regions, a C-banding procedure has been developed to identify centric rings and dicentric PCCs. Air dried chromosome preparations are placed in 0.2M HCl at ambient temperature for 15 min. Excess HCl is removed by gently blotting the slides, which are then treated with 5% barium hydroxide for 5-15 min, depending on the age of the preparations. Slides are briefly immersed in 0.2M HCl, rinsed in Sorensen's buffer (pH 6.8), and placed in hot Sorensen's buffer (60 ^{o}C) for one hour. They are then stained in 7% Giemsa for 7-10 min.

Results and Discussion

The cytogenetic approach used in this project, which allows the direct observation of chromosome damage without having lymphocytes proceed to mitosis, can be very useful for biological dosimetry purposes. Ring and dicentric analysis in PCCs provides not only confirmatory evidence of an exposure, especially when blood samples are not available soon after irradiation, but also quantitative estimates in terms of equivalent whole-body doses. Figures 1 and 2 show dicentrics (dic) and a centric ring (cr) in peripheral blood lymphocyte PCCs obtained 6h after exposure to 5 Gy X-rays.

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1. Pantelias, G.E., and H.D. Maillie (1983). A simple method for premature chromosome condensation induction in primary human and rodent cells using polyethylene glycol, Somat.Cell Genet,. 9, 533-547.

Pantelias, G.E. and H.D. Maillie (1984). The use of peripheral blood mononuclear cell prematurely condensed chromosomes for biological dosimetry. Radiation Res., 99, 140-150.

fig. 1 fig. 2

Preliminary data for X-rays suggest that the aberration yields observed in PCC preparations are similar to those in metaphase spreads. However, further experiments are required in order to establish dose response curves for the most common radiation sources, dose rates and qualities used, and compare them with those established using the conventional metaphase chromosome analysis.

2. MECHANISMS OF RADIATION ACTION

A. EFFECTS OF DNA REPAIR INHIBITORS ON REPAIR OF RADIATION DAMAGE

Methodology

The identification of the sequence of events that lead from DNA damage to chromosome damage and cell death is of particular importance for the elucidation of the action of ionizing radiation in living cells. Experiments were carried out to study the ability of compounds acting via inhibition of DNA polymerases such as araA, araC and aphidicolin, to inhibit repair of radiation-induced damage at the DNA, chromosomal and cellular levels in plateau phase CHO cells. For the experiments at the chromosomal level, the PCC technique was used. Repair of total DNA breaks was measured by the unwinding technique and repair of DNA double strand breaks by the neutral filter elution technique. The results obtained at these end points were compared with results of experiments measuring fixation of radiation-induced PLD by these compounds. When the potentiation of killing of a given araA concentration was studied, araA was added to the cells one hour before irradiation. Plateau phase cells were chilled on ice before irradiation to prevent repair. They were then irradiated on ice using a Siemens theurapeutic X-ray machine operated at 250kV, I5 mA with 2mm Al filter. After treatment, cells were analyzed either immediately or after a 24-48h incubation in araA-free condi-

tioned medium. Conditioned medium was obtained from plateau phase cultures and used after filtration to remove floating cells and debris.

Results

In agreement with the hypothesis that DNA polymerase activity is involved in cellular repair reactions, araA and aphidicolin strongly inhibited repair of radiation induced damage at the DNA and the chromosomal level. Also, araC inhibited repair at these endpoints but only to a very limited extent. The relative inhibition of repair by these compounds was similar at the various end points studied. At the survival level araA effectively fixed radiation induced PLD resulting in survival levels corresponding to an exponential survival curve. On the other hand, araC and aphidicolin had only a small effect on cell survival. DNA replication was effectively inhibited by aphidicolin, moderately by araC, and even less by araA.

- araA toxicity: Incubation of cells with various concentrations of araA for 3h resulted in enhanced cytotoxicity when plating was carried out immediately after treatment. For example, 500 µM araA reduced plating efficiency to 4% of that of untreated controls. However, cell killing diminished when plating was delayed for 24h and was essentially absent at this concentration in cells plated 48h later.

- Effect of araA on cell survival: An increase in survival was observed in cells plated sometime after irradiation, indicating repair of PLD. The postirradiation (3h) presence of 100 µM araA prevented the increase in survival observed and caused a small potentiation in cell killing. This potentiation in cell killing indicates fixation by araA of radiation-induced PLD normally repaired in cells plated immediately, and was more pronounced after treatment with 500 µM araA.
Cell survival after irradiation as a function of araA concentration rapidly decreased with increasing araA concentration. Concentration of 500 µM araA combined almost maximum potentiation of radiation-induced cell killing with maximum drug toxicity.

- Effect of araA on radiation-induced chromosome damage: A linear induction of chromosome fragments was observed as a function of the radiation dose with an induction rate of 2 fragments per cell and per Gy. After an exposure of 15 Gy x-rays, a rapid repair was observed in cells incubated after irradiation in the plateau phase occurring with a half time of one hour. This value is similar to that obtained at the cell survival level for PLD repair. In the presence of 100 µM araA repair was partly inhibited. A complete inhibition of repair was observed in the presence of 500 µM araA during the first 6h.

Experiments were performed also with cells exposed to 8 Gy x-rays and analyzed for residual chromosome damage either immediately after a 3h incubation with various concentrations of araA, or 24h later. An inhibition of chromosome repair with increasing araA dose was observed. Concentrations higher than 600 µM caused a complete inhibition of repair.

It is interesting to note that araA concentrations that caused complete inhibition of repair at the chromosome level also caused complete expression of the araA-sensitive sector of PLD.

Discussion

The results suggest that repair and araA-mediated fixation of PLD have their counterparts at the chromosome level as indicated by the similar repair kinetics and inhibition/fixation characteristics obtained for PLD and chromosome damage. Since araA is expected to inhibit the polymerization steps necessary for the completion of DNA repair, it is suggested that DNA polymerization is required for chromosome repair. Among lesions induced by radiation in the DNA, double strand breaks are the lesions more likely leading to chromosome damage. Furthermore, the results obtained demonstrate that the efficacy of a polymerase inhibitor to inhibit DNA and chromosome repair does not always coincide with its ability to fix radiation induced PLD, or with its ability to inhibit DNA replication. This finding indicated that partly different biochemical pahtways may underlie PLD fixation, DNA repair inhibition and DNA replication inhibition.

B. EFFECT OF BrdUrd INCORPORATION ON RADIATION DAMAGE

Methodology

In order to characterize the relative contribution of increased DNA damage induction and increased PLD fixation in the halogenated-pyrimidine-induced radiosensitization, experiments were carried out at the DNA level, using non-unwinding DNA filter elution, at the chromosomal level, using the PCC technique, and at the cellular level. Exponentially growing and plateau phase cultures of CHO cells, and a radiation sensitive mutant of CHO, the xrs-5 cell line, were used as biological systems in order to enable parallel experiments at DNA, chromosomal and cellular levels.

Results and Discussion

Incorporation of BrdUrd into DNA, in the place of thymidine, sensitizes exponentially growing and plateau phase CHO cells to a subsequent exposure to low LET radiation. An increase in the amount of DNA and chromosome damage induced per unit radiation dose was observed with increasing incorporation of BrdUrd into DNA that was quantitatively similar to the increase observed in the survival curve slope. Although sensitization was observed both in cells irradiated in the exponential as well as in cells irradiated in the plateau phase of growth, the degree of sensitization was significantly larger in exponentially growing cells for the same degree of thymidine replacement by BrdUrd in the DNA. It is hypothesized that this result indicates the possible importance of chromatin structure at the time of irradiation and/or the importance of

chromatin conformation changes after irradiation in the expression of radiation induced potentially lethal damage in BrdUrd containing cells. BrdUrd incorporation affected both the slope and the shoulder width of the survival curve, and increased the induction of DNA and chromosome damage per unit absorbed dose. The increase observed in the survival curve slope was quantitatively similar to the increase observed in damage induction at the DNA and the chromosomal level, suggesting a cause-effect relationship between these phenomena. Reduction in the shoulder width did not correlate with the increse in DNA and chromosome damage induction suggesting that different phenomena, probably related with enhanced fixation of radiation induced PLD in BrdUrd containing cells, underlie its modulation.

xrs-5 cells are sensitive to ionizing radiation and defective in the repair of radiation-induced DNA double strand breaks, chromosome damage, and potentially lethal damage (PLD). Compared to repair-proficient CHO 10B cells, a reduction was observed in the overall BrdU-mediated radiosensitization in plateau phase xrs-5 cells for the same degree of thymidine replacement. This finding is interpreted by a model in which two distinct components act to produce the overall radiosensitization observed. One component involves processes associated with the increase in initial damage (DNA and chromosome) production per unit absorbed dose and causes an increase in the slope of the survival curve, while the second component involves enhanced fixation of radiation-induced damage (PLD) and causes a reduction in the width of the shoulder of the survival curve. It is suggested that in plateau phase xrs-5 cells, the deficiency in the repair of radiation-induced damage compromises BrdU-mediated radiosensitization by leaving active only the radiosensitization component that is associated with an increase in damage induction. Enhancement of cell killing by BrdU resulted in a decrease in D_0, the relative value of which was similar to the relative increase in the production of chromosome damage as measured by the PCC method. The relative values for the change in D_0 and the production of chromosome aberrations were similar in plateau phase CHO and xrs-5 cells, suggesting that the physico-chemical and/or biochemical processes associated with this phenomenon are the same in the two cell lines. Radiosensitization of a magnitude similar to that observed in exponentially growing CHO cells was induced by BrdU in exponentially xrs-5 cells. This effect is attributed to a partial expression of the repair gene that permits some repair of radiation-induce damage and which is compromised by BrdU.

C. EFFECT OF HYPERTHERMIA ON INDUCTION AND REPAIR OF RADIATION DAMAGE

1. Hyperthermia

Methodology

In order to study the effect of hyperthermia on the induction and repair of radiation induced chromosome damage, as a first stage, experiments were designed to study the effects of heat (43 and 45.5 C) on chromatin

morphology and nuclear organization, as visualized by PCC, in exponentially growing and plateau CHO cells. Experiments were also carried out with exponentially growing HeLa cells.

Results and Discussion

The results obtained indicate that exposure to heat drastically reduces the ability of interphase chromatin to condense and the ability of the nucleolar organizing region to disintegrate under the influence of factors provided by mitotic cells when fused to interphase cells. The fraction of cells with non-disintegrated nucleoli increased with increasing exposure time at 45.5 C and reached a plateau at almost 100% after about 20 min. Exponentially growing and plateau phase cells showed similar response. Recovery from the effects of heat on chromatin condensation and disintegration of the nucleolar organizing region depended upon the duration of the heat treatment. For exposures up to 15 min at 45.5 C, a gradual reduction in the fraction of cells with non-disintegrated nucleoli was observed when cells were allowed for repair at 37 C. However, only a very limited amount of repair was observed after a 30 min exposure to 45.5 C. The repair times observed at the chromosome level were similar to those reported for the removal of excess protein accumulating in chromatin or the nuclear matrix, suggesting a causal

relationship between the two phenomna. It is proposed that nuclear protein accumulation on chromatin or in the nuclear matrix reduces the accessibility of chromatin to enzymes responsible for the phosphorylation reactions necessary for chromatin condensation and disintegration of the nucleolus.

2. Effect of hyperthermia on the induction and repair of radiation-induced chromosome damage

The effect of pre-exposure to heat on the induction and repair of chromosome damage was measured in plateau phase CHO cells using the PCC technique. Plateau phase cultures were obtained by growing 10^5 cells in T25 tissue culture flasks for 4 days without refeeding. Cells were exposed to heat (45.5 C) for 8 or 15 min in fresh growth medium without serum, were irradiated, either immediately or at various times thereafte (up to 16h), and were returned to the incubator at 37 C. At various time intervals after irradiation (up to 24h) flasks were trypsinized and an aliquot containing 10^6 cells were mixed with an equal amount of cells selected at mitosis using nocodazole. The cell mixture was treated with PEG to effect fusion and PCC induction. Exposure to heat prevented chromatin from fully condensing and nucleoli from disintegrating. Therefore, measurements of residual chromosome fragments were carried out after reversion of these effects. Despite these inherent difficulties in measuring chromosome breaks in interphase cells after exposure to heat, the results obtained clearly indicated a significant reduction in the ability of heated cells to repair radiation-induced chromosome damage. The experiments also indicated a larger induction by radiation of chromosome damage in heated cells.

D. INDUCTION AND REPAIR OF CHROMOSOME DAMAGE IN AN X-RAY SENSITIVE CHO MUTANT CELL LINE

Induction and repair of chromosome damage were studied in interphase xrs-5 cells by means of the PCC technique. The results obtained were compared to those previously reported for CHO cells. Induction of chromosome damage per unit of absorbed radiation dose was in xrs-5 cells larger by a factor of 2.6 than in CHO cells. Changes in chromatin structure, associated with the radiation sensitive phenotype of xrs-5 cells, that increase the probability of conversion of a DNA dsb to a chromosome break are invoqued to explain this effect. Repair of chromosome breaks as measured in plateau-phase G_1 cells was deficient in xrs-5 cells, and the number of residual chromosome breaks was practically identical to the number of lethal lesions calculated from survival data. This observation suggests than non-repaired chromosome breaks are likely to be manifestations of lethal events in the cell. The yield of ring chromosomes scored after a few hours of repair was higher by a factor of 3 in xrs-5 compared to CHO cells. This increase in ring formation suggests an increase in the probability of misrepair of chromosome damage that may stem either from the reduced ability of xrs-5 cells to repair DNA dsb, or from the higher production of chromosome fragments observed per cell and per Gy.

IV. Other research groups collaborating actively on this project:

Most of the experimental work involved in the achievement of the objectives of this project was carried out in collaboration with Professor Dr.G.Iliakis in the Laboratory of Experimental Radiation Oncology, Thomas Jefferson University Hospital, Department of Radiation Oncology and Nuclear Medicine, Philadelphia, PA 19107, USA.

V. Publications:

1. PUBLICATIONS IN SCIENTIFIC JOURNALS AND ABSTRACTS

Iliakis, G., G.E.Pantelias, R.Okayasu, and R.Seaner (1987). 125-IdUrd induced chromosome fragments, assayed by premature chromosome condensation, and DNA double strand breaks have similar repair kinetics in G_1-phase CHO-cells, International Journal of Radiation Biology, 52, 705-722.

Iliakis, G., G.E.Pantelias, and R.Seaner (1988). Effect of arabino-furanosyladenine on radiation-induced chromosome damage in plateau phase CHO-cells measured by premature chromosome condensation: Implications for repair and fixation of alpha-PLD, Radiation Research, 114, 361-378.

Pantelias G.E., G. Iliakis, R. Okayasu and R. Seaner. The use of premature chromosome condensation technique in the study of the mechanisms of radiation-induced chromosome breakage and rearrangement. 37th Annual Meeting of Radiation Research Society and 9th Annual Meeting of North American Hyperthermia Group, Seattle, March 1989.

Iliakis, G., G.E. Pantelias, R.Seaner and R.Okayasu (1989). Comparative studies on repair inhibition by araA, araC and aphidicolin of radiation-induced DNA and chromosome damage in rodent cells: Comparison with fixation of PLD. International Journal of Radiation Oncology, Biology and Physics, 16, 1261-1265.

Pantelias, G.E., G. Iliakis and R. Seaner. Effect of hyperthermia on the induction and repair of radiation induced chromosome damage as visualized by the technique of premature chromosome condensation. 37th Annual Meeting of Radiation Research Society and 9th Annual Meeting of North American Hyperthermia Group. Seattle, March, 1989.

Iliakis, G., S. Kurtzman, G.E. Pantelias and R. Okayasu. Radiosensitization by halogenated pyrimidines: Effect of BrdUrd on the induction by radiation of DNA and chromosome damage and its correla-

tion with cell killing. 37th Annual Meeting of Radiation Society and 9th Annual Meeting of North American Hyperthermia Group. Seattle, March 1989.

Iliakis G. and G.E.Pantelias (1989). Effect of hyperthermia on chromatin condensation and nucleoli disintegration as visualized by induction of premature chromosome condensation in interphase mammalian cells. Cancer Research, 49, 1254-1260.

Iliakis G., S.Kurtzman, G.E.Pantelias and R.Okayasu (1989). Mechanism of radiosensitization by halogenated pyrimidines: Effect of BrdUrd on radiation-induced DNA and chromosome damage and its correlation with cell killing, Radiation Research, 119, 286-304.

Iliakis, G. and G.E. Pantelias (1990). Production and repair of chromosome damage in an X-ray sensitive CHO mutant visualized and analyzed in interphase using the technique of premature chromosome condensation, International Journal of Radiation Biology, 57, 1213-1223.

Pantelias, G.E. and G.Iliakis. Chromosome aberration formation in cells exposed to heat in various phases of the cell cycle measured in interphase by the technique of premature chromosome condensation. 38th Annual Meeting of Radiation Research Society and 10th Annual Meeting of North American Hyperthermia Group, New Orleans, April 1990.

Pantelias, G.E. and G.Iliakis. Production and repair of chromosome damage in an X-ray sensitive CHO mutant visualized and analyzed in interphase using the technique of premature chromosome condensation. 38th Annual Meeting of Radiation Research Society and 10th Annual Meeting of North American Hyperthermia Group, New Orleans, April 1990.

Wang, Y., S.Kurtzman, G.E.Pantelias, R.Okayasu, R.Seaner, and G.Iliakis. Effect of BrdU incorporation on radiation sensitivity and induction of DNA and chromosome damage in DNA double strand break repair deficient cell lines. 38th Annual Meeting of Radiation Research Society and 10th Annual Meeting of North American Hyperthermia Group, New Orleans, April 1990.

Iliakis, G., D. Blocher, L. Metzger, and G.E. Pantelias (1990). Comparison of DNA double strand break rejoining as measured by pulsed field gel electrophoresis, neutral sucrose gradient centrifugation, and non-unwinding filter elution in irradiated plateau phase CHO cells. International Journal of Radiation Biology, in press.

Iliakis, G., G.E. Pantelias, and S. Kurtzman (1991). Mechanisms of radiosensitization by halogeneted pyrimidines: Effect of BrdUrd on cell killing and interphase chromosome breakage in radiation sensitive cell. Radiation Research, 125, 56-64.

Pantelias, G.E. and G. Iliakis. Hyperthermia-induced cellular radiosensitization: Synergetic effects that may contribute to the reduction in therapeutic radiation doses. First National Radiation Protection Congress, Athens, Greece, October 1990.

Pantelias, G.E. and G. Iliakis. Radiation as a tool for the study of intra-nuclear processes that lead to chromosome breakage and rearrangement. First National Radiation Protection Congress, Athens, Greece, October 1990.

2. THESES

Sambani, K.D. (1989). Premature chromosome condensation induction in peripheral blood cells from normal individuals and AML and CML patients: Comparative kinetic studies in G_1 phase. NRCPS "Democritos" and Medical School of the University of Athens.

Final Report

B 13

Contract: Bi6-338 Sector: ~~B21~~
Title: Cytological follow-up of individuals exposed in Goiania (Brazil) accident

1. Natarajan Univ. Leiden Sylvius Lab.

I Summary of Project and Global Objectives

A radiation accident involving cesium-137 therapy source occurred in Goiania (Brazil) in September 1987 in which more than 50 individuals were exposed to moderate to high doses (0.2 - 7 Gy) of gamma radiation. Cytogenetic technique (i.e., frequencies of dicentrics and rings in peripheral lymphocytes) was employed to estimate the absorbed radiation dose. The follow-up study extending over more than one year indicated a decline in the frequency of dicentric carrying lymphocytes, with an average half life of about 130 days, which is much lower than other published estimates.

Using chromosome-specific biotinylated library probes for chromosomes 1, 2, 8 and 19, the frequencies of chromosomal translocations and deletions as well as the incidence of aneuploidy in the lymphocytes of exposed individuals were determined. In some individuals there was a significant increase in the frequency of translocations and aneuploidy. This increase was not dose dependent. Some of the translocation bearing lymphocytes appeared to be of clonal origin exhibiting the same translocation. We also determined the frequencies of HPRT mutations in lymphocytes using BrdU labelling method and the frequencies of hemoglobin mutations using mutation specific antibodies. In both cases, some individuals showed an increase of 2 to 50 fold in mutant frequencies in comparison to unexposed controls.

INTRODUCTION

On September 13, 1987, a shielded, highly radioactive caesium-137 source (50.9 TBq or 1375 Ci) was removed from its protective housing in a teletherapy machine left in an abandoned private clinic in Goiania, Brasil. Since the aim of this removal of the source was to sell the scrap metal from the housing, the source was broken into pieces and moved to three junk yards and subsequently spread to several houses situated in different areas of Goiania. In this process many people incurred large doses of radiation due to both external and internal exposure. Four of the casualties died and twenty eight people suffered radiation burns. Residences and public places were contaminated and the decontamination process involved demolition of several residences and various other buildings, removal of top soil from large areas, generating about 3500 m^3 of radioactive waste. A full report of this accident has been published by the International Atomic Energy Agency (1988).

In this report, we summarize results of the initial estimation of absorbed dose both due to internal and external contamination as well as the follow up of the victims using cytogenetic parameters. For more details please refer to publications marked *.

METHODS:

Estimation of frequency of chromosome aberrations:

Phytohemogglutinin-stimulated peripheral blood lymphocytes from radiation victims were cultured in F-10 medium for 48h, arrested with colcemid, treated with a hypotonic solution (0.075 M KCl) and fixed in acetic acid - methanol. Metaphase spreads were made by the standard protocol (IAEA, 1986). For scoring dicentric frequencies, the slides were stained with aqueous Giemsa solution. Depending on the frequencies of dicentrics 100 to 500 metaphases were scored to make the dose estimates.

For determination of the frequencies of aneuploidy and translocations in situ hybridization with chromosome specific DNA library or probes were used. 10 to 30 ug/ml of biotin labelled DNA representing library inserts were combined with competitor DNA, ethanol precipitated and resuspended in 50% formamide, 2 X SSC, 10% dextran sulfate and hybridized in situ with cytological preparations. After hybridization the slides were washed and incubated in 5% natural non-fat dry milk for 15 min in a moist chamber at room temperature. Detection of the biotinylated probe was achieved using fluorocein labelled avidin. All detection reagents were incubated with 5 ug/ml FITC-conjugated Avidin DCS for 20 min at room temperature followed by two washes. When necessary the signals were amplified by another incubation with Avidin D and FITC for 20 min. Slides were dehydrated, dried and mounted in 20 mM Tris-HCl (pH 8.0), 90% glycerol containing 2% of antifade 1,4-diazabicyclo-(2,2,2)-octane. Preparations were examined under a Zeiss microscope equipped with DAPI and FITC epifluoresence optics. Metaphases and interphases were scored for the presence of translocations and aneuploidy respectively.

Estimation of HPRT and hemoglobin mutant frequencies:

Lymphocytes from blood samples (shipped from Brazil) were separated by Ficoll-Hipaque density centrifugation and stored at -70°C. Cryopreserved lymphocytes were grown in lectin and 6-thioguanine containing medium followed by a second round in bromodeoxyuridine (BrdU, 5uM) containing medium. Slides were prepared by cytocentrifugation of fixed lymphocytes and stained with Hoechst fluorescent stain. Labelling indices in the cells grown in the

presence (LIt) and absence (LIc) of 6 thioguanine was estimated under a fluorescent microscope. Mutant frequencies were calculated by dividing the values of LIt by LIc.(Natarajan et al., 1991 a).

The frequencies of mutations in hemoglobin locus were determined using the technique developed by Bernini et al (1990).

RESULTS AND DISCUSSION:

INITIAL DOSE ESTIMATION

Mainly two techniques were used to estimate the dose, namely (a) internal dosimetry involving bioassay and whole body monitoring and (b) estimation of doses by determining frequencies of exchange type of aberrations (dicentrics and rings) in the peripheral blood lymphocytes.

Internal Dosimetry:

Initially, attempts were made to identify who had heavy internal contamination (mainly through ingestion) and estimation of their intakes by monitoring urine and faecal samples. Some of the samples were very radioactive and needed special handling. Age specific modelling based on Oak Ridge National Laboratory reports was made for estimating the committed dose profiles of different individuals. Prussian Blue (ferric cyanoferrate) which binds to Caesium ions was administered daily. The average half-life of Caesium in the body is about 100 days and with the administration of Prussian blue, the half life is reduced to about 1/3 and thereby the body burden is also reduced. The efficacy of Prussian Blue in promoting decontamination process was determined both by whole body counting and measuring radioactivity levels in urine. There was no statistically significant difference in the estimates made using these two different methods and Prussian Blue was found to be very effective in eliminating Caesium-137 from the body, provided the dosage was more than 3 gms. per day (I.A.E.A., 1988).

During the follow up studies, in the first weeks after the accident, the radioactivity in the blood samples of some heavily exposed individuals was measured. If one considers that Caesium and Potassium behave similarly in the body, as suggested by literature data, one would expect that most of the radioactivity should be found in the serum. We found that the activity was confined to blood cells and mainly in the erythrocytes. When the erythrocytes were lysed and the activity was measured, it was found that most of the activity was retained in the membranes (Oliveira et al.,1990).

CYTOGENETICS

Blood samples for chromosome analysis arrived few days after the accident (from October 2, 1987) in Rio de Janeiro. More than 100 samples were processed using the standard protocol. Absorbed radiation dose was estimated from the yield of dicentrics and centric rings. Since no dose - response curve for a low rate Caesium-137 (simulating this accident) was available, a calibration curve generated earlier for Cobalt-60 at a dose rate of 0.12 Gy min^{-1} was used for the dose estimates. The calibration curve fitted the equation:

$Y = (2.70 + 1.03) \times 10^{-2} D + (2.20 + 0.49) \times 10^{-2} D^2$

The alpha and beta coefficients for yield of exchange aberrations agree quite well with other previously published dose response curves generated for chronic gamma radiation (Ramalho et al., 1988). As one would expect, due to the complex nature (protracted exposure, different dose rates, fractionated doses) these dose estimates are only approximations.

The distribution of individuals at different levels of estimated doses is presented in Table 1.

Table 1

Estimated dose (Gy)	Number of probands
0 - 0.49	81
0.50 - 0.90	8
1.00 - 1.99	8
2.00 - 2.99	4
3.00 - 3.99	1
4.00 - 4.99	3
5.00 - 5.99	2
6.00 - 6.99	2
7.00	1
Total	110

Of these, samples from 29 probands whose estimated doses were 0.50 Gy and above were studied in great detail with regard to distribution of aberrations among the lymphocytes. Following uniform exposure to low LET radiation the dicentric chromosomes among the lymphocytes follow a Poisson distribution. Deviation from Poisson, such as overdispersion, may indicate a partial body irradiation (I.A.E.A, 1986). Though most of the victims, by the nature of this accident must have received nonuniform exposure as evidenced by localized burns, the distribution of dicentrics was non-Poisson only in six cases. In view of the fact that these victims had a complex nature of exposure, the distribution of dicentrics was indistinguishable from Poisson (Ramalho et al., 1990a).

It is known that the scoring criteria vary between laboratories and this may have the consequence on the accuracy of estimation of absorbed dose based on dicentric frequency in peripheral lymphocytes. A small inter-laboratory comparison study was made using two chromosome preparations from one of the heavily exposed victims. The frequencies scored by four laboratories, namely, Kyoto (Sasaki), Leiden (Natarajan), Oak Ridge (Littlefield) and Rio de Janeiro (Ramalho) were very similar (Ramalho et al, 1991), reassuring that the chromosome aberration data on which dose estimates were made are reliable.

For an acute human whole body exposure to low LET radiation, a lethal dose for 50% mortality occurring within sixty days has been estimated to be 3 to 4 Gy without any medical treatment (Duncan and Nias,1977). However, the fact that victims receiving chronic exposure spread out over several days as in the case of the Goiania accident supported by medical treatment survived doses 4 to 7 Gy and this is not unexpected.

FOLLOW-UP STUDIES

Unstable Chromosome aberrations:

The frequency of chromosomal aberrations in patients who had received estimated radiation doses above 0.5 Gy was determined periodically after the

accident. The frequencies of dicentrics and rings decreased with time in most of the patients, whereas in patients who had internal contamination, the frequency increased initially up to about 100 days and then decreased. Some typical cases are presented in Table 2.

Table 2:

Proband no.	Frequency of dicentrics & rings/cell at days			
	0	30	100	470
1	1.25	0.98	0.46	0.123
2	1.03	0.94	0.46	0.066
16	0.07	0.10	0.11	0.006
21	0.05	0.07	0.10	0.016

(after Ramalho et al., 1990a)

The persistence of dicentrics in the lymphocytes of individuals exposed to low LET radiation has been studied earlier and the yield has been reported to decrease with time half lives of 530 to 1600 days after the exposure (Sasaki, 1983). In Goiania accident, the disappearance of dicentrics and rings containing lymphocytes is estimated to have a half life of about 130 days, with a range of 95 to 220 days (Ramalho et al., 1990a). The half life times reported in literature are based on few cases of acute irradiation or larger samples of partial body irradiation. The shorter half life found in this accident may be attributable to high bone marrow depletion in several patients and the chronic nature of exposure. Similar results have been observed in the recent Salvador radiation accident victims (Littlefield, G. personal communication).

Stable chromosome aberrations:

Unlike unstable aberrations which decline with time, the frequency of stable aberrations (reciprocal translocations) is essentially independent of time after irradiation. If one assumes that lymphocytes with and without stable aberrations proliferate at the same rate, then one can use the frequency of such aberrations to estimate radiation doses from past exposures. Lymphocytes carrying some specific translocations may proliferate faster and may have selective advantage. For detection of translocations, G banding of the chromosomes is usually employed. Since each chromosome has a specific pattern any translocation involving two different chromosomes can be detected. However, this technique is laborious and time consuming. Recently techniques have become available to use chromosome specific libraries and insitu hybridization to specifically stain single pairs of human chromosomes. Any translocation involving that particular chromosome can be easily discerned. Using chromosome specific biotinylated library probes for chromosomes 1, 2, 8 and 19 and in situ hybridization, we estimated the frequencies of translocations, deletions and incidence of aneuploidy in lymphocytes of 14 victims 470 days after the radiation accident (Natarajan et al., 1991a). There were high frequencies of translocations and deletions, though they were not dose related. The data pertaining to chromosome #2 in some probands are presented in Table 3.

Table 3:

No. of proband	estimated dose (Gy)	lymphocytes (%) with deletion	translocation
1.	7.0	2.3	0
2.	6.2	8.3	6.8
3.	4.4	3.0	2.0
4.	4.3	5.0	1.0
5.	3.0	0	0
6.	2.9	1.6	0
7	2.7	0	2.7

(From Natarajan et al., 1991a).

The pattern of distribution of frequencies of translocations and deletions among the different probands was very similar to that found for chromosome #2. In addition to whole chromosome libraries, we also used a probe for centromeric repetitive DNA of chromosome #1 (puc 1.77) to detect for aneuploid cells by scoring the number of fluorescent signals in the interphase nuclei. Though a high frequency of hypoploid cells was found in all cases, they were not taken into account for assessing aneuploidy as lack of signals can be due to technical artifacts. Frequency of hyperploid cells varied between 0 to 1.12 % for chromosome #1 between individuals and was not dose related. Similar pattern of distribution was observed for other chromosomes as well (Natarajan, et al., 1991a).

Mutations at the HPRT locus:

Seven months after the accident, few samples of lymphocytes from victims were processed for detecting the presence of $HPRT^-$ variants. Bromodeoxyuridine labelling in the presence of 6-thioguanine was used to detect these variants. Variant frequencies in these probands varied between 3.6×10^{-5} to 3.8×10^{-4} in the lymphocytes in comparison to 1 to 5×10^{-6} found in parallel controls, indicating an increase of 10 to 100 fold over the controls (Natarajan et al., 1991a).

Mutations at the haemoglobin locus:

A technique to detect mutations in the beta chain of haemoglobin in human erythrocytes using polyclonal monospecific antibodies (against HbS or san Jose or Leiden) has been standardized by us (Bernini et al, 1990). Since one measures changes in a single codon in this system (transversion -HbS; transition - Hb San Jose and deletion - Hb Leiden), the spontaneous frequency of these mutations is very low ranging from 1 to 5 in 10^8 cells.Seven months after the accident, frequencies as high as 11×10^{-6} were observed in the blood samples of some of the victims (Bernini et al., unpublished). Though it is generally assumed that ionizing radiation predominantly induces deletion type of mutations, the results
obtained from the follow up studies of the victims of Goiania accident indicate that in vivo chronic irradiation in man can lead to high frequencies of point mutations in somatic cells.

CONCLUSIONS:

Cytogenetic dosimetry was found to be an extremely useful technique for estimating the external whole body radiation dose and inhomogeneity in the

distribution of the dose in the exposed individual. It was found to be helpful in providing useful information to the physician responsible for diagnosis and prognosis.

The half life of dicentric bearing lymphocytes in vivo was found to be lower in this study (95 to 220 days) than the estimates (about 3 years) reported in the literature.

Chromosome painting technique was found to be very efficient in detecting chromosomal translocations and numerical aberrations in the follow up studies. Evidence was obtained for clonal proliferation of lymphocytes containing specific translocations.

Both types of somatic mutations studied, i.e., HPRT- mutations in lymphocytes and hemoglobin mutations in erythrocytes, were found to increase in the exposed individuals. The increase in hemoglobin mutations indicate that ionizing radiation can induce single base pair changes very efficiently in contrast to the belief that ionizing radiation induces mainly deletion type of mutations.

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* Publications arising from the contract Bi6-338 of the CEC Radiation Protection Research Programme.

Progress Report

Contract: B170023

1.	Seymour	NEB
2.	Riches	St Andrews University
3.	Pertusa	Valencia University

I Summary of Project and Global Objectives

There is a need for a relevant in vitro assay for studying cell transformation of human cells. In this way the molecular mechanisms and dose response relationships of carcinogenesis in humans can be studied. As about 85-90% of human tumours are of epithelial origin, it is important to utilise cultures of normal human diploid epithelial cells in transformation studies.

Studies on human epithelial systems, reviewed at the Dublin workshop on Cell Transformation, revealed that a promising approach to the investigation of oncogenic transformation in human systems was to utilise lines that had been developed from different tissues. The most promising of these are the SV40 immortalised human urothelial cell line, SV-HUC-1, developed by Reznikoff and the HPV 16 or 18 immortalised lines which can be developed routinely using the technique developed by DiPaolo from the transformation zone of the human cervix. This system has the advantage of being immortalised by a human virus rather than SV40, although there is considerable variation form line to line, and the system is technically more difficult. A third promising human system is the immortalised keratinocyte line (HaCaT) developed by Boukamp et al. This line arose spontaneously in normal keratinocyte cultures form a subject with skin carcinoma.

The overall aims of the project are to collaborate on a systematic study of radiation induced oncogenic transformation using different human epithelial cell lines and to study initiation of carcinogenesis by radiation by examining changes which occur in molecular, genetic and morphological features of normal human cells after exposure to radiation. This more longterm aim is at present being approached at a mainly qualitative level. It has proved extremely difficult to transform primary cultures of normal human epithelial cells and thus this approach provides the next logical step in developing a full understanding of radiation-induced transformation of human epithelial cells.

<u>The specific objectives for this contract are:</u>

(1) To compare available human transformation systems in terms of their ability to address radiation protection problems, particularly radiation quality and low dose and low doe rate effects.

(2) To attempt to develop new human epithelial systems capable of looking at initiation of carcinogenic damage, particulary by target specific radionuclides on the target organ in culture.

Head of Project 1: Dr Seymour

II Objectives for the reporting period

1. To establish HPV immortalised human keratinocytes (HPV-G) in the laboratory and to test their radiosensitivity and suitability for transformation assays.

2. To test the tumorigenicity of the line in nude mice and by using several other less rigorous tests involving structural changes and tumour marker detection.

3. To establish HaCaT spontaneously immortalised human/keratinocyte cells and Hela x human fibroblast CGL1 cells and test them as in 1 and 2 above.

4. To develop and characterise human normal urothelial cells in culture and to determine their long term response to radiation using a variety of tumour markers and structural change criteria.

III Objectives for the next reporting period:

1. To continue assays for transformation using the HPV-G line with emphasis on alteration of total dose and dose rate.

2. To continue to develop the normal human urothelial system and to quantify the appearance of certain changes leading to the development of foci, with respect to dose.

3. To attempt to isolate and culture foci and determine their survival and malignant potential in nude mice.

4. To attempt to model the development of transformed characteristics and resulting from this to assign a transformation probability to the culture based on the number and frequency of defined features which occur.

5. To assess the importance of lethal mutations occurring in distant progeny of human cells for quantification of transformation frequency.

Progress achieved:

1. Establishment and characterisation of the HPV-G line: The line was obtained from Professor J DiPaolo, USA and is now growing in the laboratory. Plating efficiencies and growth characteristics have been determined. These show the line to be suitable for transformation assays using the protocol developed by Reznikoff. This protocol is already being used by our partners in St Andrew's University.

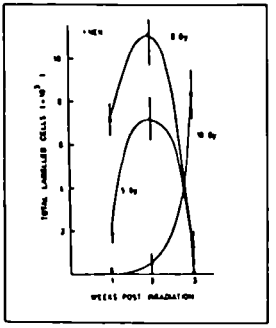
2. Tumorigenicity: Two experiments to assay the level of tumour production in nude mice are underway. The experiments take approximately three months to perform due to the slow growth rate of the cells and the need for at least 6 serial subcultures of the cells before presumptive expression of transformation can be tested. Samples of each passage are being retained for marker and morphological analysis in our

Laboratory and in Valencia University.

3. HaCaT and CGL1 cells have also been established in the laboratory. The HaCaT line is unsuitable for transformation experiments, it has a very slow growth rate and the plating efficiency is unstable with respect to initial number plated, meaning that correlation of tumour frequency or characteristics with cells initially exposed would not be possible. The CGL1 line is promising and is being used in preliminary transformation assays. The line which can become malignant as a result of a deletion in the region of an oncosuppressor gene expresses a protein marker (P75) when transformated and is thus very interesting in the context of this work. A positive control line (CGL4) which has the relevant deletion can be carried with experiments and expresses high levels of P75. High levels of this marker have been detected following irradiation and serial passaging of CGL1.

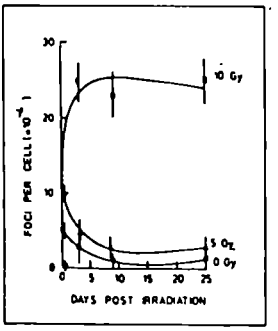
4. Initiated/immortalised lines such as those described above are important for obtaining relative effect data for different radiation qualities but where risk of initiation by radiation is important, normal human cells must be used. The system in use in this laboratory involves explanting normal human urothelial tissue and treating the cells while still in three dimensional organisation. Effects in cells growing out from the explanted tissue are then monitored. Fig. 1 shows results of attempts to link enhanced proliferation/extended life span of progeny with radiation dose. While "waves" of enhanced growth are seen at relatively low doses, very cytocidal doses are required to induce a truly non senescent population. Quantification of immortalised foci with respect to initial number of cells treated reveals (Fig 2) that there is a significant increase in number of foci with time post irradiation at 10Gy. Non significant effects occurred at 5Gy. Ultrastructural and immunochemcial analyses of the foci confirm that they have a number of nuclear and cytoplasmic characteristics associated with malignant cells and that they express high levels of cmyc which is not detectable in non focal cells. Expression of P75 protein referred to in point 3 above was also found in these cells.
Morphological analysis was performed by the University of Valencia on the surviving populations in the above experiments. These results suggest that there is a smaller nuclear area in irradiated (10Gy) cells which is consistent with the high numbers of foci present which are composed of obviously small cells.

Fig 1



Total No. of proliferating cells detected after exposure to irradiation

Fig 2



No. of foci detected on the monolayer expressed as a function of the number of epithelial cells exposed initially.

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Head of Project 2: Dr. Riches

II Objectives for the reporting period

1. To establish immortalised human urothelial cell lines (SV-HUC-1; NT11; BC16) in our laboratory and define their radiation sensitivity.
2. To characterise these lines using monoclonal antibodies to cytokeratins and SV40 large T protein.
3. To assess the genetic stability of these lines using the micronucleus assay.
4. To define whether these cell lines are tumorigenic following transplantation to nude mice.
5. To define whether cell lines NT11 and BC16 can be transformed by radiation

III Objectives for next period

1. To establish the immortalised human urothelial cell line CK2 (transfected with SV40 origin defective genome) and define its radiosensitivity using the micronucleus assay.
2. To define whether the urothelial cell lines SV-HUC-1 and CK2 can be transformed by radiation in vitro.
3. To investigate the dose effect relationships for transformation by radiation.
4. To compare the transforming ability of radiation with that of established chemical carcinogens as a baseline.

IV Progress achieved including publications

a. Characterisation of human urothelial cell lines.

Several human urothelial cell lines which have been immortalised by infection with SV40 were kindly supplied by Dr. Catherine Reznikoff, and have been established in our laboratory. These lines , designated SV-HUC-1, NT11 and BC16, grow as attached epithelial sheets. The growth rates of the lines are similar with doubling times of about 40 hours. Immunocytochemical techniques have been used to stain for human cytokeratins (including keratins 10,17 and 18) and expression of SV40 large T protein. DNA finger printing techniques have been undertaken for one of the lines (NT11). These will be useful marker systems to evaluate the origin of transformed cells.

b. Radiation responses and chemical carcinogen responses.

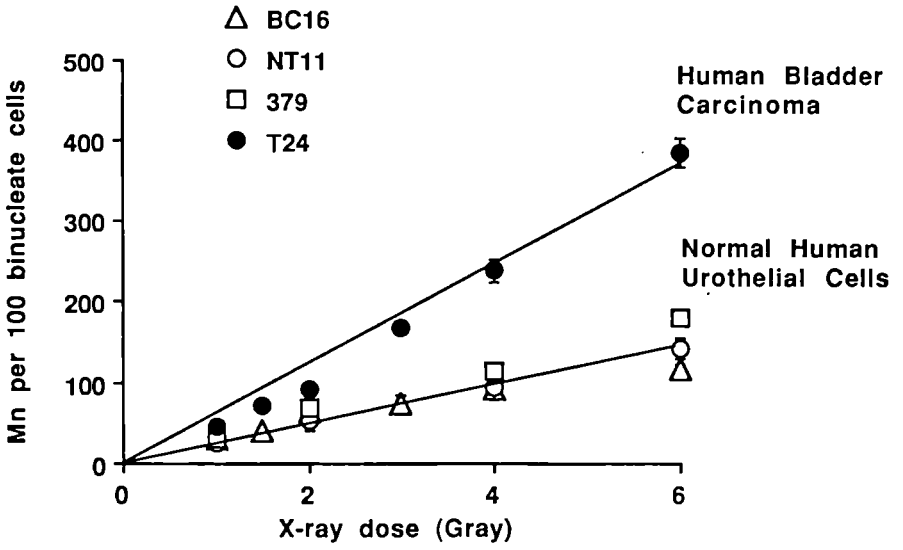
While colonies will form following plating of the cell lines using conventional colony procedures, it was extremely difficult to produce single cell suspensions from these cell lines which grow as epithelial sheets. Thus conventional survival curves could not be obtained with these cells. The micronucleus assay was thus used to evaluate the responses of these cells to irradiation. Following irradiation, the cells were exposed to cytochalasin B and cytospin preparations scored for micronuclei in bi-nucleate cells. The optimal harvesting time was defined using the binucleate index and the frequency of micronuclei per 100 binucleate cells. The normal human urothelial cell lines were compared with an established bladder tumour cell line (T24). The binucleate index fell dramatically after 48 hours and the frequency of micronuclei per 100 binucleate cells plateaued at 48 hours, so this was selected as an optimal harvesting time. A comparison of the radiosensitivity of these 3 lines with the bladder carcinoma line (T24) revealed that the normal lines

were less sensitive than the carcinoma line but exhibited little inter cell line variation (Fig. 1). The spontaneous frequencies of micronuclei in the normal urothelial cell lines varied markedly and may reflect different degrees of genetic instability. This maybe very important in defining models suitable for transformation.

Some preliminary studies using image analysis techniques were undertaken by Dr. Pertusa using the cell line NT11. This enabled area, perimeter and density measurements of Feulgen stained cells to be made. These could prove useful in characterising transformants.

Chemical carcinogens would also be used to compare with the responses of cells to radiation and to act as positive controls. Cytotoxic responses of the cell lines were evaluated following exposure to the chemicals. Treated and untreated cells were plated at the same density and the cellularity assessed after a defined growth period and compared with the untreated controls.

Fig.1. Dose response relationship between the frequency of micronuclei (Mn) observed in binucleate cells of a human bladder carcinoma cell line T24 and three normal human urothelial cell lines SV-HUC-1, NT11 and BC16 following exposure to different doses of X-irradiation. Sampling time 48 hours after X-irradiation. Error bars represent standard errors of the mean taken from at least three separate experiments. The background Mn values have been subtracted from the data shown.



c. Establishing a model to investigate radiation-induced transformation of human epithelial cells.

The human urothelial cell lines have been used to develop a model to investigate radiation-induced transformation of human epithelial cells. Sub-confluent cultures of the human urothelial cell lines were irradiated and the cells continuously passaged for 8 weeks. Each flask was kept separately and cells were finally harvested and transplanted subcutaneously into nude mice . The mice are currently being screened for tumour development over a 6 month period. Control (untreated) cells from the cell lines NT11 and BC16 passaged in the same way did not produce any tumours in the nude mice. To date no tumours have been observed in mice receiving irradiated cells from NT11 and BC16 which can be positively identified as arising from the original NT11 or BC16 lines (tumours are screened using histological, immunocytochemical and molecular biological techniques).

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Head of project: Dr. Pertusa

Objectives for the reporting period.

Our objectives are the quantification of several morphometric and densitometric parameters of the experiments carried out by the other laboratories participating in this project using computerised image analysis techniques. The parameters being measured have been nuclear area, nuclear diameter, nuclear perimeter and optical density.

Objectives for the next period.

In addition to the above objectives it is envisaged that we shall also attempt to carry out a morphological characterization of the transformed cells. If this is successful, it will allow a fast and easy determination of the presence of transformation in cell cultures.

Other new parameters have been incorporated to the measurements (convolutedness, nuclear-cytoplasmic ratio, integrated optical density, and cellular area), and these, together with previous ones will be used for the quantification of new samples.

Progress achieved including publications.

The first attempt to quantify samples sent to us by the other partners produced some difficulties due to the preparation of the samples which did not allow a successful, clear and reproducible image analysis. The main problem was that the samples were processed on a plastic substrate and without being mounted. The quality of light and contrast required were not good enough to permit the high resolution necessary to carry out the image analysis.

Since then, the preparation of the samples has been notably improved. The samples have been sent to us on a glass substrate and mounted, allowing the development of a semiautomatic procedure for the analysis of several parameters which are of great interest.

Since this modifications were made, samples from various experiments have been evaluated, both for the Radiobiology Group (Dublin) and for St. Andrew's (Scotland). The results of measurements carried out for Dublin, revealed significant differences both in nuclear area and amount of DNA per cell, in samples that had been treated with carcinogenic agents with respect to the controls. The most interesting and promising results are shown in figure 1. This shows that cultures of urothelial cells treated with two different doses of radiation exhibit a reduction on the distribution of their nuclear areas. This reduction is greater as the dose of radiation increases.

The data obtained from the measurement of the samples sent to us by Scotland are according to Dr. Riches of great interest since could provide useful information about the characterization of transformant cells. The evaluation of the results obtained in these samples are being evaluated at the moment and no further information can be supplied here about this particular.

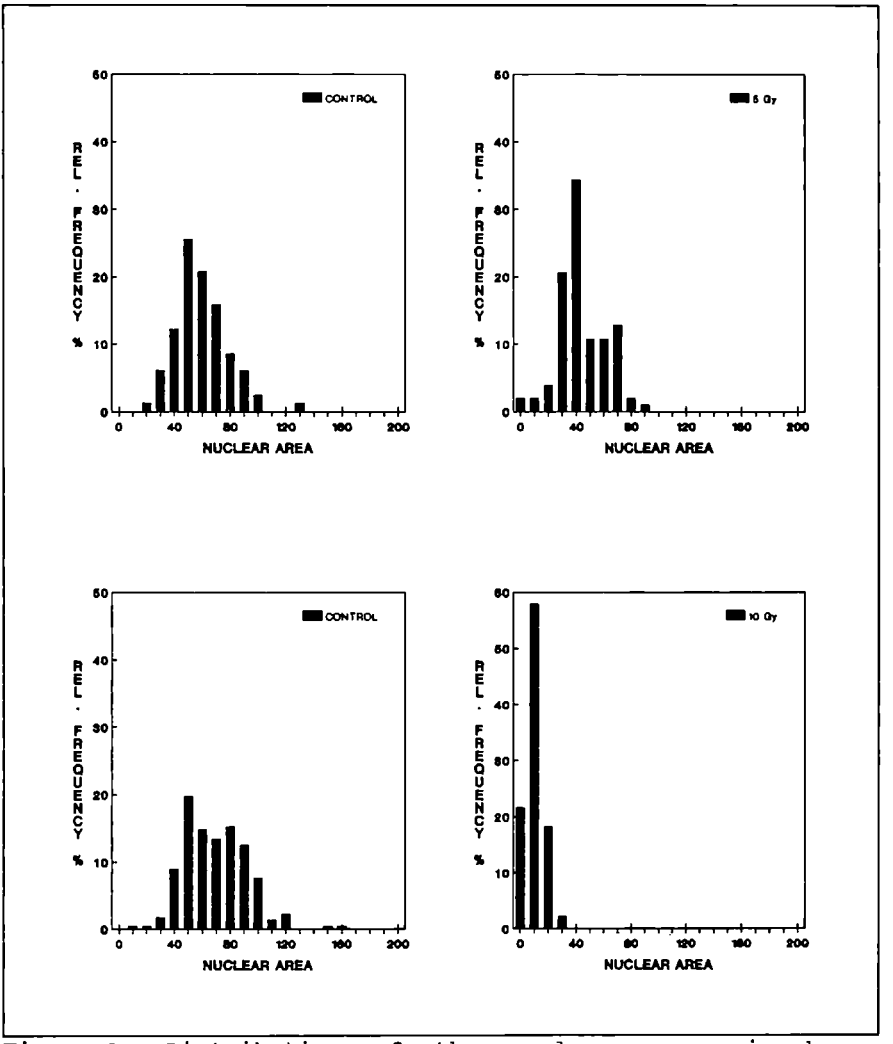

Figure 1. Distribution of the nuclear areas in human urothelial cells treated with two doses of radiation. In any case at least 200 nuclei were measured.

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Contract: Bi7-033 Sector: B13

Title: Cellular and molecular studies on radiation quality: A comparison between genetically relevant damage and cell inactivation.

1	Kraft	GSI
2	Sideris	NCSR "Demokritos"
3	Lloyd	NRPB
4	Natarajan	Univ. Leiden Sylvius Lab.

I. Summary of Project and Global Objectives

The influence of radiation quality will be measured for different biological endpoints like the induction of single and double strand breaks, chromosome aberrations, and cell cycle effects. From the analysis of the chromosome aberrations, it is expected to gain more insight the mechanism of the damage induction and interaction when the previously reported changes in the distribution over the various types of chromosomes will be scored systematically as function of particle energy and atomic numbers. Strand break induction of both, single and double strand breaks, will be measured as function of the chemical environment for extra- and intracellular DNA in order to estimate the influence of radiation quality and the absolute induction probability on the ratio between single and double strand breaks.

Finally, studies of the perturbation of cell cycle progression are important for the interpretation of the chromosome aberration measurements. But they can be also used at a fast monitor of radiation damage.

In the reporting period it was intended to use the high energetic heavy ion beam from SIS as well as lighter ions, as for instance carbon and oxygen from the Unilac. Due to the technical problems in the installation phase of the new experimental area at the SIS accelerator, it was not possible to start with high energy experiments. But at the Unilac accelerator in the range between 5 and 15 MeV/u experiments has been performed with ions ranging for neon to gold.

For the next period Ne- and Kr-ions will be available at low (5-15 MeV/u) and high (200-400 MeV/u) energies at the SIS. In addition, we asked for a O- and Ne-beam of approximately 100 MeV/u at the French accelerator Ganil.

Finally an international workshop on "Heavy Charged Particles in Biology and Medicine" will be held at GSI in September 23-25, 1991.

Head of Project I: Dr. Kraft

II Objectives for the reporting period

For the chromosome experiment it was planned to compare the occurence of disintegrations in mitosis with the occurence of normal chromosome aberrations over a wide range of LET and particles.

2. In order to study the induction of DNA damage and repair at doses comparable to doses of cell survival, a method of sensitive analysis has to be established.

III Objectives for next period

1. Chromosome measurements will be continued.
2. The sensitive deflection system of DNA breaks will be used for particle and X-ray irradiation.
3. Changes in cell cycle proliferation after heavy ion exposure will be studied in greater detail.

IV Progress achieved including publications

1. Chromosome disintegration after exposure of V79 Chinese hamster cells in mitosis has been observed at Unilac and Ganil. The data has been analysed and published.
   S. Ritter, W. Kraft-Weyrather, M. Scholz, G. Kraft: Influence of radiation quality on heavy ion induced chromosome aberrations in V79 cells. Radiation Protection Dosimetry, Vol. 31 No. 1/4 pp. 257-260 (1990).
   S. Ritter, W. Kraft-Weyrather, M. Scholz, G. Kraft: Induction of chromosome aberrations in mammalian cells after heavy ion exposure. GSI-Preprint, GSI-91-06 (1991), to be published in Advances in Space Research.
2. Using a hybride hamster-human cell line and hybridization methods to the human part of the genom, it was possible to establish a method to detect double strand breaks in sequences of several 100 kbp.
   C. Wiese: Entwicklung einer Methode zur Detektionstrahleninduzierter Doppelstrangbrüche in eukaryontischer DNA. GSI-Report, GSI-90-30 (1990).
3. DNA, double strand breaks, and single strand breaks has been measured for SV40 DNA in various chemical environments. Data has been analysed and published in Radiation Protection.
   G. Taucher-Scholz, J.A. Stanton, M. Schneider, G. Kraft: Induction of DNA breaks in SV40 by heavy ions. GSI-Preprint, GSI-91-05(1991), to be published in Advances in Space Research.
   J. Stanton, G. Taucher-Scholz, M. Schneider, G. Kraft: Comparison between indirect and direct effects for high and low LET radiations in SV40 DNA strand breaks induction. Radiation Protection Dosimetry, Vol. 31 No. 1/4 pp. 253-256 (1990).

Head of Project 2: Dr. E.G. Sideris

II Objectives for the reporting period:

Study of thermodynamic parameters being affected by exposure of Mammalian DNA to gamma rays and preliminary work of the same parameters following exposure to alpha particles.

III Objectives for next period

Study of thermodynamic parameters following exposure of mammalian DNA to alpha particles and their relationship to double strand DNA breaks and chromosome aberrations induced by the same particles. Theoretical analysis of the results towards the development of model describing the evolution of primary lesions induced on the DNA molecule.

IV Progress achieved including publication

An alpha particle source (25 MBq Am 241) was fit for exposure of mammalian cells in culture to alpha particles travelling in parallel tracks through vacuum. Using a Monte Casrlo code the dose at different distances from the source and its dependence on the geometry of radiation was investigated. The spread of alpha particles energy and corresponding LET within the irradiated cells were calculated so that these factors might be taken into account for the estimation of the absorbed energy during exposure the cells to alpha particles. This work was coupled with the computing of the specific primary ionization for alpha particles as well as protons in water so that these computations can be used as a physical parameter for the evaluation of biological damage in the subcellular level. This thought to be useful taking into consideration the limitations of the LET concept from one hand and the inaccuracies of the cross-sections, as have been used in the past for such calculations, on the other. On the basis of these assumptions the rate of the expected absorbed dose from mammalian cells exposed to an AM-241 source has been estimated.

On the experimental side the effect gamma rays on thermodynamic characteristics of DNA molecules isolated from mammalian cells continued and expanded to involve preliminary work with alpha particles. Analysis of the experimental results from Inverse Gas Chromatography studies indicate a dramatic difference of Gibbs Free Energy (ΔG_s) values (attributed on the availability of hydrogen bonds) between irradiated and non-irradiated DNA. The higher the dose the higher the elimination on the availability of hydrogen bonds on the exposed molecules. This is on line with an observed decrease on the availability of sites participating in hydrogen bond formation. The function regressing the ΔG_s values on radiation dose seems to involve at least two components. ΔG_s is rapidly decreasing up to the region of 50 Gy while from there on the phenomenon seems to reach, under our conditions, a saturation level. These results are on line

with those observed when the effect of gamma rays on mammalian DNA was studied by Thermal Transition Spectrophotometry. When the dose is increasing the T_M of the DNA is decreasing in similar fashion to that involving the ΔG_s. There is again a dramatic drop of T_M values up to the region of 50 Gy while from there on this phenomenon, too, seems to reach a saturation level. The T_M values were estimated from the second derivatives of computed simulated melting curves constructed on the basis of the experimental results and a theoretical model developed to account for the peculiarities observed when studying ΔG_s or T_M changes induced by ionizing radiation.

Our theoreticalmodel for the transgression of the DNA molecule from the double strand stage to the single strand stage is based on the acceptance of a quasi-one-dimensional lattice and attempts to correlate the "melting" of the DNA molecule with the development of single and double strand breaks due to external agents, in our case the exposure to gamma-rays. The derived function was then be used to define the DNA T_M point as well as the flexibility of the DNA molecule.

It should be noted here that as it has been found in our laboratory the frequency of the induced by gamma rays double strand breaks of the DNA molecules, as it is estimated by neutral elution as well as the frequency of radiation induced dicentrics evolves in a similar fashion as that describing the changes observed on the Gibb's Free Energy (associated with the elimination of the availability of hydrogen bonds) and those observed on the T_M values of DNA associated with the breakage hydrogen bonds on the DNA molecules.

Following the standardization of our Am-241 source the experimental work was expanded to the study of the effects of alpha particles through the use of the same physicochemical methods. Preliminary results indicate that exposure to alpha particles does not affect the molar excited states of the exposed DNA molecules the same way as in the case of gamma rays. The dramatic ranges on the hydrogen bonding observed from exposure to gamma rays were not present in our preliminary experiments with alpha particles.

Publications

PIALOGLOU P, E G SIDERIS, P PERRIS 1990 Cell survival and chromosomal aberration frequency in V79 Chinese hamster cells exposed to low energy protons, Frontiers in Radiation Biology:295-300.

PIALOGLOU P, E G SIDERIS, P PERRIS 1990 Enhancement of cellular damage induced by gamma rays after inhibition of poly-(ADP)-ribose polymerase by 3-aminobenzamide. Frontiers in Radiation Biology:459-464.

Presentations in International Meetings

LOUKAKIS G K, E G SIDERIS, C A KALFAS, B E MAZOMENOS, A ANAGNOSTOPOULOU-KONSTA 1990 Measurements of structural characteristics of damaged DNA through the use of inverse gas chromatography. Meeting on Chemistry and Properties of Biomolecular Systems, Loutraki, Greece, June 10-13, 1990.

ANAGNOSTOPOULOU-KONSTA A, P PISSIS, G LOUKAKIS, E G SIDERIS 1990 Dielectric study of DNA-water systems by thermally stimulated curents method. International Discussion Meeting on Relaxation, Heraklion, Greece, July 18-29, 1990.

Head of Project 8: Dr. Lloyd

II Objectives for the reporting period

a) To produce sample holders so that sufficient volumes of blood held as a thin layer can be irradiated with accelerated charged particles at the Darmstadt facility.

b) To undertake at least one visit to Darmstadt to irradiate specimens and to grow cultures so that dose response curves for chromosomal damage in lymphocytes can be constructed for ^{3}He and ^{12}C ions.

III Objectives for next period

a) To complete the irradiations and microscope evaluation of the cultured cells.

b) To collate and analyse the results.

IV Progress achieved including publications

Progress has been very slow. The specimen holders have been constructed and tested to ensure that good yields of metaphases may be obtained.

No visit to Darmstadt has yet taken place. Access to an appropriate beam has not yet been possible. A suggestion from Darmstadt is that we would be more successful if we were prepared to accept a beam of particles of a higher atomic number. A beam of 10-20 MeV per AMU neon ions would be acceptable. An alternative suggestion is that we try to do part of the work at Ganil, France instead.

It seems likely that the project will not be completed in the contract time.

Head of Project: Prof. A. T. Natarajan

II Objectives for the reporting period

a) To test the sample holders produced by NRPB for irradiation of blood samples and isolated lymphocytes to be carried out at Darmstadt, by mock experiments using different X-rays.

b) to undertake atleast one visit to Darmstadt to irradiate specimens and grow cultures as well as to make premature chromosome condensation preparations in order generate dose response curves for chromosomal damage in metaphases and interphases following irradiation with ^{3} He and ^{12}C ions.

III Objectives for next period

a) To complete the irradiation and microscope evaluation of the metaphase chromosomes and prematurely condensed interphase chromosomes (PCCs).

b) To study the influence of DNA repair inhibitors on the yield of aberrations as judged in PCCs.

c) To collate and analyze the results.

IV Progress achieved including publications

We have checked the feasibility of using sample holders for radiation experiments, both for mitotic chromosomes and interphase chromosomes (PCCs) using isolated lymphocytes. It was found that one could do successful experiments using these sample holders.

No visit to Darmstadt has yet taken place and access to appropriate beams has not yet been possible. We may have to start with a beam with a higher particle number such as 10-20 MeV per AMU neon ions would be acceptable. Alternatively, part of the work can be carried out in Ganil, France. It is unlikely that the project will be completed with in the contract time.

Progress Report

Contract: Bi7-034 **Sector: B13**

Title: Radiation induced processes in mammalian cells: principles of response modification and involvement in carcinogenesis.

1	Van der Eb	Univ. Leiden Sylvius Lab.
2	Sarasin	CNRS
3	Devoret	CNRS
4	Rommelaere	Université Libre de Bruxelles
5	Bertazzoni	Consigli Nazionale delle Ricerche
6	Thomou-Politi	Greek Atomic Energy Commission

I. Summary of Project and Global Objectives

In the present research contract 6 different laboratories collaborate on a theme involving the induction of stress responses in mammalian cells. Stress responses are defined as the phenomena occurring after exposure of cells to DNA damaging agents, including UV- and ionizing radiation. These responses influence the subsequent fate of the cells, giving rise to mutation, recombination, DNA amplification and cancer.

The laboratory of Devoret focuses on the identification of RecA-like genes in mammalian cells. In *E.coli* the RecA gene plays a central role in a variety of processes that protect the cells from the deleterious effects of radiation. The group has identified in mammalian cells a cDNA from a gene (*KIN17*) which exhibits partial homology with the E.coli recA gene. Further work is concentrated on the identification of the functions of this gene.

The relationship between DNA repair, mutagenesis and cancer is most clearly illustrated in the human DNA repair-deficient syndrome Xeroderma pigmentosum (XP). Such patients show a high incidence of skin tumors in sunlight-exposed areas of the body. The group of Sarasin has found that an unusually high percentage of skin tumors in these patients contain mutated *ras* oncogenes. This shows that unrepaired lesions in DNA can give rise to point-mutations. A small proportion of XP patients, however, does not develop skin tumors, a phenomenon which was found to be correlated with abnormal expression of the stress response Enhanced reactivation (Van der Eb), Elucidation of the molecular events occurring during induction and expression of several ("SOS-like) stress responses is the subject of research of the groups of Rommelaere, Sarasin, Bertazzoni and Van der Eb. The following phenomena are investigated: Enhanced reactivation, the mechanisms of induction by UV of UV-inducible genes, gene amplification, induction of mutations (in particular at a-basic sites) and the role of poly (ADP-ribose) polymerase during recovery following radiation damage. Furthermore, biochemical and genetic analysis of repair-deficient cell lines is carried out by Bertazzoni. Finally, the group of Thomou-Politi investigates the effect of radiation on cell surface expression of the transfected human CD2 gene (coding for a cell surface antigen that binds sheep erythrocytes).

Head of Project 1: Prof. Dr. Van der Eb

II Objectives for the reporting period

1. Search for suitable *in vitro* cell culture systems to assay the carcinogenic effects of radiation (ionizing and UV-irradiation).

2. Characterization of UV-inducible phenomena in human cells: a) from normal humans and XP-patients; b) from "non-cancer-prone" XP- and TTD-patients; c) from cancer-prone genetic syndromes.

III Objectives for next period

The objectives for the next period are identical to those of the reporting period. If it is not possible to obtain suitable cell culture systems for assaying the carcinogenic effects of radiation, we will attempt to construct suitable cell culture models from established rodent cell lines in which an oncogene has been introduced (see progress report).

IV Progress achieved including publications

Induction of *in vitro* cell transformation by radiation

The objective of this study is to develop *in vitro* assay systems for the assessment of the contribution of ionizing and UV-irradiation to the process of radiation-induced carcinogenesis. The aim is to derive an assay system consisting of primary or secondary cell cultures from mice transgenic for an activated oncogene. Such cells will be non-oncogenic, since conversion to a fully oncogenic phenotype requires activation of at least 2 complementing oncogenes, and possibly inactivation of tumor suppressor genes. However, since the cells have already made 1 step in the process of carcinogenesis, it should be possible to convert them to a (more fully) oncogogenic phenotype with lower doses of radiation than when the cells are completely unchanged. In the latter case very high doses of radiation would be required to bring about in the same cell the 2 or 3 genetic events necessary for complete transformation. The following types of transgenic mice were available: mice transgenic for the pim-1 oncogene (regulated by the H2 promoter) showing pim-1 expression in the kidney; mice transgenic for the polyoma large-T antigen; and mice transgenic for the polyoma middle-T antigen, the latter genes regulated by the polyoma promoter/enhancer.

None of the primary or secondary embryo cultures nor the cultures of baby mouse kidney cells from these transgenic animals developed any sign of morphological transformation upon irradiation with UV-light or X-rays. The possible cause of this negative result is that expression of the transgene in the cells was very low or absent. It was decided, therefore, to discontinue these attempts and to try and create suitable tester cells from established swiss mouse 3T3 cells which, in contrast to NIH 3T3 cells, are completely non-oncogenic in nude mice. If introduction of a single activated oncogene does not yet render the cells tumorigenic, they might be suitable for our screening test. In

addition, a proposal is being submitted elsewhere to create transgenic mice expressing selected oncogenes specifically in the skin. These mice will be used as a model for UV-carcinogenesis. If successful, it will complement the EEC project.

Inducible repair processes in human cancer-prone syndromes

Exposure of human cells to UV-light or ionizing radiation results in induction of a number of stress or SOS-like phenomena, including Enhanced Reactivation (ER) of UV-treated virus and Enhanced Mutagenesis (EM) of untreated virus. We have recently noted that the ER phenomenon was absent in Xeroderma pigmentosum patients which, for unknown reasons, lacked tumors in sunlight-exposed areas of the skin, in contrast to most other XP-patients. This suggests that the ER phenomenon might be co-regulated with events that give rise to oncogene activation, or activation of certain types of oncogenes. Since EM was unchanged in these ER^- XP patients, it seems likely that the ability to induce point mutations in response of DNA damage, and hence the ability to activate oncogenes by point mutations, is not affected.

Further work showed that cells from UV-sensitive Trichothiodystrophy (TTD) patients, which are also "non-cancer-prone", show no ER-response, and that cells from hereditary cancer-prone syndromes (e.g. Polyposis coli, Dysplastic naevus syndrome, Wilm's tumor, etc.) respond to UV irradiation with super-induction of ER. This confirms the notion that cancer induction is somehow correlated with induction of ER.

Work in the reporting period has concentrated on identification of molecular "defects" in ER^- cells or in ER^{super+} cells. Sofar it was found that induction by UV-irradiation of UV-inducible genes (c-*jun*, c-*fos*, metallothionine, HSP70, collagenase, ornithine decarboxylase) is not affected in ER^- and ER^{super+} cells. However, an abnormality was found for UV-induced stabilization of the tumor-associated protein p53. in ER^- cells, no UV-induced stabilization of p53 was found. However, it turned out that p53 was already constitutively stabilized in such cells. The phenomenon is investigated in more detail.

Publications

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P.J. Abrahams, A. Houweling and A.J. van der Eb. High levels of Enhanced Reactivation of HSV-1 in UV-treated skin fibroblasts from various hereditary cancer-prone syndromes. Submitted to Cancer Research.

Abstracts

P.J. Abrahams, A. Houweling, R. Schouten and A.J. van der Eb. The induction of SOS-like responses and the stabilization of p53 oncoprotein in UV-exposed skin fibroblasts derived from Xeroderma pigmentosum, Trichothiodystrophy and various hereditary cancer-prones syndromes. Workshop on DNA repair, Noordwijkerhout, April 14-19 1991.

Libin Ma, G. Weeda, A.G. Jochemsen, J.J. Hoeijmakers and A.J. van der Eb. Characterization of a promoter of the human XPBC/ERCC-3 gene. Workshop on DNA repair, Noordwijkerhout, April 14-19-1991

Head of Project 2: Dr. Sarasin

II Objectives for the reporting period

1. Construction and mutagenic properties of shuttle vectors containing a unique DNA lesion.

2. Development of EBV-based shuttle vectors for gene amplification studies.

3. Oncogene activation in skin tumors from Xeroderma pigmentosum and non-Xeroderma pigmentosum patients.

III Objectives for next period

- Mutagenic properties of abasic sites as a function of local DNA sequence.
- Fidelity of the amplification process.
- Differences and similarities between the DNA repair-deficient syndromes where only one is associated with cancer-proness.

IV Progress achieved including publications

1) Abasic sites have been proposed as cryptic DNA lesions to explain most of the "spontaneous" mutagenesis and also as intermediates due to depurination of bases modified by chemicals. Their mutagenic characteristics are relatively well known in bacteria but nothing has been done in cultured mammalian cells.

The mutagenic properties of a true unique abasic site located opposite a guanine residue were studied. An oligonucleotide containing a chemically-produced abasic site was inserted into a shuttle vector able to replicate both in simian cells and in bacteria. Plasmid DNA was rescued from simian cells and screened in bacteria by differential hybridization with a labelled oligonucleotide probe. Using this technique, mutations were easily detected without selection and then sequenced. Results showed that opposite a guanine, the abasic site was repaired error free or replicated by mammalian cells with an efficiency of 99%. Point mutations occurred at a frequency of approximately 1% in control host cells and at more than 3% in UV-pre-irradiated host cells. Adenine, cytosine or thymine were found to have been inserted opposite the abasic site. No preferential insertion of a particular base was observed in contrast to the situation reported in bacteria. This work is being continued by analysing the mutagenic properties of an abasic site inserted opposite A, T and C.

The effect of local sequence on the type of bases inserted opposite a true Ap site is probably important to explain mutational hot spots. We are therefore developing a shuttle vector system which will allow us to look at mutation specificities on mutational hot spots of the Ha-ras oncogene (codons 12 and 13).

2) The EBV-based shuttle vectors can be maintained stably and episomally for a long period of time in cultured human cells. We have used this system in order to determine if such vectors can be amplified *in vitro* as a response to stress. These vectors carry a target gene for mutagenesis study (*lac*Z') which should allow us to determine the fidility of the

amplification process in mammalian cells, this amplification being a characteristic of some tumour cells. We also constructed hybrid plasmids containing both the EBV and the SV40 replication origins. These molecules are able to replicate episomally either like an EBV vector or like SV40 if the SV40 large T antigen is provided at the same time. UV irradiation of both human adenovirus-transformed 293 or SV40-transformed MRC5 host cells leads to vector amplification irrespective of the type of replication origin used for the episomal maintenance. Our results clearly show that the EBV latent replication origin (OriP), in the presence of the Epstein-Barr virus Nuclear Antigen-1 (EBNA-1) and the SV40 large T antigen, is sensitive to overreplication in UV-irradiated human cells. Since the UV doses were small enough to induce very few damages, if any, on the plasmid sequences, this amplification should be mediated through a cellular factor acting *in trans*. The interest in using shuttle vectors for this kind of study lays in the easy analysis of the amplified vectors in rescued bacterial colonies. The accuracy of the amplification process can be monitored by studying restriction maps of individual plasmid molecules or more precisely the integrity of a target gene, such as the *lac*Z' sequence, carried by our vectors.

3) Xeroderma pigmentosum is one of the best examples of the relationship between unrepaired DNA lesions, mutations and carcinogenesis in man. This rare, autosomal recessive hereditary disorder is deficient in excision repair of DNA lesions after UV irradiation. This results in a high incidence of cancers of the skin, particularly in sun-exposed parts of the body. Using the 3T3 transfection assay we have detected an activated N-*ras* gene in two XP tumours due to a T to A transversion in codon 61 and a transforming Ki-*ras* gene in a third XP tumour by mutation at codon 12 resulting in the substitution of a C to an A. Southern analysis of genomic DNA from XP tumours has shown high levels of Ha-*ras* gene amplification and rearrangement not found in skin tumours from normal individuals. Screening for *ras* mutations in skin tumours from XP and normal patients by PCR followed by differential hybridization, resulting in detection *ras* mutations in 55% of XP tumours compared to 20% in controls. Hence, unrepaired DNA lesions persisting in XP skin results in higher levels of *ras* mutations compared to the same type of carcinomas from individuals with normal repair capacities .

This result indicates clearly that XP cells are hypermutagenized by UV-light in patients as they are *in vitro*. The high level of oncogene amplification found in XP cells agrees with the model of overreplication due to blockage of DNA polymerase at unrepaired lesions. The presence in the same cell of activated oncogene and mutated oncogene can easily explain the higher cancer fequency in XP patients compared to normal individuals.

Publications

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Head of Project 3: Dr. Devoret

II Objectives for the reporting period

The work preceding this contract has shown that recA protein, which plays an essential role in repair, recombination and mutagenesis is a natural radioprotector. In the present period, we wanted to isolate and characterize a similar protein in mammalian cells.
Our objectives were: 1) to identify a mouse protein serologically related to RecA protein (kin protein); 2) to clone into an expression vector the cDNA coding for the kin protein; 3) to map, by hybridization with a cDNA fragment, the gene coding for the kin protein on a mouse chromosome; 4) to establish whether the gene was present on a human chromosome. The four defined objectives have been achieved.

III Objectives for next period

Our objectives are:
1) to clone into a convenient vector the *KIN17* cDNA in order to express kin17 protein in *E.coli*. 2) to purify kin17 protein; 3) to determine *in vitro* if kin17 protein is a protein involved in DNA transactions; 4) to establish kin17 functions *in vivo*, and in collaboration with Van der Eb's and Sarasin's groups, to determine its effect on SOS in mammalian cells; 5) to clone the mouse or the human *KIN17* gene.

IV Progress achieved including publications

1. Demonstration that in several mammalian species (mouse and man), there are some proteins that share antigenic determinants with *E.coli* recA protein.

2. Isolation of a mouse cDNA fragment, 601 nucleotides long, that codes for a polypeptide expressing the strongest immunoreactive recA epitope. This cDNA fragment was used as a probe in the next step.

3. Cloning of a mouse complete cDNA, 1413 nucleotides long, called *KIN17* cDNA, encoding a protein of 44kDa.

4. Determination of the kin17 amino acid sequence that has (i) a zinc-finger motif, (ii) nuclear localization signals, and (iii) a motif homologous to recA protein. Kin17 appears to be a nuclear protein which may bind to DNA.

5. Identification of *KIN17* mRNA (1.8 kb), which is expressed in transformed mouse neuroendocrine AtT-20 cells at a high level.

6. Localization by cytogenetic mapping indicating that the *KIN17* gene is located in band A of mouse chromosome 2.

7. Demonstration that genomic sequences homologous to *KIN17* are present in human DNA.

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Head of Project 4: Prof. Rommelaere

II Objectives for the reporting period

Analysis of radio-induced processes in human cells.

a) **Subproject a** aims at identifying mediators of radiation-induced mechanisms. We investigated whether factors secreted by irradiated cell cultures can mimic radiation by triggering radio-induced processes in unirradiated human cells.

b) **Subproject b** concerns the mechanism by which radiation induces a defined set of genes in eukaryotic cells. This question was tackled by analyzing the levels at which gene expression is up-modulated in irradiated human cells.

III Objectives for next period

Further studies will be achieved to determine the short- and long-term effects of radiation on the expression of cellular genes related to the initiation or maintenance of malignant transformation of human cells.

a) During the reporting period, we observed that UV light can modulate gene expression in human cells by extending the life-span of certain transcription products. Attempts will be made to unravel how UV light modulates the stability of mRNAs by determining, in particular, the translatability of affected transcripts. X-rays will also be tested for their ability to regulate gene expression at a post-transcriptional level in human cells.

b) We shall attempt to identify and characterize cellular functions associated with radiation-induced neoplastic transformation. Parvoviruses will be used as probes to isolate cadidate protein markers of radio-transformation by means of their binding to the viral genome.

IV Progress achieved including publications

Radiation is known to confer on mammalian cells a transient "stress" phenotype that is characterized by distinct cellular responses including Enhanced reactivation (ER) and Enhanced mutagenesis (EM) of damaged viruses and altered DNA replication. The early events triggering these radio-induced phenotypes remained elusive until now. Yet it has been shown recently that irradiation of mammalian cells enhances the expression of a set of proteins. Some of these proteins appear to be involved in the progression of the cell cycle or in various protective, degradative and infammatory processes and might also be involved in the above-mentioned conditioned phenotypes.

Our work aims at:

-identifying the nature of the signal triggering the inducible ER recovery process; and

-investigating the radiation-induced disturbances of gene expression.

1) Mediators involved in the radio-induction of the ER recovery process.

Pretreatment of mammalian cells with subtoxic doses of UV light, ionizing radiation or various chemical agents results in a greater survival of incoming damaged single-stranded (SS) or double-stranded (DS) DNA viruses. This phenomenon was denoted Enhanced reactivation (ER). ER is expressed transiently and in a dose dependent fashion, requires *de novo* protein synthesis and may be accompanied by Enhanced mutagenesis (EM) of the damaged virus. The nature of the signal triggering ER and EM is not yet defined. Most of the physico-chemical agents inducing ER and EM appear to have the capacity to damage DNA and/or to inhibit its synthesis. It has been shown previously that supernatants of irradiated cell cultures, transferred to untreated cells, can mimic radiation in the induction of specific genes. Irradiated cells appear to secrete one or several factors that are able to induce the synthesis of some proteins. This extracellular factor(s), called EPIF (Extracellular Protein Synthesis Factor) or also UVIS (UV Induced Secreted protein) is not yet fully defined and may belong to the family of growth factors. In order to undestand the

mechanism of ER induction in human cells, we investigated whether the factor(s) secreted by irradiated cells is (are) able to trigger ER of SS and DS DNA viruses (H-1 and HSV-1, respectively) in unirradiated cells. Medium of human or mouse cells exposed to increasing doses of radiation has been transferred onto unirradiated human cells, 24h after irradiation. These conditions are optimal to achieve induction of EPIF. Unirradiated human cells were infected with damaged H-1 and HSV-1 15h (H-1) or 24h (HSV-1) after their exposure to the conditioned medium. We determined whether the transfer of these conditioned media or intact human cells increased the capacity of these cells to reactivate damaged H-1 or HSV-1. This treatment did not increase significantly the level of H-1 reactivation but enhanced HSV-1 reactivation to a significant extent relative to that in unirradiated cells. However, ER of HSV-1 observed under these conditions was lower than ER obtained after direct irradiation of the cells. This disparity of the data obtained with SS and DS DNA viral probes is reminiscent of a situation described in the previous CEC report: irradiated fibroblasts from ataxia telangiectasia (AT) patients acquire an enhanced capacity to reactivate the DS DNA virus HSV-1 but not the SS DNA virus H-1.

Together these observations lead us to propose that the induction of the conditional recovery process depends on a gradual signal. The signal provided by extracellular factors may be sufficient to partially activate ER of HSV-1 but may be too low to stimulate H-1 reactivation.

2) Control of Gene Expression in Irradiated Cells.

As mentioned above, cell irradiation stimulates the expression of a defined set of genes. Activation of these genes takes place at the level of initiation of their transcription and was recently related to the post-translational modification of transcription factors in irradiated cells. Yet, transcription is not the only level at which gene expression may be up-modulated. It was therefore of interest to determine whether modulation of gene expression by irradiation also includes a post-transcriptional component. This prompted us to compare the fate of specific RNAs in mock- and UV- or X-irradiated cultures of NB-E human kidney cells. In order to minimize the interference of transcription, advantage was taken of the fact that production of a series of mRNAs can be turned on by exogenous agents (such as interferon-α (IFN-α), interleukin-1α (IL-1α) or the double-stranded RNA poly(I)- poly(C), and rapidly fades upon removal of the inducer. These mRNAs therefore constitute suitable substrates to seek for post-transcriptional effects of radiation inflicted on cells after a period of incubation with above-mentioned agents. This analysis concerned, in particular, six transcripts (hereunder termed according to their translation products) that were induced by IFN-α (2',5'-oligoadenylate synthetase, 6-16, 1-8, IFI-15K), IL-1α (IL-1b) or poly (I)-poly(C) (IFI-54K). Northern blotting experiments showed that the steady-state levels of cytokine- and poly(I)-poly(C)-induced transcripts were higher in irradiated cells compared with mock-treated cultures. Moreover, this stimulation appeared to be dose-dependent. The enrichment of irradiated cells in cytokine- and poly(I)-poly(C)-induced mRNAs may reflect either sustained transcription of corresponding genes after removal of the inducer or stabilization of preformed transcripts.

Data from run-on experiments strongly argued against the possibility that UV-irradiation may enhance the expression of IFN-induced genes upon removal of the cytokine, by keeping their transcription on. Pulse-chase experiments provided further evidence for a posttranscriptional mode of regulation of the cytokine-induced genes by radiation.

For the time being, the mechanism responsible for the inhibition of RNA decay in irradiated cells is a matter of speculation.

Further experiments are needed to unravel how radiation modulates the life-span of

mRNAs, in particular with regard to the translatability of affected transcripts. This investigation should contribute te evaluating mRNA stabilization as a putative adaptive response that may compensate for at least part of the inhibition of gene expression in cells esposed to genotoxic stresses.

Publication

G. Hilgers, I. Clauss, G. Huez and J. Rommelaere (1991) Posttranscriptional efffect of ultraviolet light on gene expression in human cells: stabilization of cytokine- and poly(I)-poly(C)-induced messenger RNAs. Eur.J.Biochem. (in press).

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Head of Project 5: Dr. Bertazzoni

II Objectives for the reporting period

Cellular and genetic characterization of Trichothiodystrophy (TTD) and Xeroderma pigmentosum (XP) patients. Study of the TTD/XP association. Chromosomal instability in homozygous and heterozygous carriers of XP mutations. Cellular and genetic characterization of mutagen-sensitive rodent mutants (F. Nuzzo and M. Stefanini).

Study of the role of poly(ADP-ribose)polymerase (pADPRP) during proliferating activity of mammalian cells occurring in rat liver regeneration. Identification of inhibitors of poly(ADP-ribose)polymerase with low cytotoxicity (U. Bertazonni and A.I. Scovassi).

III Objectives for next period

Analysis of the relationship between DNA damage, immediate cellular responses and long-lasting consequences in human and rodent cell lines showing normal or enhanced sensitivity to mutagens. Biochemical analysis of the DNA repair defect in Chinese hamster mutants. Localization on the human genome and cloning of genes involved in DNA repair processes. Study of chromosomal proteins ADP-ribosylated *in vivo* before and after treatment of mammalian cells with DNA damaging agents. Study of involvement of ADP-ribosylation in rat liver carcinogenesis during initiation, promotion and progression steps of neoplastic transformation.

IV Progress achieved including publications

Chromosome analysis in 8 XP patients and 14 family members demonstrated the presence of structural chromosome changes in cultured fibroblasts from unaffected skin of all the individuals. Furthermore, multiple unrelated clonal chromosome rearrangements, some of which present in the primary explant and in the early culture passages, were observed in 4 subjects carrying the XP-C mutation (2 homozygotes and 2 heterozygotes) and in 1 XP-D patient. The anomalies were apparently balanced translocations, deletions and inversions. These results suggest that the occurrence of cytogenetically abnormal clones represents an initial step in the process leading to neoplastic transformation.

Consanquinity studies in three Italian families with members showing the complex phenotype due to the association of XP-D with TTD, demonstrate the presence of multiple remote inbreeding within and among the families. These observations suggests that the mutations responsible for TTD and XP-D should be at closely linked loci or affect the same gene (Nuzzo et al., 1990). DNA repair investigations were extended to 8 TTD patients from different countries (referred to us by Dr. Lehmann, MRC Cell Mutation Unit, Falmer, UK, and by Dr. A. Sarasin, IRSC, Villejuif, F) and confirmed the association of TTD with XP-D in unrelated patients.

The characterization with respect to UV sensitivity and human chromosome content of hybrids obtained by fusing the mutagen-sensitive Chinese hamster clone CHO7PV (carrier of a DNA repair defect genetically different from the eight so far identified in rodent mutants) with normal human lymphocytes indicates the human chromosome 7 as the best candidate for the localization of the human gene able to correct the repair defect in CHO7PV cells.

The nuclear enzyme pADPRP, which is activated in response to DNA damage, modifies chromosomal proteins and plays a key role in DNA repair processes. To further clarify the function of pADPRP in the regulation of different cellular processes of DNA

metabolism, and in particular during proliferation, we have followed the activity and mRNA levels of the enzyme during the early and the late phases of rat liver regeneration. When the endogenous activity of the enzyme was measured in isolated liver nuclei obtained at different times after hepatectomy, two different peaks were observed: a very early increase which returned to control level before the onset of DNA synthesis, and a second one, which reached its maximum after the peak of DNA synthesis and returned to control level at later times. In order to correlate the observed variations of the enzyme activity with the extent of gene transcription, we measured the levels of pADPRP mRNA during rat liver regeneration, using a specific cDNA probe for the enzyme. Evidence was obtained that the transcription rate is significantly enhanced during cell proliferation, slightly preceding the increase in DNA synthesis.

In conclusion, these observations suggest that the early increase results from the activation of preexisting pADPRP molecules, whereas the second phase can be associated with de novo synthesis of the protein, thus demonstrating that pADPRP is involved both in the early and in the late events of the cell proliferation process (Cesarone et al., 1990).

The inhibitors of pADPRP are known to potentiate the action exerted by DNA-damaging agents; in particular, 3-aminobenzamide has been utilized by most investigators, although the specificity of this compound has been questioned. The objective of our study was to increase the knowledge on the relationship between chemical structure and biological activity of a series of compounds substituted in different ways at the level of the ring of benzamide or modified at the amidic function of the benzene ring. By means of *in vivo* and *in vitro* assays, we have found that the most powerful inhibitors are characterized by substitution in position 3 of the NH2 function and are devoided of cellular cytotoxicity. A good correlation was also found between similarity index and experimental inhibitory capacity (Sestili et al., 1990).

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E. Botta, R. Riboni, C. Mondello and M. Stefanini (1990) Mapping of the human gene complementing the DNA repair defect in a UV sensitive Chinese Hamster mutant. Association for radiation research/DNA repair network joint meeting, January 3-5 Andrews (UK).

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A.I. Scovassi, R. Izzo, C. Mariani, M. Negroni, C. Negri and U.B. Bertazonni (1990) DNA topoisomerase II is ADP-ribosylated in HeLa cells. Poly(ADP-ribosyl)ation reactions, Workshop, September 19-20, Strasbourg (F).

Head of Project 6: Dr. Thomou-Politi

II Objectives for the reporting period

1. CHO cells deficient in HPRT (HAT-sensitive) were selected by growing them on 2-Amino-6-Mercapto-purine (final conc. 20 μg/ml). Resistant colonies appeared after 10 days.
2. The above mentioned cell-line was used for the introduction, propagation and maintenance of selected genes in mammalian cells. Thus, after cotransfection with pSV2-gpt vector and πH3-CD2 vector using the calcium phosphate coprecipitation technique, surviving cells were grown selectively in HAT medium. So, recombinant DNAs containing pSV2-gpt as a selective marker may be useful for cotransformation of nonselectable genes.

III Objectives for next period

The stably transformed CHO cells with the πH3-CD2 vector carrying the human CD2-cDNA and pSV2-gpt vectors express the cell surface antigen CD2. The transfectants will be analyzed by Southern blotting. Our approach will be the titration of the bioresponse of these cells to low doses of radiation affecting the quantitative expression of the CD2 gene. Especially, we are going to study the increase or decrease of the cell surface antigen CD2, responsible for binding sheep erythrocytes and rosette formation. This antigen is implicated mainly in the immune response of the human T lymphocytes.

IV Progress achieved including publications

Stable genetic transformation of mammalian cells after transfection with DNA is a relatively rare event; consequently the recognition and recovery of rare transformants requires a selection for the appropriate phenotype. It has been previously reported that transfection of cultured mammalian cells with recombinant DNAs containing the Ecogpt segment induces the synthesis of bacterial XGPRT. Moreover, HPRT-negative cells transfected with appropriate vectors containing the Ecogpt gene synthesize XGPRT and grow selectively in HAT medium (Mulligan and Berg, 1981).

Cells. CHO-K1 cells (Flow lab) were grown in plastic flasks in McCoy's 5A medium supplemented with 10% FCS, glutamine and antibiotics. The cultures were incubated at 37°C in a CO_2 incubator. SRBC, used in rosette formation, were prepared from freshly drawn blood by 4 washes in Hank's Balanced Salt Solution (HBSS) and stored as a 0.5% cells/ml solution in HBSS supplemented with 10% FCS at 4°C for a maximum of 1 week.

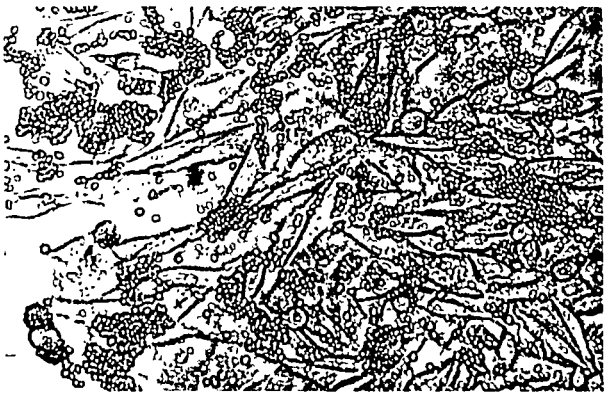
Transfection and Selection of stable transformants

The method of calcium phosphate transfection of cells with purified plasmid was employed (Sambrook, Fritsch and Maniatis, 1989) with some modifications. Briefly 22 hr before transfection, CHO cells (HPRT-) were removed from adherent growth with trypsin, inactivated with trypsin inhibitor and plated in T-25 flasks at $3x10^5$ cells per flask in complete medium containing 2-Amino-6-Mercapto-purine. 2 hours prior to transfection medium was removed from flasks and 5 ml of complete medium was added and the cells were incubated at 37°C.

$CaPO_4$/DNA precipitate (containing 10 μg πH3-CD2 and 10 μg pSV2-gpt plasmid DNAs/$1x10^6$ cells) was prepared and the mixture was incubated for 30 min at room temperature. The precipitate was mixed by pipetting and added very slowly to plated cells.

The culture was incubated for 4 hours in a humidified CO_2 incubator. After that cells were shocked by glycerol for 3 min at 37°C, then washed once with PBS and the cells were grown in complete medium for 72 hr at 37°C in a humified CO_2 incubator.

Given that the selection is necessary for stable transformants, cells were plated in a selective medium (1XHAT) after trypsinization. The culture fluid was replaced with fresh selective medium (1XHAT) every 3 days. Colonies were readily visible in 7-10 days and clones were isolated with cloning cylinders on the basis of their ability to express human CD2 receptor and consequently sustain rosette formation with SRBC (Figure) (Thomou et al., 1991). These isolated clones, which consistutively express the human CD2-cDNA, have been plated in complete medium for propagation and further manipulation (analysis of transfectants by Southern blotting, titration of their bioresponse to low doses of radiation etc.).



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Progress Report

Contract: Bi7-035 Sector: B13

Title: Methodology for the analysis of radiation carcinogenesis studies and application to ongoing experiments.

1	Broerse	Academisch Ziekenhuis Leiden
2	Chmelevsky	GSF Neuherberg
3	Masse	CEA - FAR
4	Morin	CEA - FAR
5	Zurcher	TNO
6	van Bekkum	TNO-ITRI

I. Summary of Project and Global Objectives

Large scale animal experiments at various European institutes have been undertaken in the past decade. The information from different laboratories can be meaningfully combined if the experimental procedures and the pahology are closely coordinated and similar requirements apply to the design of an experiment with regard to animal numbers, dose groups and other factors and also to the methods of statistical analysis which need to be defined at the outset of the experiments. It is accordingly necessary to develop standards of expermimental planning and methods of statistical analysis in animal experiments. Analysis of the tumor induction data reveals differences, which may be partly explained by a diversion of the cohorts under study, but are also believed to result from the employed analysis methods. In particular the different mathematical models, e.g. the parametric Weibull model versus the non parametric proportional hazards model have been under discussion. A unified approach to the analysis of experimental data of animal carcinogenic studies is the aim of this contract. To this aim a framework will be laid down for the analysis of dose-effect relationships in a concorded way for the existing data in Europe. A number of studies in experimental animals is included in the contract, to provide a database on stochastic effects after low dose irradiation.

The results of the work will be assembled in a laboratory handbook and accompanying software. Essential points will be:

- A set of rules and recommendations concerning the choice of the animal strains, the number of animals required in an experiment and their distribution into groups with different doses and time schedules, and the necessity of control groups.
- A set of rules defining the conduct of an experiment to ensure comparability and repeatability of the results. Special emphasis will be on the necessity of correct randomisation.
- Recommendations on the co-ordination of the pathology and on the recording of tumors.
- A set of rules on the extent of data end their format which permits efficient exchange between laboratories for comparison of results.

The department of Clinical Oncology of the Academic Hospital Leiden supports the project through the co-ordination of the proposed methodological approaches and the associated software development for the comprehensive analysis program. This program will be produced in such a way that it can be distributed among various European institutes.

Head of Project 1: Dr. J. Davelaar, Prof.dr. J.J. Broerse

II Objectives for the reporting period

The computer program for the analysis of animal carcinogenic data from various institutes (CEN-FAR, TNO), which was developed under this contract, needed adaptations according to discussions, both on the previous progress meeting at Leiden (June 19 and 20, 1990) as well as a separate meeting at GSF (April 11 and 12, 1991). Other models for the analysis of the time distribution of tumor appearances needed to be considered. In addition extensive changes to the lay-out of the program was requested. The conversion program for the data from CEN-FAR and TNO needed to be finalized.

III Objectives for the next period

New database structures, both at TNO and other institutes, need to be interfaced to the program, which will include the pathology results. Preliminary analysis of CEN-FAR data show satisfactory results, however the pathology data needs meaningful grouping before an intended publication. The existing prototype of the program manual and handbook needs to be completed. The Knudson-Moolgavkar model will be implemented in the program and the log-normal model updated. Other changes to the program, resulting from the meeting at GSF, will be made.

IV Progress achieved including publications

The latest version of the computer program was sent in April 1991 to a number of institutes for evaluation. The analysis of animal carcinogenic data was discussed at the Workshop "The relevance of animal experiments in view of developments in molecular biology" at TNO on October 22 and 23, 1990 (1).

A preliminary analysis of the CEN-FAR data show a distinct dose-effect relationship for tumor induction after irradiating the animals by both neutron and gamma sources. The animals are selected for analysis on the basis of instant lethality of the induced tumor. A further refinement in selecting these animals on the bases of the pathological classification is required, but a publication of these results is presently in preparation (2).

The analysis methods, developed under this contract, have also been applied to data from the long-lasting monkey experiment performed at TNO-Rijswijk since 1961. The monkeys were subjected to total body irradiation up to relatively high doses of both X-rays and neutrons, followed by a bone marrow transplantation. The late effects observed in the animals surviving more than 3 years include a number of neoplasms. Up to now the prevalence of animals with these induced tumors was quoted to be nine out of twenty over an observation period of 227 monkey-years for X-ray irradiated monkeys (average dose 6.7 Gy) and seven of the nine monkeys exposed to fission neutrons (average dose 3.4 Gy) during 87 monkey-years. In a control group of 21 animals two animals were observed with lethal neoplasms. The pathology findings showed a variety of neoplasms including osteosarcomas, kidney, thyroid and glomus tumors. An apparently shorter latency period was found for fission neutrons. The RBE was found to be between 4 and 7 for these relatively high doses of fission neutrons.

A more detailed analysis with Lifestat serves a dual purpose: (i) a correction for the animals dying intercurrently from causes unrelated to the endpoint, being the induction of a tumor, (ii) the analysis of the distribution of tumor induction times will provide the most information on carcinogenicity for these two cohorts of monkeys. Figure 1 shows the

cumulative hazard of the X-ray and neutron irradiated monkeys, corrected with the Kaplan-Meier product limit for intercurrent deaths, as a function of the follow-up time of the monkeys in years. The full drawn lines represent maximum likelihood fits to the data on the basis of a Weibull function, which proves to be a good model for both the neutron and X-ray data. No common slope of the Weibull fits is however observed and the cumulative hazard has a steeper increase for X-rays with ultimately a crossover point at around twenty years. The derived RBE will vary markedly depending on the time of follow-up: close to 17 at ten years follow-up, decreasing to 5 at fifteen years and around 2 at twenty years.

(1) Davelaar, J., Weeda, J., Broerse, J.J., "Analysis of animal carcinogenesis data by various mathematical methods", Rad.Env.Biophys., to be published.
(2) Chmelevsky, D., et al, in preparation.

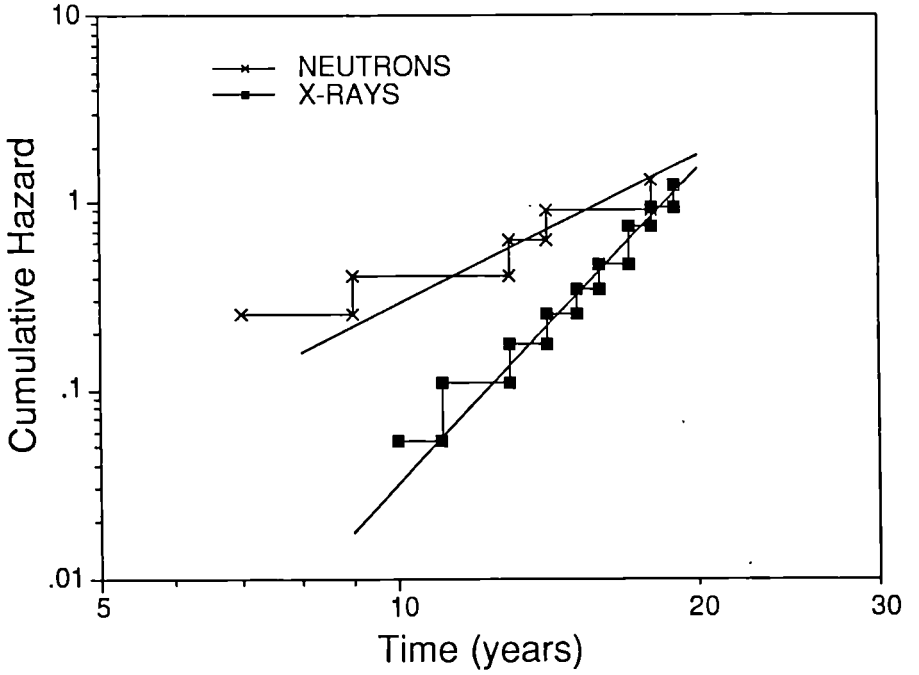


Figure 1.

Head of Project 2: Dr. D. Chmelevsky

II Objectives for the reporting period

To further develop statistical methods for the analysis of animal experiments and to apply them to the carcinogenesis experiments performed in the Nuclear Center of Fontenay aux Roses (France).

Another aspect of the work was the planning of a new experiment in Fontenay aux Roses. One important unsolved problem in radiation protection is the question whether protraction of irradiation with high LET particles leads to a reduction of the effect or to an increase (a reverse time factor has been repeatedly observed). Animal experiments in this respect could be a very important tool in elucidating this question. The experiments recently started at Fontenay aux Roses were planned to answer this question.

III Objectives of the next period

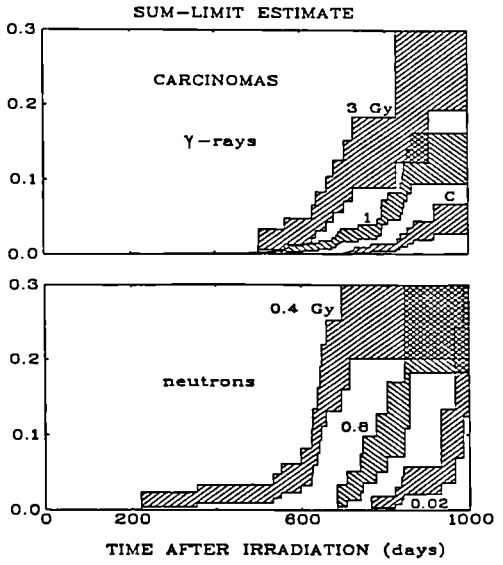
Preparation, together with the other contractors, of the monograph containing a review of the requirements in planning animal experiments as well as in analysing the results. In particular the mathematical methods implemented in the Lifestat package will be described in detail.

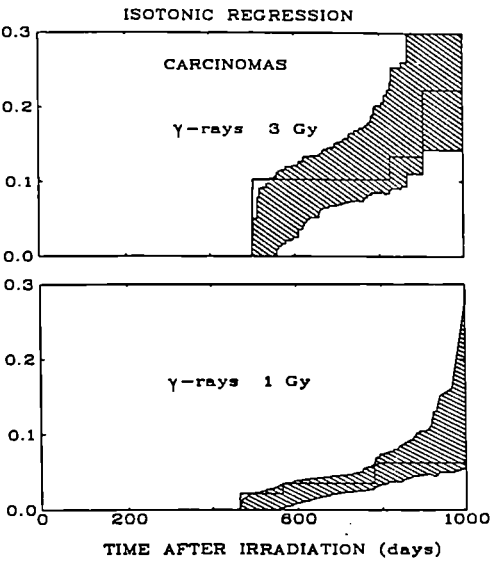
IV Progress achieved including publications

A detailed comparison of the efficiency of neutrons versus gamma-rays whole body irradiation in Sprague Dawley rats in inducing a broad spectrum of neoplasms has been performed. The results show differences in the time course of the various types of neoplasms (sarcomas as compared to carcinomas) and in the relative efficiency of neutrons and gamma-rays. The analysis shows very high RBE for neutrons compared to X-rays, again in the range of those found for pulmonary carcinomas. These results will be published as the illustrative part of the monograph which is planned within this contract.

Another aspect of the work was the further development of methods of analysis for incidental tumors. The isotonic regression has been used to estimate the incidence of incidental tumors. The drawback of such a method was the absence of confidence intervals for the estimated function. In the absence of an analytical answer we have introduced a modification of the wellknown "bootstrap method". The figure gives an illustration drawn from the non parametric analysis of experiments performed at Fontenay aux Roses. Groups of Sprague Dawley rats were irradiated either with gamma-rays or neutrons with the doses as given in the figures. For a selection of tumors (carcinomas of the unrinary and of the digestive tract) are compared with results obtained under the assumption of non lethality (isotonic regression). The hatched zone gives the confidence intervals. For the isotonic estimate they were obtained with the bootstrap. The purpose of this comparison is to show that, for the same data set, the two assumptions of lethality or non-lethality lead to quite different results.

Publication: Mathematical Methods in the analysis of Animal Experiments:
D. Chmelevsky, M. Morin, Radiation and Evironmental Biophysics: in print.


SUM-LIMIT ESTIMATE
CARCINOMAS
γ-rays
3 Gy
1
C
neutrons
0.4 Gy
0.6
0.02
0.0
0.1
0.2
0.3
0
200
600
1000
TIME AFTER IRRADIATION (days)


ISOTONIC REGRESSION
CARCINOMAS
γ-rays 3 Gy
γ-rays 1 Gy
0.0
0.1
0.2
0.3
0
200
600
1000
TIME AFTER IRRADIATION (days)

Head of Project 3/4: Dr. M. Morin

I Summary of Project and Global Objectives

Assessment of cancer risk following exposure to ionizing radiation at low dose and low dose rate is a problem of major concern. Epidemiological data from human groups, occupationally medically or naturally exposed, are of little help due to confounding factors and uncertainties on exposure levels. Extrapolation from groups exposed at higher doses and dose rates rely upon the hypothesis on the shape of the dose-response curves, mainly based on concepts applicable to the early event of interaction between ionizing radioation and DNA targets. This has led to the popular linear-quadratic model. Whether these early events are limiting steps, governing the dose-response curves, is not established and data from transformation work seem to indicate that initiating events were more frequent than expected, that environment strongly influenced clone outgrowth, and that the probability for an initiated cell to grow into a clone increased with the density of initiated cellls among their normal counterparts. All these observations may indicate that linearity for dose response at low dose and dose rate may not be the consequence of single events in single cells.

From this standpoint it appears that there is a need for more information from experimental data in animals exposed to low doses and low dose rate. Several drawbacks of this approach have to be overcome, which are notably:

1. Number of animals is limited to a few hundred, therefore design of the protocol should be optimized and take advantage of all the refinements of statistics.
2. Dose range for single events in appropriate targets is probably not explorable with low LET radiation, we proposed to look at dose response for fission neutrons at very low dose rate; radiobiological concepts do not support that the induction for cancers by particles such as high LET neutrons should depend on dose-rate, therefore if an attenuation of the risk coefficient is observed in the low dose range, we may expect that linearity does not preclude absence of a Dose Rate Effectiveness Factor (DREF).
3. The biology of tumors has to be clarified for an appropriate use of the statistics or for shedding some light on the extrapolation process from animal to man.

In previous experiments in Sprague Dawley male rats we observed that DREF for gamma rays (dose rate between 80 en 2 mGy/h) varied with tumor types. In the 1 - 3 Gy range an average factor of 5 was observed for carcinomas whereas no attenuation was observed for sarcomas. Preliminary results with fission neutrons indicated that the doubling dose for all cancers was less than 20 mGy and some evidence for an inverse effect of dose rate was provided between flash exposure and 1mGy/h. A decision was made to explore the response of rats exposed to a californium source at very low dose rate, leading to the lowest detectable cumulated incidence, as compared to a control group of 500 animals.

II Objectives for the reporting period

The first objective was to select appropriate exposure levels to design the exposure facility and to decide on the protocol of exposure. Review has to be made on the spectrum of cancers and benign tumors found in the strain, with consideration to time to tumor onset and criteria for malignancy and lethality.

III Objectives for next period

Checking individual dosimetry

Irradiation and observation of animals, starting with exposures at intermediate dose-rate with ETCA facilities, according to the following protocol:

Neutron Exposure

Start exposure	Total dose (cGy)	Exposure Dose Rate (μGy/h)	Age of rats at begin of exposure	Number of rats
05.1991	2.5	a few hours	3 months	150
05.1992	2.5	id.	15 months	250

Performing histopathology study on available samples; standardization of results within a panel of pathologists.

IIII Progress achieved including publications

A californium source has been sealed up and installed at the top of a well pole at 2 meters above the basement in a circular room of 12m in diameter. Animals are being continuously exposed with a daily interruption for cleaning and feeding of about 1h per day, 6 days per week.

Dosimetric measurements performed by Dr Nguyen van Dat, whom we wish to acknowledge, provided the following results in air.

Dosimetry

Dose rate (μGy/h - 01.1991)

Distance from the source (meter)	Total dose (CIRCE)	Neutron (Bubble dosimeter)	Gamma (Panasonic)
2.4	5.49	3.58	1.66
1.4	11.21	7.72	3.46

Five male rats were housed in each aluminium cage at a given distance of the source in order to reach the following neutron doses and rotated once a week.

Neutron Exposure

Start exposure	Total dose (cGy)	Exposure	Dose rate (μGy/h)	Age of rats at beginning of exposure	Number of rats
01.1991	2.4	1 year	3.5	4 months	250
01.1991	5.3	1 year	7.7	4 months	50
1990	control				500

This protocol has been selected for the following reasons:
1. Dose and dose rate should be kept as low as possible, and allow for the observation of an effect either in the case of an increased or a decreased incidence due to protraction. Owing to the relatively small number of cancers in excess, we decided to have a life span survey of all the groups, which will not allow for discriminating effect on latency time in the case of non fatal tumors: This has been justified according to the small effect to be expected on life shortening at such low doses. Analysis of our previous results in rats exposed to fission neutrons at 2mGy per hour provided the following results for 754 historical controls and 300 exposed rats:

Age at the diagnosis of tumor(days)

Dose in Gy	0.0	0.016
Carcinomas in		
Lung	992	870
GI tract	796	888
Liver	901	708
Pancreas	921	990
Skin	778	863
Kidney and bladder	922	943
Pituitary	960	1059
Adrenals	857	867
Thyroid	900	942
Sarcomas in		
Brain	735	845
Bone	943	904
Blood vessels	817	798
Soft tissues	743	813
Leukemias and lymphomas	778	745

It did not appear a significant trend for a difference between tumors which have been considered as lethal: carcinomas in pituitary, adrenals, pancreas, liver, sarcomas in bone, vessels and brain and the rest of tumors which are possibly (kidney, thyroid, soft tissues) and probably (lung) non lethal, when a possible dose dependant shift for the time of appearance is considered.

2. Aging was shown to influence strongly tissue susceptibility to tumor induction, after exposure to gamma rays at 3 Gy, (fetus 66, 3 months old 120, 9 months old 120, controls 754) as shown in the following graph. This led us to take into account that protracted exposure include one major component, not related to dose rate but to aging, that has to be corrected for.

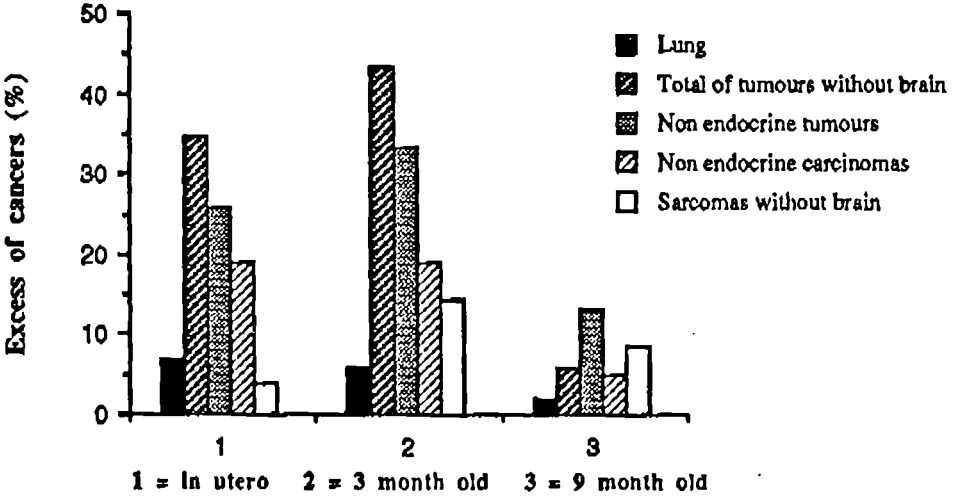
Publications

Chmelewsky D., Morin M.
Mathematical models in the analysis of animal experiments.
Radiation and Environmental Biophysics, in press.

Masse R.
Lung cancer in laboratory animals.
Radiation and Environmental Biophysics, in press.

Morin M., Masse R, Lafuma J.
Role de l'âge au moment de l'irradiation sur l'induction des tumeurs;
CR. Acad. Sc. série 3, in press.

Excess of cancers in function of age at irradiation (3 Gy), in comparison with control rats.



Head of Project 5 : Dr. C. Zurcher

i Summary of Project and Global Objectives

The general goal of this part of the project is to gather survival and pathology data from rats, that received different doses of total body-irradiation (as single dose or fractionated) of different radiobiological effectiveness. (ie. X irradiation and 0.5 MeV neutrons)

The following experimental groups will be examined:

1. Single dose X-ray up to a dose of 4 Gy.
2. Single dose 0,5 MeV neutrons in doses of 0,1 and 0,2 Gy.
3. Single dose 1,2 Gy X-ray compared with fractionated 1,2 Gy X-ray.
4. Single dose 1,2 Gy X-ray given at the age of 8 weeks (whose susceptibility to the carcinogenic effect of radiation is maximal) compared with single dose of 1,2 Gy X-rays given at the age of 17 weeks (whose susceptibility has subsided considerably).

ii Objectives of the reporting period and progress achieved

Background tumor incidence in untreated control groups may vary considerably. It is therefore necessary to compare data obtained from experimental groups with those from a corresponding untreated control group from the same period. As the experiment stretched over almost a decade, several control groups had to be included to serve as a reference. Furthermore the spectrum of age related pathology, characteristic for lifetime experiments, is influenced by intercurrent disease. The rats used for our experiment were derived from specific pathogen free stock and kept there after under conventional conditions.
Regular health programme results indicated that a Sendai virus infection occurred during the later part of one of our earliest experiments. It was therefore of paramount importance to check whether this influence could have disturbed the results of this experiment. It appeared that in the control groups, except for interstitial pneumonia as described earlier no life threatening complications occurred and that survival was not decreased. We therefore decided not to exclude those experiments from our pathology and survival survey. Later experiments were free of complicating disease.

Part of the histopathological examination of complete necropsies of the dose group receiving 4 Gy X-rays and the corresponding control group has been finished.

iii Objectives for the next reporting period

Continuation of the histopathological examination. Consultation of the group of dr. Masse in order to devise common criteria for histological diagnoses for lumping or splitting of diagnostic categories and for the discrimination between fatal and non-fatal lesions.

Head of Project 6: Dr. D.W. van Bekkum

I. Summary of Project and Global Objectives and II. Objectives for the reporting period.

Many uncertainties remain concerning the carcinogenic effects of very low doses of gamma-radiationtion and the effects of fractionation. The problem is that tumor incidence following exposure to total dose below 0.50 Gy cannot be measured accurately, because the number of animals required is prohibitive. We attempt to solve this dilemma by employing a large number of very low dose fractions (fraction dose 2.5 and 10 mGy) with intervals of 12 hrs to a total dose of 1 and 2 Gy. In view of the scarcity of data on induction of all types of tumors by low doses of photon radiation and the limited availability of facilities to carry out such studies it was proposed to add 4 groups of 40 rats (table 1) to ongoing exposures for other experiments. Single dose exposures and unirradiated groups (all without estrogen) are included (table 2). Apart from the identification of mamary tumors all other tumors occurring in the groups listed in tables 1 en 2 will be recorded. For this purpose mammary tumors are surgically removed to extend the life span of the rats. This study offers a unique opportunity to collect data on the carcinogenic effects of very low dose fractionation as compared to a single high dose. It is envisaged that the results will provide experimental evidence concerning the question of the existence of a threshold for radiation carcinogenesis.

TABLE 1.

Fraction	Nr. of fr.	Total dose	Exposure period	Age of rate at end of exposure
2.5 mGy	400 x	1 Gy	28 wks	36 wks
2.5 mGy	800 x	2 Gy	56 wks	64 wks
10 mGy	100 x	1 Gy	7 wks	15 wks
10 mGy	200 x	2 Gy	14 wks	22 wks

TABLE 2.

Single dose Age at exposure	Total dose
8 wks	1 and 2 Gy
15 wks	1 and 2 Gy
22 wks	1 and 2 Gy
36 wks	1 and 2 Gy
64 wks	1 and 2 Gy
non-irradiated controls	
8 wks	2 groups

III. Objectives for next period.

Continuation of observations, collection of mammary tumors and complete autopsies on all animals showing signs of terminal disease.

IV. Progress achieved including publications

The irradiation exposures have been completed according to schedule.

Contract: Bi6-004 **Sector: B13**
Title: Study of radiobiological effects at low doses

1 Coppola ENEA-CRE Casaccia

I. Summary of Project and Global Objectives

The present contract is a prolongation of the one expired in 1989. Therefore, the research programme follows the lines of the previous one, and is intended to provide information useful to enlarge our knowledge and understanding of the biological action of radiation and to tackle specialized problems relevant in the context of radiological protection. Shortly, the general aims of the work carried out in this contract are the study of the biological effectiveness of low radiation doses *in vitro* and *in vivo*, also in terms of interaction mechanisms. For this, the ENEA Casaccia Centre has in progress an extended programme aiming at studying the shape of the dose-effect relationship at low doses of different radiation qualities for various modes of irradiation, and in particular, the influence of dose rate and radiation quality, as well as of important biological host factors, using appropriate experimental model systems for well identified endpoints, including neoplastic transformation of immortalized cell lines, and life-shortening and tumor induction in experimental animals.

In this context, fast neutrons represent a particularly important problem, as, in the present situation, no useful estimate of RBE can be obtained from available human data of cancer mortality. Differences in RBE for tumor induction by radiation in various organs and tissues also call for careful consideration. Furthermore the variation of carcinogenic and mutagenic effectiveness consequent to a protraction of exposure deserves particular attention.

Because of our long lasting scientific experience in the field of late somatic effects of radiation, and the existence at our research centre of adequate animal housing, as well as of well equipped laboratories for cell cultures and irradiation facilities, we have already started an active collaboration with several European groups interested in the study of radiation carcinogenesis.

Finally, since animal experiments require long planning times and have very long duration, the research lines cited in this programme will extend of necessity well beyond the limit of end 1991 covered by the present RST Framework Programme of the Commission.

Head of Project 1: Prof. Coppola

II Objectives for the reporting period

- Analysis of liver tumor induction in mice after acute whole-body irradiation at different ages.
- In vitro study of the effect of dose fractionation of neutrons with various radiation qualities on neoplastic transformation of C3H10T1/2 cells.
- In vivo study of the carcinogenic effect of fractionated doses of fission neutrons for various tumor types in $BC3F_1$ mice.
- Experimental and theoretical studies of the microdosimetric characteristics of the neutron irradiation field of the fast research reactor RSV TAPIRO at Casaccia Research Centre.

III Objectives for next period

- In vivo study of the carcinogenic effect of fractionated doses of fission neutrons for various tumor types in $BC3F_1$ mice.
- In vitro study of neoplastic transformation of C3H10T1/2 cells, using monoenergetic neutrons.
- Acute irradiation of male CBA/Cne mice for studies of myeloid leukemia induction.
- Studies of hormonal unbalance influence on the incidence of ovarian tumors in partially shielded mice.

IV Progress achieved including publications

The present programme is mainly based on the use of low doses of different radiation qualities and various modalities of irradiation on different experimental model systems, for suitable endpoints, including life-shortening and tumor induction in experimental animals.

We have shown that liver tumors can be induced in mice exhibiting either a high or a low spontaneous incidence, namely 67% in CBA/Cne, and 11% in $BC3F_1$. Based on these findings we have also investigated the influence of radiation quality and age-at-irradiation (17.5 days *post coitum*, 3 months, 19 months) on the induction of such tumors in $BC3F_1$ male mice. A marked age-dependence was demonstrated for radiation-induced liver tumors, with a much higher susceptibility in young than in old mice. In the dose range where the risk appears to increase as a function of the dose, the data points after fission neutron irradiation were well fitted assuming a linear dependence of the incidence on the dose, with the values of the linear term coefficient ranging from a maximum of 1.18 per Gy for prenatal irradiation to a minimum of 0.03 per Gy for mice irradiated at 19 months of age, with an intermediate value of 0.35 per Gy for young-adult mice (Fig. 1). In the case of X-ray irradiation the dose-response data were adequately described assuming a quadratic dose dependence. Here the values of the coefficient of the quadratic term are fairly close to each other for the *in utero* and 3-month-old irradiated animals (1.7 and 1.2 per Gy^2, respectively), and not significantly different from zero for old mice.

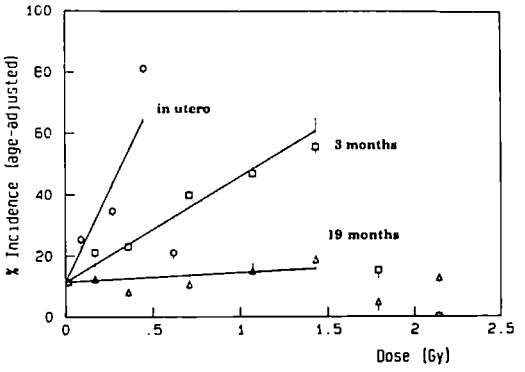


Fig. 1. Percentage incidence of liver tumors in mice irradiated with fission neutrons at various ages. Bars are standard errors.

Age-dependence also appears to affect neutron RBE relative to X rays for the induction of liver neoplasms. In fact, the RBE value for prenatal irradiation (28 at 0.09 Gy) is about two times higher than for young adult animals irradiated at comparable low doses (13 at 0.17 Gy).

In order to obtain experimental evidence on the influence of exposure prolongation (low dose-rate or fractionation) on the effectiveness of low neutron doses, an *in vivo* study of the carcinogenic effect of fractionated doses of fission neutrons and, for comparison, also of X rays, was carried out. About 2000 $BC3F_1$ male mice, subdivided in 16 groups, were given each five equal daily dose fractions, corresponding to cumulative doses of 2.5 to 70 cGy for fission neutrons from the reactor RSV TAPIRO (average neutron energy 0.4 MeV), and of 25 to 300 cGy for X rays from a deep therapy machine. Irradiated and control animals were followed-up for their entire life span. Soon after spontaneous death a complete autopsy was performed. The necropsy included a complete external and internal gross examination. Tissue masses as well as sections of the major organs were taken and processed for histological analysis. Experimental data are still being analyzed both with respect to life-span shortening and the induction of a number of selected tumor types, including malignant lymphoma and myeloid leukaemia. Data treatment will include the correction for competitive risks and the analysis in terms of cumulative mortality, death-rates for specific causes, and trend. A comparison will be carried out with data for acute exposure of $BC3F_1$ mice at comparable doses of fission spectrum neutrons, for critical endpoints.

In vitro studies of neoplastic transformation induced by radiation and chemicals are frequently carried out using different cell lines. In particular, the C3H10T1/2 mouse embryo fibroblast system represents a very useful tool for these studies, since it allows a closely quantitative determination of the dose-effect relationships for this end point. Recently, this system has yielded very interesting information on the effect of

radiation dose-rate. In particular, some experiments with neutrons indicated the possibility of an enhanced transforming potential of fractionated doses or low dose-rates in the dose range of 0 to 1 Gy, in comparison to single acute exposures.

A series of experiments using C3H10T1/2 cells are on their way. Cells are inoculated at low density 48 to 72 hours prior to irradiation with fission neutrons from the fast reactor RSV TAPIRO or with X rays at Casaccia, and with monoenergetic neutrons from the Van de Graaff accelerator of the TNO Institute at Rijswijk. Neutron doses are either single acute or fractionated. Dose fractionation follows a five fractions four days protocol, with equal doses delivered at 24 hour intervals. Following an incubation period of 6 weeks with weekly refeedings, transformed foci are identified by morphological criteria. Only type II and III foci are scored as transformants. The partial results obtained so far are for fission neutrons and show that: a) the survival curves after fission neutron irradiation are very nearly exponential for both acute and fractionated exposures and there is no appreciable effect of dose fractionation; b) dose fractionation does not modify significantly the transformation rate compared to acute irradiation; c) fission neutrons are more effective than X rays both to inactivate and to transform C3H10T1/2 cells at all neutron doses of this experiment (from 0.1 Gy up to about 1 Gy; d) in the low-dose region, cell transformation frequency is very nearly linear with the dose for exposure either to neutrons or X rays; e) maximum values of fission neutron RBE relative to X rays determined from survival and transformation data are in the region of 14 to 16, and are in close agreement with those obtained by other laboratories.

Finally, further investigation was conducted on the dependence of the frequency ratio of acentrics to dicentrics produced in human lymphocytes on treatment with radiation, misonidazole, a cell sensitizing chemical compound, and the combination of the two agents. This confirmed the previous finding that this ratio is markedly influenced by the presence of the chemical substance, especially at low radiation doses.

V Publications

1) V. Di Majo, M. Coppola, S. Rebessi, V. Covelli. Age-related susceptibility of mouse liver to induction of tumors by neutrons. Radiat. Res. **124**, 227-234, 1990.

2) A. Saran, S. Pazzaglia, M. Coppola, S. Rebessi, V. Di Majo, M. Garavini, and V. Covelli. Absence of a dose-fractionation effect on neoplastic transformation induced by fission spectrum neutrons in C3H10T1/2 cells, Radiat. Res. In press.

3) V. Covelli, M. Coppola, V. Di Majo, S. Rebessi. The dose-response relationships for tumor induction after high-LET radiation. Proceedings of the International Symposium on "Radiation Carcinogenesis in the Whole-Body System", Tokyo, December 4-7, 1990.

4) V. Covelli, V. Di Majo, M. Coppola, and S. Rebessi. Neutron carcinogenesis in mice: a study on the dose-response curves. Proceedings of "International Colloquium on Neutron Radiation Biology", Rockville, November 5-7, 1990.

5) A. Saran, S. Pazzaglia, M. Coppola, S. Rebessi, V. Di Majo, and V. Covelli. Neoplastic transformation of C3H10T1/2 cells following single or fractionated doses of fission spectrum neutrons and X rays. 38th Annual Meeting of Radiation Research Society, New Orleans, April 1990 (Book of Abstracts, Abstract Eo-3, p. 183).

6) S. Pazzaglia, A. Saran, M. Garavini, M. Coppola, S. Rebessi, V. Di Majo, and V. Covelli. Absence of a dose-fractionation effect in the transformation of C3H10T1/2 cells by fission spectrum neutrons. III Italian-Yugoslav Symposium, Plitvice, June 1990.

7) V. Di Majo, S. Rebessi, M. Coppola, V. Covelli. Induction of liver tumors by X rays and fission neutrons in hybrid mice (BC3F1). 38th Annual Meeting of Radiation Research Society, New Orleans, April 1990 (Book of Abstracts, Abstract Cw-3, p. 102).

8) A. Saran, S. Pazzaglia, M. Coppola, S. Rebessi, V. Di Majo, M. Garavini, V. Covelli. Neutron dose-fractionation does not enhance neoplastic transformation of C3H10T1/2 cells. 23rd Annual Meeting of the ESRB, Dublin, September 1990.

9) V. Covelli, V. Di Majo, M. Coppola, and S. Rebessi. Dose Response Relationships for Lymphoma and Skin Cancer in Mice after Total Lymphoid Irradiation (TLI). 23rd Annual Meeting of the ESRB, Dublin, September 1990.

10) N. Vulpis, and M. Coppola. Ratio of acentrics to dicentrics in human lymphocytes exposed to X rays and misonidazole. Mutation Research **245**, 107-110, 1990.

Contract: Bi6-075 **Sector:** B13

Title: Late effects in rhesus monkeys after whole-body irradiation with X-rays and fission neutrons

1 Broerse. TNO-ITRI

I. Summary of Project and Global Objectives

Specific information related to the risk of radiation induced tumours and other late effects in man is limited. Studies on acute and late effects in non-human primates are relevant for man since the radiation effects in both species do not seem to show significant differences. This type of studies with larger animals are valuable for risk assessment in man and for estimation of the relative biological effectiveness (RBE) for tumour induction by neutron irradiation of human patients. In addition, the induction of deterministic effects in various tissues and the RBE of neutrons for these effects are of increasing importance for radiation protection problems. The response of rhesus monkeys after exposure to relative high doses of fission neutrons has been investigated. The protective effect of autologous bone marrow transplantation was initially demonstrated, however, new research has shown an increasing importance of the application of haemopoietic growth factors. The study on longevity, tumour induction and other late effects of total body irradiation of rhesus monkeys with fission neutrons and X-rays was initiated in the time period 1963 - 1973. In the irradiated groups 4 out of 29 animals are still alive, whereas the group of untreated controls still comprises 11 out of 21 monkeys. Experiments on the efficacy of haemopoietic growth factors were performed since 1988. It is too early to expect already radiation induced neoplasms in the latter group. It has been realized, however, that both cohorts will be of interest for the study of deterministic effects. In particular, attention will be given to the occurrence of radiation cataract relatively early (a few years) or late (20 years) after exposure.

Head of Project 1: Dr. Broerse.

II Objectives for the reporting period

Stochastic effects, such as tumour induction and deterministic effects, such as cataract formation has been studied in a long term surviving monkeys. After total body irradiation with large doses of X-rays (average dose 6.7 Gy) or fission neutrons (average dose 3.4 Gy) and autologous bone marrow transplantation, the animals were kept under observation for a period of more than 25 years. The average latency period for carcinogenesis amounted to 11 years for 7 out of the 9 neutron irradiated animals and to 13 years for 10 out of the 20 X-irradiated monkeys. A variety of neoplasms, in many cases multiple tumours were observed in the irradiated animals, whereas in the control group only 2 out of 21 animals developed single malignancies. In the reporting period the histology results have been examined in detail.

III Objectives for next period

- Observation of the four remaining irradiated animals for possible incidence of malignancies.
- Investigation of deterministic and stochastic effects in the control group.
- Studies on cataract formation either early (a few years) or late (20 years) after exposure.

IV Progress achieved including publications

The treated and untreated monkeys are regularly checked for the occurrence of neoplastic and non-neoplastic late effects of radiation and are kept for life span studies. Every half year, at the time of tuberculosis testing, they are weighed and a physical examination with routine haematological and blood chemistry testing is performed. When moribund, the animals are euthanized followed by a complete necropsy and histological examination. Descriptions of the malignant and benign neoplasms observed in the irradiated groups are given in Tables 1 and 2.

- J.J. Broerse, D.W. van Bekkum, J. Zoetelief and C. Zurcher (1991). Relative biological effectiveness for neutron carcinogenesis in monkeys and rats. Radiat. Res. in press.
- J.J. Wielenga (1990). Hemopoietic stem cells in rhesus monkeys - surface antigens, radiosensitivity and responses to GM-CSF. Thesis, Rotteram.
- C. Zurcher, M.J. van Zwieten, C.F. Hollander and J.J. Broerse (1991). Radiation carcinogenesis in large animals. Radiat. Environm. Biophys. in press.

Table 1: Neoplasms observed in X-irradiated Rhesus monkeys.

Sex	Post irrad. interval (y)	Average whole body dose (Gy)	Neoplastic disease	
			Malignant	Benign
m	10	3.7		parathyroid adenoma
m	13	7.1	renal cortical papillary adenoma.	
f	14	7.1	Mal. schwannoma (popliteal space)	thyroid: follicular adenoma, renal cortical adenoma.
f	15	7.5		multiple uterine fibroleiomyomas, endocervical polyps
m	16	7.5	thyroid follic. CA., ileum adenoCA.	splenoma, subcut. fibroma and neuro-fibroma
m	10	7.9	colon: mucinous cyst - adenoma, renal cort. papillary ad. CA.	
m	8	7.9	renal cortical papillary cyst adeno-CA.	
f	7	7.9	renal cortical papillary cystadenoCA.	
m	14	7.9	multiple myeloma renal cortical CA	
m	12	8.0	mal. glomus tumour (elbow), metastatic to lung, kidney, meninges, osteosar-coma maxilla.	renal cort. adenoma.
m	15	8.0	well diff. renal cortical papillary adenocarcinoma	renal papillary cyst adenomas, thyroid follicular adenoma, peritoneal lipoma, subcut. neurofibroma, pituitary: p. dis. adenoma
m	11	8.0	osteosarc.(supra-orbital), metastatic to lungs	pituitary: small adenoma., p. distalis

Table 2: Neoplasms observed in Rhesus monkeys after irradiation with fission neutrons.

Sex	Post irrad. interval (y)	Average whole body dose (Gy)	Neoplastic disease	
			Malignant	Benign
m	10	2.3	Mal. glomus sacral area metastasis	fibroma lip. pancreatic islett cell adenoma.
m	20	2.6	scrotal mal. glom. tumour renal cort. CA.	cavernous hemangioma subcutis, subcut. neurofibroma, adrenal pheochromocytoma, multiple insulinoma's, schwannoma
m	18	3.5	synovial sarcoma humerus renal cort. CA. metastatic to lung, liver, lnn, liver cell CA, thyroid foll. CA.	fibrous epulis.
m	12	3.5	renal cystic papill. cort. carc., osteosarc. os frontale	splenoma
m	4	3.8	teleangiectatic osteosarc. (humerus) mandibular mal. glomus tumour, metastatic to liver	
m	6	4.1	right frontotemporal astrocytoma	ossifying fibroma skin
f	4	4.4	glioblastoma temporal lobe	

Contract: Bi7-036 **Sector: B13**

Title: Molecular and cellular effects of protons, deuterons and alpha-particles.

1	Moschini	Istituto Nazionale di Fisica Nucl.
2	Goodhead	MRC Radiobiological Unit
3	Belli.	Istituto Superiore di Sanità

I. Summary of Project and Global Objectives

It has been well demonstrated in independent experiments on accelerators that the biological effectiveness of low doses of protons for producing stochastic effects in cells can increase substantially in the LET region 10-30 kev/μm. This can represent a considerable deviation from the behaviour of alpha-particles of corresponding LETs, and it has substantial implications for understanding mechanisms of radiation action and the hazards from exposure to neutrons and alpha-particles.

The present project is intended to extend the existing data by measuring genetic ($HGPRT^-$ mutation) and molecular (DNA dsb) damage and then to investigate the generality of this phenomenon, its extension to deuterons of yet higher LET, its possible dependence on the dose-rate, and its causes in terms of the microscopic track structure. For this purpose deuteron beams will be used as a surrogate for protons having, at a given LET, a greater range with respect to protons, but almost identical differential track structure. Design and construction of small size systems for cell irradiation with alpha sources during incubation to perform comparative experiments of the effectiveness of higher LET alpha-particles at different dose-rates. Monte Carlo track structure simulations will be carried out for all the radiations and analysed, to seek microscopic features which correlate with the particle- and energy-dependence of the experimentally observed biological effectiveness at low doses.

In particular, the objectives of the project are:

(1) measurement of the effectiveness of low doses of protons in inducing $HGPRT^-$ mutations in mammalian cells, as a function of LET;

(2) measurement of the initial production of DNA double strand breaks in mammalian cells, irradiated with protons, as a function of LET;

(3) set-up of a beam line for irradiation of cell monolayers with deuterons to extend to higher LETs, that is not practically possible with protons;

(4) measurement of the effectiveness of deuterons for inactivation and mutation induction in mammalian cells, as a function of LET;

(5) performance of comparative experiments with low energy alpha-particles radionuclide sources at different dose-rates;

(6) analysis of the microscopic track structure of the radiations to seek features, which correlate with their observed biological effectiveness.

Head of Project 1: Prof. G. Moschini

II Objectives for the reporting period

1. Measurement of the effectiveness of low energy protons in inducing HGPRT$^-$ mutations in V79 cells, as a function of LET;
2. Measurement of the effectiveness of low energy protons in inducing DNA double strand breaks in V79 cells, as a function of LET;
3. Set up of a beam line for irradiation of cell monolayers with deuteron beams to extend to higher LET values, that are not practically possible with protons;
4. Measurement of the effectiveness of deuterons for cell inactivation in V79 cells, as a function of LET;
5. Design and construction of small size system for cell irradiation with alpha sources during incubation.

III Objectives for next period

1. Measurement of the effectiveness of deuterons for cell mutation in V79 cells, at the HGPRT locus, as a function of LET;
2. Start of comparative experiments with low energy alpha-particles radionuclide sources at different dose-rate.

IV Progress achieved including publications

(in collaboration with the ISS group)

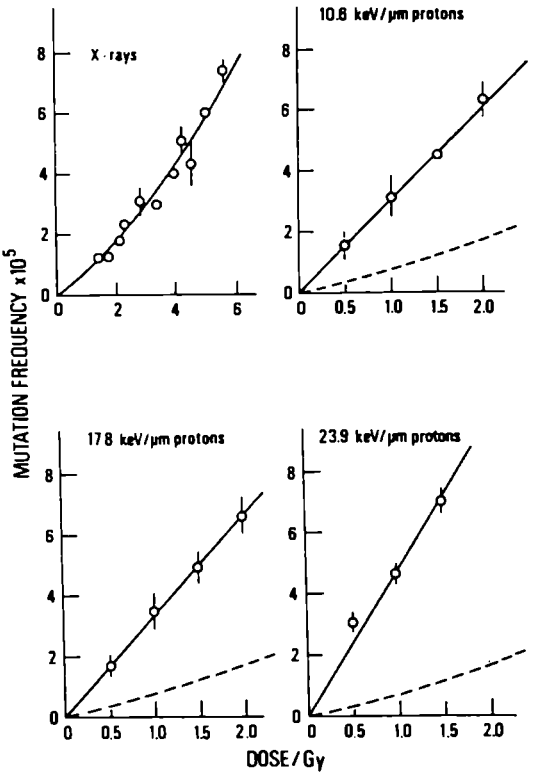
In previous studies on cell inactivation of V79 cells caused by low-energy protons, we have shown that the RBE-LET relationship is in poor agreement with those values predicted by the currently assumed curve, mainly based on alpha particle data.

In an attempt to extend these studies to endpoints other then cell inactivation, we have analysed the mutation induction at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in V79-753B Chinese hamster cells, after irradiation with protons having incident energies of 3.36, 1.70 and 1.16 MeV in the dose range 0.5 - 4.0 Gy.

The mutation curve obtained with 200 kV X-rays was used for comparison.

The figure shows the mutation frequency for cells irradiated with protons and X-rays as a function of the dose. Each data point represents the mean of three to six independent experiments, and the error bar denotes one standard error of the mean. The dashed line represents the X-ray curve in the same dose range used for protons.

The proton effectiveness increases with the LET, as shown by the slope increase. The comparison between protons and X-rays shows a substantial difference in the number of induced mutants at the same dose.



In the LET range we have considered, low energy protons appear to have a higher effectiveness in mutation induction than other charged particles with the same LET. This finding is similar to that observed for cell inactivation.

Recently, irradiation experiments have also been performed with protons of 7.6 keV/µm (5.12 MeV) in order to extend the range of LET studied for inactivation and mutation induction. Preliminary results show that for both biological endpoints the RBE values are intermediate between that for X-rays and that for 10.6 keV/µm protons..

Proton beams, for LET higher than 35 keV/μm, were not used due to the limited range of these particles with respect to cell thickness.

To perform cell irradiations in air with deuteron beams, that have virtually the same track structure but twice the range of protons with the same LET, we improved the existing facility at the Van de Graaff CN accelerator of the LNL with a remote controlled multisample holder housing up to 20 Petri dishes that can be irradiated at a prefixed temperature.

The multisample holder is provided on a lateral wall with a circular port serving as entrance for the beam. The cylindrical copper container is thermically connected to a Peltier cooling system, which permits to maintain costant a chosen temperature during the irradiation (in the range 20 - 0°C).

Preliminary results have been obtained for inactivation of V79 cells irradiated in air with 17.4 and 24.0 keV/μm deuterons in the dose range 0.5 - 4.0 Gy.

Besides we have studied the yields of DNA double strand breaks in V79 cells with protons of 3.36, 1.70 and 1.16 MeV, in the dose range 10 - 120 Gy. For all energy values considered, we found linear dose-response relationship and a preliminary data analysis shows that there are only little differences among the four radiation qualities (see ISS report for further details).

Publications:

M. Belli, F. Cera, R. Cherubini, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron
"LET dependence for cell inactivation, mutation induction and DNA DBS on V79 cells irradiated with high LET protons", Thirty-Eighth Annual Meeting of the Radiation Research Society, Tenth Annual Meeting of the North American Hyperthermia group, New Orleans, Lousiana, 7-12 April, Abstract Book: Ek-7, p. 164, 1990

M. Belli, F. Cera, R. Cherubini, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron
"RBE-LET relationschip for V79 cells irradiated with low energy protons", Radiation Protection Dosimetry, vol.31, n.1/4, 309-310, 1990

F. Barone, M. Belli, F. Cera, R. Cherubini, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron
"Initial yield of DNA DSB in V79 cells irradiated with low energy protons and X-rays", 23rd Annual Meeting, Dublin, Ireland, 23rd-26th September, Abstract, 1990

M. Belli, F. Cera, R. Cherubini, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron
"Mutation induction and RBE-LET relationship of low energy protons in V79 cells", International Journal of Radiation Biology, (1991) vol. 59, n. 2, 459-465

G. Simone, M. Belli, F. Ianzini, O. Sapora, M.A. Tabocchini, F. Cera, R. Cherubini, P. Tiveron and G. Moschini
"Lethal and mutagenic effects of 7.6 keV/μm protons on V79 cells", To be presented ICRR 91 Toronto, Canada;

R. Cherubini, F. Cera, A.M.I. Haque, P. Tiveron, G. Moschini, G. Galeazzi, G. Simone, M. Belli, F. Ianzini, O. Sapora and M.A. Tabocchini
"The biological effectiveness of deuterons: description of the facility at LNL and preliminary results on V79 cells", To be presented ICRR 91 Toronto, Canada;

M. Belli, F. Cera, R. Cherubini, D.T. Goodhead, F. Ianzini, T.J. Jenner, G. Moschini, H. Nikjoo, O. Sapora, G. Simone, D.L. Stevens, A. Stretch, M.A. Tabocchini and P. Tiveron
"Relevance of experiments with different charged particles having the same LET for biophysical modelling of radiation effects" To be presented Workshop of Biophysical Modelling of Radiation Effects DOE-CEC 1991, Padua, Italy.

Head of Project 2: Dr. Goodhead

II Objectives for the reporting period

Jointly, with partners, analyse experimental data on biological effectiveness of protons compared to α-particles of the same LET. Generation, by Monte Carlo methods, of simulated tracks of protons and α-particles corresponding to energies for which the LETs of two particles are the same and for which radiobiological data are available on their effectiveness; randomly sample these to obtain absolute frequency distributions of energy depositions in microscopic target volumes corresponding approximately to dimensions of DNA, nucleosomes and chromatin fibre.

III Objectives for next period

Compare the frequency distributions of energy deposition in microscopic volumes by protons and α-particles of the same LET and compare these with the relative effectiveness of the particles as observed in biological experiments.

Generate frequency distributions for deuterons of similar LET and extend data to other LETs and volumes for all 3 particles, within the region of experimentally observable effects.

IV Progress achieved including publications

Analysis of experimental data

Together with others in this coordinated contract, we have analysed raw experimental data obtained previously in a direct comparison of the biological effectiveness of protons and α-particles of the same LET. Experiments had previously been carried out on the Variable Energy Cyclotron at Harwell to measure inactivation of V79, HeLa and C3H 10T½cells, $HPRT^-$ mutations in V79 cells and double-strand breakage of DNA, by protons and α-particles each of 20 and 23 keV μm^{-1}. In this contract the data were analysed and prepared for publication.

For cell inactivation it was found that the ratio of the linear (low dose) terms of protons to α-particles, for 20 and 23 keV μm^{-1}, respectively, were as follows: for V79-4 cells 1.75 ± 0.38 and 1.45 ± 0.32; for HeLa cells 1.32 ± 0.40 and 1.33 ± 0.20; for HeLa S3 cells 1.35 ± 0.28 and 1.32 ± 0.18; and for C3H 10T½cells 1.08 ± 0.25 (both LETs combined) [1]. The probability that these seven ratios, taken together, are greater than unity by statistical chance alone is < 1%. Hence it may be concluded that, overall, protons are more effective at cell inactivation than α-particles of the same LET. The largest ratios were obtained for V79-4 cells. In a different line of V79 cells Belli *et al.* (1989) had previously reported a high effectiveness of protons, apparently larger than the relative effectiveness of α-particles as available in the literature.

For mutations at the hprt locus in V79-4 cells it was found that protons were more effective than α-particles of the same LET by ratios 1.85 ± 0.31 (at 20 keV μm^{-1}) and 2.07 ± 0.19 (at 23 keV μm^{-1}) [2].

By contrast, the initial yield of DNA double-strand breaks in V79-4 cells, as measured by neutral sucrose sedimentation was smaller for protons than α-particles (ratios 0.73 ± 0.11 at 20 keV μm^{-1} and 0.86 ± 0.11 at 23 keV μm^{-1}). By the Olive method of DNA precipitation the relative yield of breaks was only slightly higher with proton than with α-particles (ratios 1.08 ± 0.13 at 20 keV μm^{-1} and 1.23 ± 0.17 at 23 keV μm^{-1}). Hence, it would appear that the residual damage which leads to cell inactivation or mutation is not a random sample from the initial double-strand breaks [3].

Track scoring: Frequency distributions of energy deposition

The above differences in biological effectiveness of protons and α-particles of the same LET must originate from their differences in track structure. Thus, the experimental data provide a useful new constraint on the biologically critical microscopic properties of radiations.

To quantify differences between the tracks over dimensions similar to DNA, nucleosomes and chromatin fibre, statistically representative tracks of protons and α-particles have been simulated by the Monte-Carlo code MOCA14 of Wilson and Paretzke. These were then scored for energy deposition in small cylindrical volumes positioned randomly with respect to the tracks. In this way, absolute frequency distributions of energy deposition in the target volumes have now been generated for the following conditions:

Particle	Energy (MeV)	Energy (MeV/μ)	LET (keV/μm)	diameter (nm)
alpha	35.56	8.89	20.8	2
"	35.56	8.89	22.2	10
"	35.56	8.89	22.2	25
proton	1.380	1.38	20.7	2
"	1.380	1.38	20.8	10
"	1.380	1.38	21.3	25
alpha	30.48	7.62	23.3	2
"	30.48	7.62	24.0	10
"	30.48	7.62	24.0	25
proton	1.16	1.16	23.9	2
"	1.16	1.16	24.0	10
"	1.16	1.16	24.0	25
alpha	21.84	5.46	30.9	2
"	21.84	5.46	30.9	10
"	21.84	5.46	30.6	25
proton	0.79	0.79	31.0	2
"	0.79	0.79	30.7	10
"	0.79	0.79	30.7	25

The last column indicates the diameters of the target cylinders. For each diameter (d), the cylinder length was varied from 0.5d to 8d.

The next stage will be to compare the distributions for the corresponding particles and seek regions which may, or may not, correlate with the observed ratios of biological effectiveness.

Publications

[1] D.T. Goodhead, M. Belli, A.J. Mill, D.A. Bance, L.A. Allen, S.C. Hall, F. Ianzini, G. Simone, D.L. Stevens, A. Stretch, M.A. Tabocchini and R.E. Wilkinson. "Direct comparison between protons and α-particles of the same LET: I. Irradiation methods and inactivation of asynchronous V79, HeLa and C3H 10T½ cells." (Submitted to International Journal of Radiation Biology).

[2] M. Belli, D.T. Goodnead, F. Ianzini, G. Simone and M.A. Tabocchini. "Direct comparison between protons and α-particles of the same LET: II. Mutation induction at the HPRT locus in V79 cells." (Submitted to International Journal of Radiation Biology.)

[3] T.J. Jenner, M. Belli, D.T. Goodhead, F. Ianzini, G. Simone and M.A. Tabocchini. "Direct comparison between protons and α-particles of the same LET: III. Initial yield of DNA double-strand breaks in V79 cells." (Submitted to International Journal of Radiation Biology.)

Head of Project 3: Dr. M. Belli.

II Objectives for the reporting period

1. Analyze the experimental data obtained at Harwell on biological effectiveness of protons compared to α-particles of the same LET.
2. Measure the effectiveness of low energy protons in inducing HPRT mutations in V79 cells, as a function of LET.
3. Measure the effectiveness of low energy protons in causing DNA double strand breaks (initial) in V79 cells, as a function of LET.
4. Develop an irradiation facility for deuteron beams and measure their effectiveness for inactivation of V79 cells, as a function of LET.

Objective 1 is in collaboration with the MRC partner, and objectives 2 through 4 with the LNL partner.

III Objectives for next period

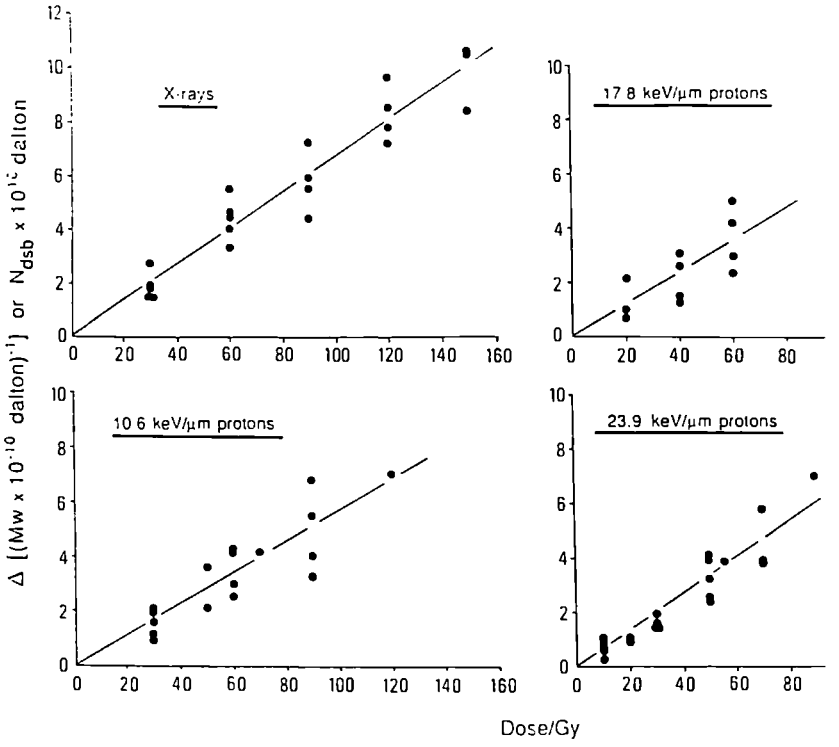
1. Measure the effectiveness of deuterons for V79 mutation at the HPRT locus, as a function of the LET.
2. Start comparative experiments with low energy α-particle radionuclide sources at different dose-rates.

IV Progress achieved including publications

1. We have analysed and prepared for publication, together with the MRC group, the experimental data obtained with the Variable Energy Cyclotron at Harwell on inactivation, HPRT- mutations an DNA double strand breakage in V79 cells irradiated by protons and α-particles, each of 20 and 23 keV μm-1. We found that protons are more effective at cell inactivation and mutation induction than α-particles of the same LET. These results are in qualitative agreement with the finding obtained at Legnaro (Belli et.al, Int. J.Radiat. Biol.1989, 1991) that indicated a relative effectiveness of protons apparently larger than that of α-particles, as available in the literature. By contrast, the initial yield of DNA dsb did not reproduce the differences observed for the cellular effect, suggesting that the residual damage responsible for cell inactivation or mutation is not a random sample from the initial dsb. (See the MRC report for further details).

2. We have studied the frequency of HPRT$^-$ mutations induced in V79-793B cells by proton beams having incident energies of 3.36, 1.70 and 1.16 MeV. The mutation curve obtained with 200 kV X-rays was used for comparison. The mutation frequency induced by all the proton beams is considerably higher than that induced at the same dose by X-rays and it is linearly related to the dose. Moreover, the RBE for protons increases with the LET, and has values higher than those reported in the literature for other ions of comparable LET. This finding parallels what we have previously found for cell inactivation, and indicates that for mutation induction, also, the RBE-LET relationship may depend on the type of radiation. (See the LNL report for further details).

3. We have studied, in collaboration with the LNL group, the initial yields of DNA dsb in V79 cells irradiated with protons of 3.36, 1.70 and 1.16 MeV, in the dose range 10-120 Gy. The sucrose gradient sedimentation technique was used for such determinations, and irradiation with X-rays was used for comparison. We found linear dose-response curves in all cases. A preliminary data analysis, that is being to be refined, shows that there are only little differences among the four radiation qualities, so that the RBE's for protons result very close to 1 for all the conditions studied.



This finding is consistent with the results obtained at Harwell (see p.1) on direct comparison between protons and α-particles,and indicates that the variations with the LET, observed in the RBE for cellular effects, are not reproduced for the initial production of DNA dsb. This could be explained by differences in the nature and/or the spatial distribution of dsb on changing the radiation LET.

4. We have improved, in collaboration with the LNL group, the facility already established at LNL for proton irradiation in order to obtain deuteron beams, that are expected to have a similar track structure but twice the range of protons with the same LET (see the LNL report for further details). Preliminary results have been obtained for inactivation of V79 cells irradiated in air with 17.4 and 24.0 keV/μm deuterons in the dose range 0.5-4.0 Gy.

The experimental data reported in points 1, 2, 3 provide useful new constraints on the biologically critical microscopic properties of radiations. The observed dependence of biological effectiveness on the LET and radiation type should originate from changes in the radiation track structure. The validity of this interpretation is under study in collaboration with the MRC partner, whose main objectives are track simulation and evaluation of the frequency distributions of energy deposition in microscopic volumes by the particles used in the experimental work.

Publications:

Belli, M., Ganesh, A.N., Goodhead, D.T., Ianzini, F., Jenner, T.J, Simone, G., Stevens, D., Stretch, A., Tabocchini, M.A., Wilkinson, R.E. "Inactivation, mutation induction and DNA dsb in V79 cells irradiated with protons and alpha particles of the same LET". 38th Annual Meeting of the Radiation Research Society, New Orleans, Lousiana, April 7-12, 1990. Abstract Book: Ek-7, p.164.

Belli, M., Cera, F., Cherubini, R., Ianzini, F., Moschini, G., Sapora, O., Simone, G., Tabocchini, M.A. Tiveron, P. "LET dependence for cell inactivation, mutation induction and DNA dsb on V79 cells irradiated with high LET protons.". 38th Annual Meeting of the Radiation Research Society, New Orleans, Lousiana, April 7-12, 1990. Abstract Book: Ek-8, p.165.

Belli, M., Goodhead, D.T.Ianzini, F.Jenner, T.J., Simone, G., Tabocchini, M.A. "The use of DNA precipitation assay for evaluating dsb induced by high and low LET radiation: comparison with sedimentation results.". NATO Advanced Research Workshop. Interdisciplinary Meeting on "The Early Effects of Radiation on DNA". San Miniato, Italy, May 7-11, 1990, Abstract p.43

Belli, M., Cera, F., Cherubini, R., Ianzini, F., Moschini, G., Sapora, O., Simone, G., Tabocchini, M.A. and Tiveron, P. "RBE-LET relationship for V79 cells irradiated with low energy protons.". Radiation Protection Dosimetry, vol.31, n.1/4, 309-310 (1990)

Belli, M., Cera, F., Cherubini, R., Ianzini, F., Moschini, G., Sapora, O., Simone, G., Tabocchini, M.A. Tiveron, P.."Mutation induction and RBE-LET relationship of low energy protons in V79 cells", International Journal of Radiation Biology, (1991) vol.59, n.2, 459-465

Belli, M., Goodhead, D.T., Ianzini, F., Jenner, T.J., Simone, G., Tabocchini, M.A. "Radiation induced double strand breaks in V79 cells: comparison between sedimentation and precipitation assays". European Society for Radiation Biology 23rd Annual Meeting. Dublin, Ireland, September 23-26, 1990. (Abstract)

Barone, F.,Belli, M., Cera, F., Cherubini, R., Ianzini, F., Moschini, G., Sapora, O., Simone, G., Tabocchini, M.A., Tiveron, P. "Initial yield of DNA dsb in V79 cells irradiated with low energy protons and X-rays". 23rd Annual Meeting European Society for Radiation Biology, Dublin, Ireland, September 23-26, 1990. (Abstract)

Goodhead, D.T., Belli, M., Mill,A.J, Bance,D.A., Allen, L.A., Hall,S.C., Ianzini,F., Simone,G., .Stevens,D.L,.Stretch, A,.Tabocchini M.A and Wilkinson,R.E. "Direct comparison between protons and α–particles of the same LET: Irradiation methods and inactivation of asynchronous V79, HeLa and C3H 10T1/2 cells". (Submitted to International Journal of Radiation Biology)

Belli, M., D.T.Goodhead, F.Ianzini, G.Simone and M.A.Tabocchini "Direct comparison between protons and α-particles of the same LET:II Mutation induction at the HPRT locus in V79 cells". (Submitted to International Journal of Radiation Biology)

Jenner,T.J., M.Belli, D.T.Goodhead, F.Ianzini, G.Simone and M.A.Tabocchini. "Direct comparison between protons and α–particles of the same LET:III Initial Yield of DNA double-strand breaks in V79 cells". (Submitted to International Journal of Radiation Biology)

Simone,G., M.Belli, F.Ianzini, O.Sapora, M.A.Tabocchini, F.Cera, R.Cherubini, P.Tiveron and G.Moschini. "Lethal and mutagenic effects of 7.6 keV/μm protons on V79 cells". To be presented to the ICRR 91 Toronto, Canada.

Cherubini,R., F.Cera, A.M.I. Haque, P.Tiveron, G.Moschini, G.Galeazzi, G.Simone, M.Belli, F.Ianzini, O.Sapora and M.A.Tabocchini. "The biological effectiveness of deuterons; description of the facility at LNL and preliminary results on V79cells". To be presented to the ICRR 91 Toronto, Canada.

Belli,M., F.Cera, R.Cherubini, D.T. Goodhead, F.Ianzini, T.J.Jenner, G.Moschini, H.Nikjoo, O.Sapora, G.Simone, D.L. Stevens, A.Stretch, M.A.Tabocchini and P.Tiveron. "Relevance of experiments with different charged particles having the same LET for biophysical modelling of radiation effects" To be presented to the Workshop "Biophysical Modelling of Radiation Effects" DOE-CEC 1991, Padova, Italy.

Progress Report

Contract: Bi7-037 Sector: B13

Title: Cellular and molecular mechanisms of radiation-induced myeloid leukaemia in the mouse.

1 Janowski CEN - SCK
2 Cox NRPB

I. Summary of Project and Global Objectives

Myeloid leukaemia can be induced with a 20-25 per cent incidence in male CBA/H or SLJ/J mice with a single X-ray dose of 3 Gy. Deletions/ rearrangements of chromosome (ch) 2 in radiation-induced mouse acute myeloid leukaemia (AML) is an extremely consistent cytogenetic feature. Evidence was provided that ch 2 rearrangement can be induced in vitro by X-rays in multipotential haemopoietic cells and, in some cases, that it generated a cellular phenotype associated with preferential recruitment and/or proliferative advantage. However, none of the clones studied during post-transplantation repopulation of recipient animals progressed to overt AML. Similarly, it was shown that ch 2 events may be observed in bone marrow cultures established from irradiated animals after only 4 months, long before the time at which overt AMLs may be expected. Overall, it seems likely that certain classes of ch 2 rearrangements may be an initiating event for AML, but are insufficient for the development of overt leukaemia.

There is evidence that ch 2 rearrangements in AMLs might involve a homeobox gene, Hox 4.1, and the cytokine gene, interleukin (IL)-1ß, in a proportion of those events. It may be argued that the deletion of one copy of a morphogenetically important homeobox gene may be associated with abnormal tissue development, or that deregulation of a specific cytokine gene uncouples haemopoietic regulation. However the existing data on the involvement of either Hox 4.1 or IL-1ß in the premalignant process are not yet compelling. Clinical reports showing autocrine production of IL-1 in AML justify further investigations in this field. Another important issue is the possibility of indirect leukaemogenesis. Indeed, a few whole body irradiated patients have developed leukaemia from healthy, transplanted bone marrow. This situation is easily reproduced in mice for lymphoid, but not yet for myeloid leukaemia.

Later events in the multistage leukaemogenesis were not investigated so far for mouse radiation AMLs. However, activation of a ras oncogene, considered with few exceptions as a late carcinogenic event, was observed in 113 out 412 (27 per cent) of human AMLs, as revealed by a recent compilation of the literature. This certainly is a minimal estimate, the reported studies having not dealt with all the possible oncogenic mutations at codon 12, 13 or 61 of all three H-, K-, and N-ras genes. Therefor, the question may be asked if such a mutation could not be present in most if not all the AMLs.

Finally, the lack of monoclonal antibodies for the murine myeloid lineage justifies the search for other biochemical markers. Membrane receptors for neurotransmitters could be useful in this regard.

Head of Project 1: Dr.Janowski

II Objectives for the reporting period

- Screening mouse radiation AMLs for all possible oncogenic point mutations in codons 12, 13 and 61 of the H-, K- and N-ras genes.
- Measurement of IL-1 in culture supernatants from leukaemic cells.
- Search for (donor) sex chromosomes in leukaemic cells from chimaeric mice given bone marrow from the opposite sex after irradiation.
- Screening for receptors for neurotransmitters coupled to adenylate cyclase in leukaemic cells.

III Objectives for next period

- In addition to ras mutations in mouse radiation AMLs, studies on other possible events related or not to ch 2 translocations: abnormal expression of IL-1 or can gene products, and of various cytokines that induce growth and differentiation of myeloid precursor cells : MGI-1M (CSF1), MGI-1G (G-CSF), MGI-1GM (GM-CSF), IL-3, MGI-2 (IL-6), LIF, DIF (TNF).
- Characterisation of NFS myeloid cell lines, obtained from AMLs that develop in irradiated mice subsequently grafted with healthy bone marrow.
- Effect of differentiation-inducing agents on leukaemic cells.

IV Progress achieved including publications

Male CBA/H mice were irradiated at the age of 3 months with a single X-ray dose of 3 Gy. AML was diagnosed on the basis of haematological and pathological examination. The spleen, lymph nodes, femoral bone marrow and "Lymphoprep" purified circulating white blood cells were stored in liquid nitrogen.

Screenings for oncogenic ras mutations were performed on the following samples:

- 11 radiation-induced CBA/H AMLs (spleen or both spleen and bone marrow; occasionally, also white blood cells).
- 5 radiation-induced SLJ/J AMLs (sub-cutaneous passages), kindly provided by N. Haran-Ghera, Weizmann Institute, Rehovot, Israel.
- 4 radiation-induced NFS AMLs (spleen), obtained from irradiated NFS mice.
- 4 cell lines established from radiation NFS AMLs. Only one was derived from one of the investigated primary tumours.
- the N122 cell line, established at the MRC from a 229-Ra-induced mouse AML
- the WEHI3 cell line, derived from a mouse myelomonocytic leukaemia, obtained through J. Van Snick, Free University of Brussels.
- 10 control CBA/H spleens

The search for mutations was performed by oligodeoxyribonucleotide mismatch hybridisation with ras gene segments (exons 1 and 2) that had been amplified by the polymerase chain reaction (PCR). The oligodeoxyribonucleotides (19 or 20-mers) PCR primers and molecular probes representative for all the possible H-, K- and N-ras point mutations in codons 12, 13 and 61, were synthesised in our laboratories.

Only one mutation was detected among the 26 AML samples examined, comprising 23 mouse radiation-induced AMLs. It was a GGC (gly) to TGC (cys) transition of K-ras codon 13 in a CBA/H radiation AML. It was detected in both the spleen and the bone marrow, where at least 50 and 25 per cent of the cells were involved, respectively. It thus appears that classical oncogenic ras mutations are much less frequent in mouse radiation AMLs than in human AMLs. Moreover, it should be noted that an overwhelming majority of the mutations in human AMLs involve N-ras, while the only mutation found here was located in K-ras.

At the cellular level, we have characterised myeloid neoplasms in the NFS strain of mice, chosen for its lack of endogenous ecotropic proviruses. Striking properties of myeloid cells obtained from such mice were:
- their rapid adaptation to growth in culture. No exogenous factors were required in addition to fetal calf serum and 2-mercaptoethanol. Clonal lines were also easily established.
- their differentiated phenotype: granulocytic features indicated that this murine system is a model for human chronic myeloid leukaemia (CML).
- the expression, in cell cultures, of membrane receptors for the neurotransmitter calcitonin gene (CGRP). Such receptors are not commonly found on blood cells but were also found on the human HL60 myelomonocytic cell line. Remarkably, however, HL60 cells express receptors for CGRP only after treatment with differentiation inducing agents.

Receptors for neurotransmitters were also studied in other myeloid lines. N122, a 229-Ra-induced myeloid leukaemia, expresses receptors for isoproterenol and prostaglandin E1 and CGRP. Stimulation of adenylate cyclase decreased after treatment with differentiation inducing agents. Responses to isoproterenol and to prostaglandin E1 were, however, not affected. In the WEHI3 murine myeloid line, except for a weak response to prostaglandin E1, none of the agonists tested stimulated adenylate cyclase.The identification of receptors for CGRP on some myeloid cells is most promising. Their relationship to differentiation and leukaemogenesis is now being investigated. In particular, could CGRP be an autocrine growth or differentiation factor for myeloid cells ?

We have obtained several myeloid leukaemias in chimaeric NFS mice given bone marrow after whole body irradiation. In each case however, karyotypic analysis has confirmed the host origin of the leukaemic cells. So far, we thus have no indication that indirect myeloid leukaemogenesis can be induced in mice. All the leukaemias obtained had the features of CML.

Publications

M. Janowski, R. Cox and P.G. Strauss: The molecular biology of radiation-induced carcinogenesis: thymic lymphoma, myeloid leukaemia and osteosarcoma. Int. J. Rad. Biol. 57, 677-691, 1990.

M. Janowski: The ras proteins and the ras-related signal transduction pathway. Rad. Env. Biophys., in press.

R. Hooghe, P. Robberecht and M.P. Defresne: Receptors for the calcitonin gene-related peptide (CGRP) on leukemic cells. Special Conference on Chromosomal and Growth Factor Abnormalities in Leukemia, Chatham, MA, USA, October 1990 (abstract).

Related studies:

C. Damien, P. Robberecht, J. Abello, R. Hooghe and J. Christophe: VIP-helodermin receptors in the murine virus-induced T lymphoma cell line BL/VL3 recover less rapidly than ß-adrenoreceptors after down regulation. J. Receptor Res., 9, 441-449, 1989-1990.

E.A. Bruyneel, M. De Smets, C.H. Dragonetti, R.J. Hooghe, S. di Virgilio and M.M. Mareel: Effect of glycosylation inhibitors on N-glycosylpeptides and on invasion of malignant mouse M04 cells in vitro. J. Cell Sc., 95, 279-286, 1990.

Head of Project 2: Dr. Cox

II Objectives for the reporting period

- to gain further information on the relationship between radiation-induced chromosome (ch) 2 changes in irradiated CBA/H haemopoietic cells and those which characterise radiation-induced murine acute myeloid leukaemia (AML).
- to analyse the structure of the ch 2 F sub-region encoding the haemopoietic cytokine genes interleukin (IL)-1α and ß.

III Objectives for next period

- to determine whether damage is expressed on only one copy of ch 2 of irradiated CBA/H haemopoietic cells.
- to investigate ch 2 radiosensitivity in other mouse strains.
- to study DNA sequence losses from ch 2 in AMLs.
- to explore the relationship between ch 2 radiation-sensitive sites and telomeric DNA repeat sequences.

IV Progress achieved including publications

Cytogenetic studies: Using random number Monte-Carlo simulations statistical concordance was sought between 63 ch 2 breakpoints scored in irradiated haemopoietic cells. These analyses revealed highly significant breakpoint clusters in the ch 2 sub-regions B, C1, C2, F1, F3 and G of haemopoietic cells, thus supporting the existence of radiation-sensitive sites (RSS) on this chromosome. Concordance between this sites and breakpoints in AMLs was highly significant for C2, F1 and F3 and marginally significant for G, thus linking specific induced chromosome damage to leukaemogenesis. Analysis of ch 2 breakpoints in AMLs and haemopoietic cells carrying deletions and translocations implies that there are two regions (C and F) where chromosome breakage and DNA losses may be critical to leukaemic initiation. These analyses also provide preliminary evidence that one copy of ch 2 preferentially expresses RSS and that these sites interact with telomeric ends of other chromosomes at an expectedly high frequency. These latter observations suggest: a) that complex heritable factors, including a possible germ line AML-predisposing mutation in the CBA/H mouse, may be influencing the leukaemogenic process and b) that RSS on ch 2 may represent highly recombinogenic (DNA repeat ?) sequences that exhibit a strong affinity for the tandem TTAGGG repeat sequences present at chromosomal termini.

Molecular studies: Pulse field gel electrophoresis (PFGE) analyses established the close linkage (<70 kb) and orientation of the IL-1α and ß genes in a ca. 800 kb sub-region of the F segment of ch 2. The pattern of SalI restriction site methylation in this chromosomal region of normal cells was suggestive of differential methylation in two sub-populations of DNA, perhaps representing differences between the two autosomal copies of this region. This proposal was supported by the analysis of six ch 2 rearranged AMLs. Three of these showed consistent loss of hyper-methylated IL-1 hybridising sequences and, within the limits of cytogenetic resolution, these losses appeared to coincide with the ch 2 deletions and

F region breakpoints carried by the AMLs. De novo methylation changes during leukaemic development provide, however, an alternative explanation for these PFGE data.

Publications

G. Breckon and R. Cox: Alpha particle leukaemogenesis. Lancet, 335, 656-657, 1990.

A.R.J. Silver, G. Breckon, W.K. Masson, J. Adam, J. Boultwood and R. Cox: Radiation-induced chromosome 2 rearrangements and initiation of murine acute myeloid leukaemia. Radiation Res., 121, 233-234, 1990.

M. Janowski, R. Cox and P.G. Strauss: The molecular biology of radiation-induced carcinogenesis: thymic lymphoma, myeloid leukaemia and osteosarcoma. Int. J. Rad. Biol. 57, 677-691, 1990.

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G. Breckon, D. Papworth and R. Cox: Murine radiation myeloid leukaemogenesis: A possible role for radiation-sensitive sites on chromosome 2. Genes, chromosomes and Cancer (accepted for publication).

A.R.J. Silver, A.M. George, W.K. Masson, G. Breckon, J. Adam and R. Cox: DNA methylation changes in the IL-1(2F) chromosomal region of some radiation-induced acute myeloid leukemias carrying chromosome 2 rearrangements. Genes, Chromosomes and Cancer (accepted for publication).

G. Breckon, A.R.J. Silver and R. Cox: Radiation-induced chromosome 2 breakage and the initiation of murine acute myeloid leukaemogenesis. J. Radiat. Res.(Japan) (accepted for publication).

Progress Report

Contract: Bi7-038 **Sector:** B13

Title: Automated detection of radiation induced chromosome aberrations by slit-scan flow cytometry.

1	Barendsen	Univ. Amsterdam
2	Green	MRC Human Genetics Unit
3	Nüsse	GSF Neuherberg
4	Bauchinger	GSF Neuherberg
5	Aubele	GSF Neuherberg

I. Summary of Project and Global Objectives

The development of techniques which allow rapid automated assessment of chromosome aberrations will enable the determination of doses which human individuals or groups have received as a result of occupational or accidential exposures. Protection and intervention measures require dose assessments which in most instances cannot be based on physical dosimetry alone, but require also a biological method to be applied to individuals. These biological methods are most relevant if damage to blood elements, in particular lymphocytes, can be measured.

At the Laboratory for Radiobiology of the University of Amsterdam a method is developed for rapid analysis of karyotype abnormalities in large numbers of cells by automated techniques using slit-scanning of fluorescent chromosomes prepared from irradiated cells. Using high speed electronics the two centromeres in dicentric chromosomes are detected and counted in real-time, to allow also sorting and collection of abnormal chromosomes for fixation on microscope slides. These chromosomes are then checked to distinguish and correct for chromosome aggregates and other artefacts.

Immunofluorescence labelling of human chromosome centromeres and the detection of centromere fluorescence coupled with chromosome DNA measurements is applied at the MRC Human Genetics Unit in Edinburgh. DNA probes for centromere and telomere sequences are currently available and work on cloning human telomeres is in progress. The use of a probe recognising sequences at the X-chromosome centromere has led to improvement in the manual and automatic detection of fragile X-chromosomes. Experiments will be directed at the fluorescent labelling of isolated chromosomes using CREST antibodies and in situ hybridised DNA probes specific to chromosome centromeres or telomeres.

The aims of the projects at the Gesellschaft für Strahlen und Umweltforschung at Munich are to develop automated micronucleus (MN) assays applied specifically to human lymphocytes, using flow cytometry. Doses as low as 0.1 Gy should be detectable with good statistical precision and reproducibility. Also variation among human individuals and the dependence on radiation quality are studied. In addition studies on dose effect curves for chromatin texture assay (CTA) of in vitro irradiated lymphocytes will be performed. The experiments shall also elucidate the role of varying chromatin texture during the cell cycle.

Head of Project 1: Prof. Dr. Barendsen

II Objectives for the reporting period

The slit scanning technique will be applied to chromosomes for the automated detection of karyotype abnormalities in large numbers of cells. To achieve this end we planned three types of studies:

A. Investigation of the suitability of the slit-scanning system for recognizing human chromosomes.
B. Testing of the method for the detection of dicentric chromosomes by automated centromere counting.
C. Adaptation of the procedure for chromosome isolation.

III Objectives for next period

Based on the results obtained in the reporting period we will continue our studies by:

A. Incorporation of additional selection criteria in the electronic analysis system developed for the automated detection of dicentric chromosomes. In this way we intend to increase its sensitivity and to reduce the number of false positive signals.
B. Investigation of the advantages of state of the art chromosome labelling techniques for the analysis procedure.
C. Application of the procedure for the analysis of radiation induced chromosome aberrations.

IV Progress achieved including publications

We have applied the method of slit-scanning flow cytometry to the analysis of chromosomes. The chromosomes are isolated from metaphase cells and stained with a DNA specific fluorochrome. In the analysis region the chromosomes are alligned parallel to the direction of flow by hydrodynamic forces and guided one by one through a ribbon-shaped laser beam. During the analysis the fluorescence intensity profiles and total fluorescence intensities of the chromosomes are detected. The data are processed to yield information on chromosome morphology and DNA content.

Slit-scanning analysis of human chromosomes

Chromosome profiles are characterized by the centromeres that appear as dips in the pulse shapes. Normal chromosomes produce biomodal profiles but dicentric chromosomes, the most commonly registered type of radiation induced aberration, produces trimodal profiles. We developed a slit-scanning system based on commercially available flow cytometry equipment with optical elements that are used in standard flow cytometry sys tems, and we tested the system on Chinese hamster chromosomes. For radiation protection purposes the method has to be applied to human chromosomes, this is a more challenging task than the analysis of Chinese hamster chromosomes due to the smaller size of the human chromosomes.

During the reporting period we have extended the slit-scanning method to analyse the shapes of human metaphase chromosomes. The shape of the chromosomes as expressed by their centromeric index (CI) and their DNA content have been used as para-

meters in bivariate flow karyotyping, c.f. Fig.1. The resolution of the DNA vs CI flow karyogram of the larger chromosomes up to chromosome 13, is much higher than the resolution obtained in the DNA-based monovariate flow karyogram. Several chromosomes cannot be distinguished or are difficult to discriminate in the DNA-based karyogram. Most of these chromosomes can now be distinguished as individual peaks, e.g. chromosomes 1 and 2. The peak representing the chromosomes 9-12 can be separated into two peaks formed by chromosomes 9 and 11, and 10 and 12, respectively.

Detection of dicentric chromosomes by automated centromere counting

For these experiments we have extended our method of slit-scanning analysis to detect dicentric chromosomes. This was achieved by introducing a system for counting the number of centromere dips in the slit-scan profiles. The device consists of an analog electronic circuit, the pulse dip counter (PDC), which is interfaced with our cytofluorograph. The advantage of an analog system is the easy interfacing of the circuit with the existing electronics of the flow cytometer. The system is also very fast, generating an output signal within 30 us. The shape analysis can therefore be performed on-line, allowing chromosomes to be sorted on centromeric characteristics. Pulse shapes of the selected chromosomes can be recorded simultaneously with the transmission of the sorting command.

When analyzing dicentric chromosomes, it is necessary to determine the number of centromeres of each chromosome, but dips in the slit-scan profile are not caused by centromeres only. Aggregates of chromosomes or irregularly stretched chromosome arms can also result in tri-modal profiles. To investigate the relation between chromosome morphology and the corresponding slit-scan profile, we sorted chromosomes yielding trimodal profiles, one by one on separate slides for micro photography. For further analysis we digitized the negatives with a microdensitometer and constructed density profiles. An example of a sorted chromosome together with its profile is presented in Fig.2. This type of data provides valuable clues that can help to distinguish between true di-centrics and artefacts. Profiles with deep dips for example are always generated by chromosome aggregates. Using the information provided by these experiments we will improve the selectivity and sensitivity of the pulse dip counter.

Procedure for chromosome isolation

Chromosome length appears to be an important factor in the resolution of centromeres. For the isolation and staining of the chromosomes we used propidium iodide because its intercalating action results in long chromosomes. After isolation the length of the chromosomes was increased by incubating the unfixed chromosome suspension with trypsin. Pulse length and pulse shape analysis with the slit-scanning flow cytometer was used to determine changes in morphology and mean length of the isolated chromosomes. In these experiments we found that the treatment has two effects. In addition to increasing the total length of the chromosomes, the trypsin appeared to stretch the centromere more that the chromo-

some arms. This allows a more efficient detection of dicentric chromosomes from irradiated cells.

Publications

Boschman GA, Rens W, Manders E, van Oven C, Barendsen GW, Aten JA. On line sorting of human chromosomes by centromeric index, and identification of sorted chromosomes by GTG-banding and fluorescent in situ hybridization. Human Genetics 85 (1990) 41-58.

van Oven C, Aten JA. Instrument for real-time pulse shape analysis of slit-scan flow cytometry signals. Cytometry II (1990) 630-635.

Hausmann M, Zuse P, Aten JA, Rens W, Männer R, Cremer C. High speed analysis of slit-scan profiles of normal and aberrant metaphase chromosomes. In: Advances in Analytical Cellular Pathology. Eds: Burger G, Oberholzer M and Vooijs GP. Elsevier Publ., Amsterdam. (1991) pp. 67-68.

Fig. 1: Bivariate slit-scanning analysis of metaphase chromosomes isolated from human skin fibroblasts. The relative fluorescence intensity (DNA-content) and the "centromeric index" were used simultaneously as parameters; 100.000 particles were analysed in 10 min. The dark spots represent the tops of the peaks and are used to indicate the peak positions.

Fig. 2: Slit-scanning analysis and sorting of a dicentric Chinese hamster chromosome.
A. Slit-scan profile and corresponding dicentric chromosome detected by the "pulse dip counter" and sorted by the cell sorter system.
B. Digitized image of A and the resulting profile.

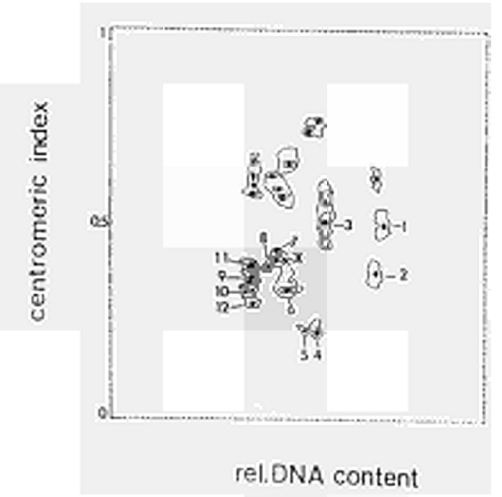


Fig. 1

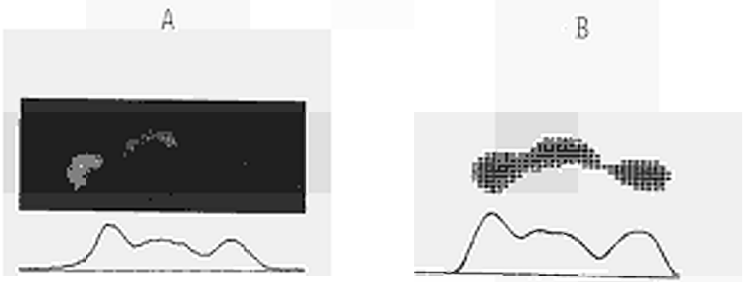


Fig. 2

Head of Project 2: Dr. Green

II Objectives for the reporting period

There were three main objectives, all of which relate to the detection of radiation induced chromosome aberrations by fluorescence labelling.

a) Development of *in situ* hybridisation technology in order to "paint" human chromosomes with fluorescently labelled DNA probes derived from sorted chromosomes.

b) Establishment of fluorescence imaging hardware capable of screening microscope slide samples of "painted" chromosomes.

c) Re-design of flow cytometry equipment to handle multiple colour fluorescently labelled chromosomes.

III Objectives during next reporting period

It is intended to establish an efficient strategy for labelling human chromosomes in order to detect radiation damage. This will involve *in situ* hybridisation development using a whole range of chromosome painting techniques in parallel with experiments using a combination of telomere and centromere probes. Image processing hardware for fluorescence microscopy will be further developed for automatic scanning of large numbers of metaphase chromosomes. Flow cytometry approaches will be in the first instance concentrated on the CREST antibody labelling technique. Following this, *in situ* hybridisation labelling will be developed for suspension chromosomes.

IV Progress achieved including publications

Chromosome Painting:

We have been investigating various sources of DNA libraries for chromosome painting which we want to use to improve the detection of radiation damage initially on metaphase slide preparations. Chromosome painting uses labelled DNA sequences from a specific chromosome to paint or cover completely that chromosome with a fluorescence signal.

There are various sources of DNA sequences from a specific chromosome:

1. Lambda DNA libraries from ATCC. We have developed a PCR based system to amplify only the insert DNA; this cuts out many tedious purification stages. We have used this successfully with chromosome libraries from 12 and 9, as is seen in the attached photographs (Fig.1 & 2). The library from chromosome 9 fails to paint the tip of the long arm of chromosome 9 and was probably derived from a deleted chromosome in a somatic cell hybrid. However it shows the power of the technique to recognise small deletions.

2. Somatic cell hybrids containing one single human chromosome. We have used the Alu-PCR technique to amplify unique DNA sequences in the vicinity of Alu repeats. Oligonucleotide primers are available for both ends of the Alu sequence and the best approach is to use each primer individually and pool the contents of each reaction. Alu repeats are clustered mainly in Giemsa light bands (R bands) and the products of Alu-PCR reactions give a slight R banding pattern (Fig.3). Another repeat, L1, is concentrated in the Giemsa dark bands (G bands) and the PCR reaction products using L1 primers give a slight G banding pattern. The best approach here may be to mix the L1 and Alu PCR products.

3. Small quantities of sorted chromosomes. We are optimising the L1/Alu technique described above for sorted chromosomes, initially using small quantities of chromosome 18, to ensure that enough amplified DNA is produced.

Our final choice of source material will depend on both a complete chromosome coverage and low background as well as ease of production.

Additionally we are implementing a second labelling system, digoxygenin, which can be detected by an antibody to digoxygenin coupled to Texas Red or FITC.

Fluorescence Imaging:

Laser scanning con-focal microscopy was available at the laboratory for examining and recording the results of chromosome painting experiments. Work was begun on assembling scanning hardware, based on CCD (Charge Coupled Device) technology, aimed at recording and analysing large numbers of fluorescence images in a partially automatic mode. Previous experience with linear CCD cameras showed that fluorescence images produce too little light intensity to make that device practical. Two dimensional CCD cameras however, which, because they are multi-lined devices, can integrate light for longer periods without loss of slide scanning speed, have a much greater potential for achieving an acceptable signal to noise within a reasonable scanning time. A two-dimensional (Pulnix) CCD camera has been used to record digital images of both bright fluorescence objects, dapi stained chromosomes for example, and weakly labelled fluorescence objects, such as those prepared by *in situ* hybridisation of small DNA probes to metaphase chromosomes. In the bright fluorescence case light was integrated for 0.1sec. and in the weak fluorescence case for 12sec. Peltier cooling of the camera head was used at both light levels to maintain a low camera dark current. The fluorescence intensity of "painted chromosomes" lies somewhere between bright DNA specific fluorescence and single unique sequence probe fluorescence depending on the quantity and size of the painting probes. Camera scanning times of a few seconds will not inhibit the development of a viable automatic aberration scoring system based on fluorescence labelled chromosomes.

Flow Cytometry:

Major changes to the chromosome sorting and analyses flow system have occurred with a view to multi-colour excitation and emission anticipated from samples of painted chromosomes. A Motorola 68030 based computer, with signal processing hardware, has been assembled and software has been written for flow cytometry control. A suitable optical bench, which will carry two lasers and has multiple fluorescence detection channels has been acquired from a former Orthocytofluorograf user. Sorting specific human chromosomes for creating chromosome painting probes has begun and will continue uninterrupted during the change to the reconfigured flow cytometer.

Fig. 1

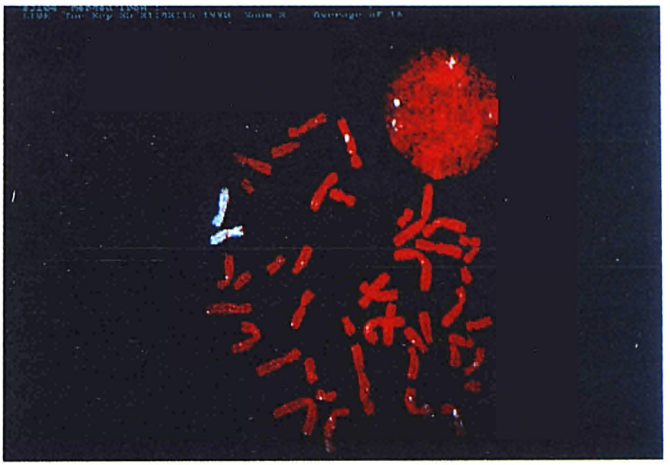


Fig. 2

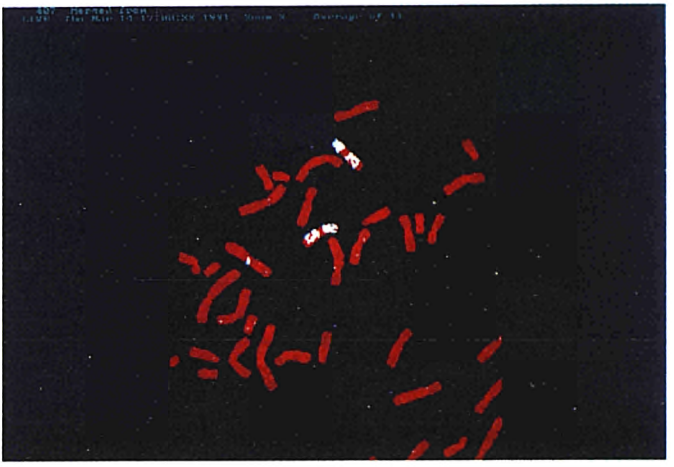
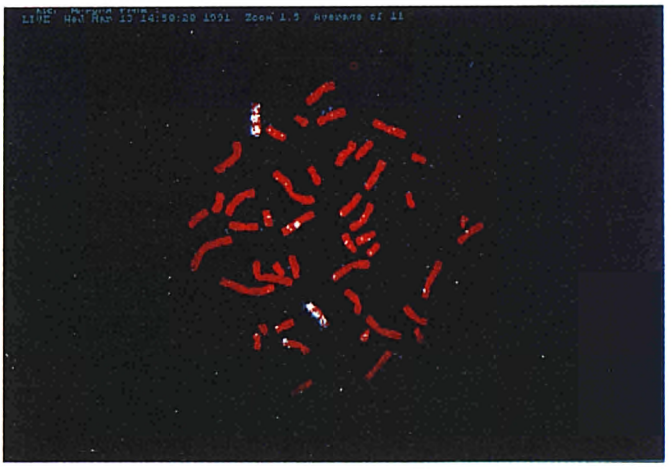


Fig. 3



Head of Project 3: Dr. Nüsse

II Objectives for the reporting period

The primary goal of our investigations during the first year of the project was the establishment and application of a new flow cytometric method to measure radiation induced micronuclei in human lymphocytes with high precision for a possible detection of low doses in irradiated humans. For this purpose a preparation technique had to be developed to obtain a suspension of nuclei and micronuclei stained with fluorescent dyes. An unambiguous discrimination between micronuclei and unspecific debris particles should be possible using simultaneous measurement of several flow cytometric parameters. Additionally, a flow cytometric technique had to be developed to measure the fraction of lymphocytes in the first and second cell cycle after irradiation.

III Objectives for next period

Application of the new flow cytometric method to measure micronuclei in irradiated human lymphocytes: Establishment of dose effect curves for human lymphocytes irradiated in vitro and comparison of results obtained by flow cytometry with microscopic observations. Flow cytometric measurements of the freqency of micronuclei in lymphocytes of unexposed, healthy persons. Analysis of factors (age, sex, etc) possibly influencing the variable frequency of micronuclei in lymphocytes of different unexposed persons. Analysis of the the factors influencing the DNA distribution of radiation induced micronuclei using flow cytometry in combination with immunofluorescence methods using antibodies (anti-BrdUrd, anti-kinetochore) and DNA-probes against telomeric and centromeric regions of chromosomes.

IV Progress achieved including publications

The induction of micronuclei in cells exposed to ionizing radiation can be used as a measure for both structural and numerical chromosome aberrations. Micronuclei represent genetic material that is lost from the genome during mitosis. Scoring of micronuclei provides therefore a quantitative measurement for the degree of cytogenetic damage in cells and is therefore increasingly used for a dose estimation of humans exposed to ionizing radiation. However, although scoring of micronuclei should be faster than the established chromosome analysis, it cannot yet provide the capacity needed to screen larger human populations. We have therefore developed a new flow cytometric technique for scoring of micronuclei in human lymphocytes using multiparametric flow cytometry, because the high measuring rates of flow cytometers could supply the capacities to screen larger groups of persons exposed to ionizing radiation.

The primary objective of our investigations was the development and application of a new flow cytometric technique to measure the frequency of radiation-induced micronuclei in cell cultures and human lymphocytes. We have previously published a method to obtain a suspension of micronuclei and nuclei for measurements of the DNA content and the frequency of radiation-induced micronuclei in a mouse tumor cell line using flow cytometry (Nüsse and Kramer, Cytometry 5, 20-25, 1984). The advantages of this technique were that a rapid scoring of micronuclei and main nuclei from irradiated cells could be achieved and additionally that DNA distributions of micronuclei could be measured. A disadvantage of this technique is that it cannot always be applied to other cell lines, for example attached growing cells or human lymphocytes due to unspecific debris particles present in the suspension of micronuclei and main nuclei. This debris was found to overlap especially with micronuclei in the low DNA content region so that only these micronuclei with a DNA content larger than about 2 % of the G1-nuclei could be registered. Because of the different amount of debris in various samples the precision of dose effect curves of micronucleus induction can be hampered by this effect. The modified technique deals especially with the discrimination of debris particles from micronuclei so that frequency and DNA distribution of micronuclei could be measured precisely by flow cytometry.

A careful and easy preparation of a suspension of micronuclei and main nuclei needed for flow cytometric measurements of micronuclei was developed using a modification of the two-step method published earlier. The DNA containing particles were stained with the two fluorescent dyes ethidium bromide and Hoechst 33258. The two dyes were excited with two lasers (488 nm and UV) and forward scatter intensity (FSC), ethidium bromide fluorescence (EB^{488}), Hoechst 33258 fluorescence (HO^{360}) and ethidium bromide fluorescence excited by the Hoechst 33258 fluorescence via energy transfer (EB^{HO}) were measured simultaneously in micronuclei, main nuclei and debris particles (Fig. 1).
At first, forward light scatter signals in combination with EB-fluorescence alone were used for a rough discrimination of debris particles showing different light scattering properties compared to micronuclei. This was demonstrated by sorting of the particles indicated in the windows of Fig. 1. With this technique, however, a well defined dose dependence could not be obtained because of different amount of debris in various samples still overlapping the micronuclei in the low fluorescence region (window 4 in Fig. 1).
Even after gating according to forward scatter, the sorted particles in the low EB-fluorescence region were found to contain both debris particles and micronuclei. Therefore, the assay was further developed to discriminate the small unspecific debris particles from micronuclei. For an optimal micronuclei versus debris detection a highly specific staining technique had to be worked out to stain only double stranded DNA (ds-DNA). The topologic properties of ds-DNA in main nuclei or micronuclei can be used to discriminate between DNA binding and unspecific binding. Energy transfer between two dyes is depending on the topologic properties. Binding sites of both donor and acceptor molecule as well as their mutual orientation and distance influence to a high degree the amount of energy transfer. One major prerequisite of energy transfer is the overlap between the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. We have used the two DNA binding dyes Hoechst 33258 and ethidium bromide as donor and acceptor. EB has even more the advantage to be excitable by the 488 nm laser emission of the first laser which allows a precise determination of the total amount of EB in main nuclei and micronuclei not influenced by energy transfer from HO. Cellular debris, which may even bind one or both of the used dyes to a certain amount, may hardly fulfill the necessary spatial conditions to fully confirm to the rules for energy transfer.
Micronuclei show a similar structure compared to main nuclei; they have a nuclear membrane and perform DNA synthesis in phase with the cell nucleus (Kramer et al., 1990). Therefore, the ratio of the different fluorescence signals measured by flow cytometry should be the same in micronuclei and main nuclei. This assumption was used here to discriminate micronuclei from debris particles measured simultaneously in the same fluorescence intensity region. Two ratios of fluorescences, EB^{488}/HO^{360} and EB^{488}/EB^{HO} were calculated for all data and plotted as function of EB^{HO} and HO^{360}, respectively. After a first gating procedure according to the forward scatter for a rough discrimination of debris and micronuclei (Fig. 1), only those particles were considered to be micronuclei that showed the same two ratios of these fluorescence signals compared to the ratios of the nuclei.
Using this new technique the dose effect relationships for radiation-induced micronuclei were analysed for Ehrlich ascites mouse tumor cells growing in suspension and attached growing 3T3 cells (Fig. 2). Both agree quite well with independent measurements by fluorescence microscopy. The courses of these dose responses were cell line specific but independent on the different amount of debris in various samples.
Also the time dependence of the micronucleus induction by ionizing radiation could be measured for these two cell lines with high precision. Since micronuclei are expressed only in cells that have divided at least once in culture, cell cycle

progression after irradiation plays an important role in the analysis of radiation induced micronuclei. In cell cultures most cells will divide at least once after irradiation with lower doses. However, the radiation-induced dose dependent G_2-block has to be considered additionally, if the time dependence of the micronucleus induction is measured. In the cell lines studied here, the frequency of micronuclei has reached a plateau when nearly all cells have divided once. It was therefore unnecessary to correct our data for the presence of undivided cells. This correction is, however, especially necessary, if micronuclei in irradiated human or mouse lymphocytes have to be analysed. Using the cytochalasin B-technique the cell kinetic problems can be solved, if micronuclei are analysed by microscopic observation. With the flow cytometric BrdUrd/Hoechst quenching technique this problem could, however, also be solved for the flow cytometric analysis of radiation-induced micronuclei in human lymphocytes. With this technique, the fraction of cells in the second cell cycle can be measured easily (Fig. 3).

A second question dealing with the size distributions of micronuclei could also be answered using the new technique presented here: Is there a smallest size of radiation-induced micronuclei? The analysis of the data obtained by flow cytometry shows that most of the debris particles are found in the low fluorescence region (« 1% of the DNA content of nuclei). Most of radiation-induced micronuclei have a relative DNA content measured by their fluorescence intensity that is larger than 1% of the DNA content of main nuclei. This agrees well with microscopic observations of the size distribution of micronuclei in mouse bone marrow cells or in human lymphocytes. By image analysis of the size distribution of micronuclei, the smallest micronuclei that can be detected have relative DNA contents of about 0.5% - 1% of the main nucleus. However, the errors of these measurements that are based on measurements of the area of micronuclei are rather large. Since the smallest chromosome of a mouse Ehrlich ascites tumor cell has a DNA content of about 1% of the G1-nucleus as demonstrated by flow karyotyping, it can be concluded that micronuclei could have a smallest size that corresponds with the size of the smallest chromosome in these cells. A radiation-induced acentric fragment could therefore only produce a visible micronucleus if it has a DNA content larger than about 1% of the main nucleus.

With the new flow cytometric technique presented here, the frequencies of radiation-induced micronuclei can easily be measured in cell cultures and human lymphocytes irradiated in vitro. In the case of human lymphocytes the fraction of cells in the first and second cell cycle has to be measured additionally using the flow cytometric BrdUrd/Hoechst quenching technique (Fig. 3). The results agree with microscopic measurements, if the number of micronuclei per main nuclei is calculated and if only those micronuclei are measured that have a DNA content between about 0.5% and about 10% of the nucleus. Larger micronuclei are usually not found after irradiation, they could, however, be induced by chemicals that interfere with the spindle apparatus. In this case, the flow cytometric technique will give results that do not agree with microscopic observation. This effect will be studied in the next period of the project.

Schreiber, G.A., Beisker, W., Bauchinger, M., Nüsse, M.: Multiparametric flow cytometric analysis of radiation induced micronuclei in mammalian cell cultures. Cytometry, submitted 1991.

Kramer, J., Schaich-Walch, G., Nüsse, M.: DNA synthesis in radiation induced micronuclei studied by bromodeoxyuridine (BrdUrd) labelling and anti-BrdUrd antibodies. Mutagenesis 5, 1990, 491-495.

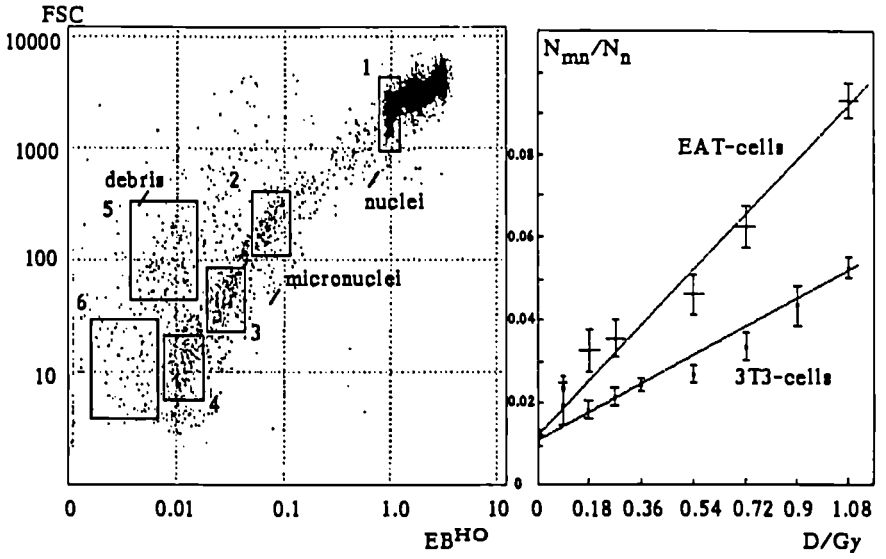


Fig. 1: Flow cytometric measurement (FSC/EB^{HO}) of a suspension of micronuclei and nuclei showing the position of G1-phase nuclei (1), micronuclei (2,3,4) and debris particles (5,6) as verified by sorting.

Fig. 2: Fraction of micronuclei N_{mn} per nuclei N_n, N_{mn}/N_n, as function of dose for irradiated Ehrlich ascites tumor cells and 3T3-cells.

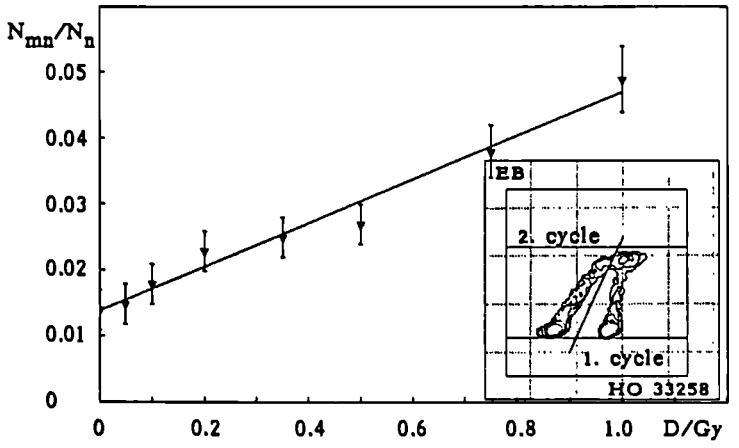


Fig. 3: N_{mn}/N_n as function of dose for human lymphocytes irradiated in vitro. The insert shows a EB/HO 33258 distribution of lymphocytes treated with BrdUrd for 55 h. From such measurements the fraction of cells in the 2. cell cycle is calculated.

Head of Project 4: Prof. Bauchinger

II Objectives for the reporting period

1. Dose-response experiments for micronuclei induced by sparsely and densely ionizing radiations in human lymphocytes.
2. Analysis of intra- and inter-individual variation of background and induced micronucleus frequencies.

III Objectives for next period

1. Based on results in II/2 representative calibration curves will be prepared.
2. Analysis of the content of micronuclei by means of nonradioactive in situ hybridization with satelite DNA probes.

IV Progress achieved including publications

Compared to conventional dicentric analysis, scoring of micronuclei (MN) is a faster and easier approach of measuring radiation-induced chromosome damage in human lymphocytes. It has therefore been suggested as an alternative biological dosimeter system. Provided an automated MN scoring e.g. by flow cytometry can be performed with a similar precision as microscopic scoring, this would enable to apply the system even on larger populations. For comparison of data sets derived with flow cytometry the present experiments were carried out to establish dose effect curves for MN induced by different radiation qualities.

Lymphocyte suspensions obtained from freshly drawn whole blood were exposed to ^{60}Co Y-rays (dose rate 0.3 Gy.min^{-1}), 220 kV X-rays (dose rate 0.5 Gy.min^{-1}) and fission neutrons (converter neutron beam, dose rate 0.23 Gy.min^{-1}). To account for cell proliferation kinetics, cytochalasin B (3 ug/ml) was added to the lymphocyte cultures after 44 h to block cyto-kinesis (Fenich and Morley 1985). After a total culture time of 68 h, hypotonic treatment, fixation, cell preparation and staining were carried out according to our standard protocol (Huber et al. 1989). Exclusively cytokinesis blocked (CB) binucleate cells were scored. The dose effect curves comprise 16.000 CB cells from two donors for ^{60}Co Y-rays, 8.000 CB cells from one donor for X-rays and 8.000 CB cells from one donor for neutrons. An iteratively weighted least squares method was applied for curve fitting. Each observation was weighted by the number of analysed cells and the inverse yield Y, assuming that the intercellular variance σ_Y^2 of the number of MN is proportional to Y, i.e. $\sigma_Y^2 = d.Y$ with a mean dispersion index d slightly greater than 1. No specific dose dependence of the dispersion index d was

recognizable. MN data of lymphocytes exposed to ^{60}Co Y-rays and 220 kV X-rays fitted the linear-quadratic model $Y= c + aD+ bD^2$. The dose response curves are shown in fig. 1 and 2, the corresponding estimated curve parameters are given in the respective legends. The dose response curve for neutrons is shown in fig. 3, the corresponding curve parameters are presented in the legend. The curve reveals a decreasing slope with increasing dose. In the linear-quadratic model the quadratic term was negative. Already the preliminary results of the present dose-response expements show that they are in line with basic radiobiophysical expectations on the effectiveness of different radiation qualities.

Additionally to the dose-response study, intra- and interindividual variations of background and radiation-induced MN frequencies were analysed. For this purpose venous blood was taken from 4 donors aged between 26 - 51 years in three-monthly intervals during one year (5 examination stages).

After preparing a lymphocyte suspension, one half was exposed to 3 Gy of ^{137}Cs Y-rays, the other half remained as control. Cell culture and preparation conditions were performed as described before. 800-1000 CB cells per donor were analysed at each examination stage from control (fig. 4a) and irradiated samples (fig. 4b). A significant overall variation of MN frequencies was found among the 20 control samples as well as among the 20 exposed samples. Significant intra-individual variations revealed for background MN levels of donor A (5-fold) and for induced MN levels of donors A, C, D (2-fold). When the 5 observed MN frequencies were averaged, a significant inter-individual variation between the averaged frequencies of the 4 donors revealed only for background MN levels. Provided that the CB MN assay in human lymphocytes is used as a biological dosimetry system to quantify radiation exposure, the results of our serial examinations of different donors should be taken into account. To reduce the influence of intra- and interindividual variations of induced MN frequencies, a standard dose-effect curve should be based upon data of 3 5 donors. Due to the considerable variation of background MN frequencies observed in samples from a single donor at different examination stages, it seems difficult to derive a reliable individual low-dose estimate below 0.5 Gy. A statistical analysis shows that such an estimate cannot be substantially improved by scoring a cell number in excess of 500.

Legends to the figures

Fig. 1.
Dose response curve for ^{60}Co Y-rays

$(Y = 1.6 \times 10^{-2} + 5.9 \times 10^{-2} D\ Gy^{-1} + 3.4 \times 10^{-2} D^2\ Gy^{-2})$

Fig.2.
Dose response curve for 220 kV X-rays

$(Y = 2.2 \times 10^{-2} + 2.0 \times 10^{-1} D\ Gy^{-1} + 3.9 \times 10^{-2} D^2\ Gy^{-2})$

Fig.3.
Dose response curve for fission neutrons

$(Y = 1.9 \times 10^{-2} + 5.9 \times 10^{-1} D\ Gy^{-1} - 5.9 \times 10^{-2} D^2\ Gy^{-2})$

Fig.4a.
Diagram of micronucleus frequencies in control samples from 4 donors A-D (age and sex are given in brackets) analysed at examination stages I-V. (Time interval between successive stages: three months). Error bars represent s.e.m. of micronucleus frequency per CB cell. Broken lines are group means. Data arranged by donors.

Fig.4b.
Diagram of micronucleus frequencies in irradiated samples from 4 donors A-D (age and sex are given in brackets). Data arranged by donors. For explication see legend to fig. 1a.

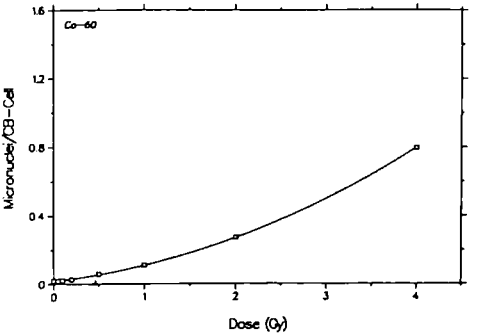


Fig.1.

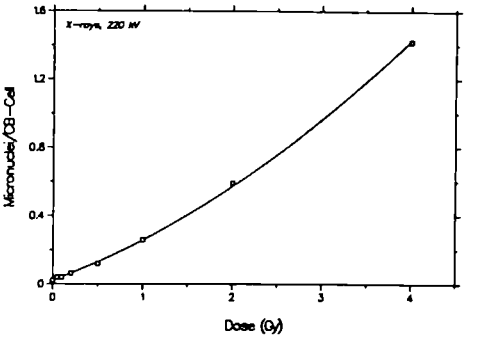


Fig.2.

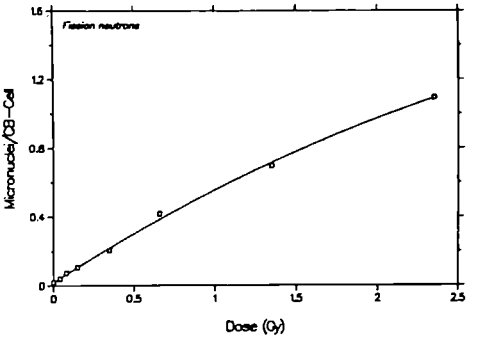


Fig.3.

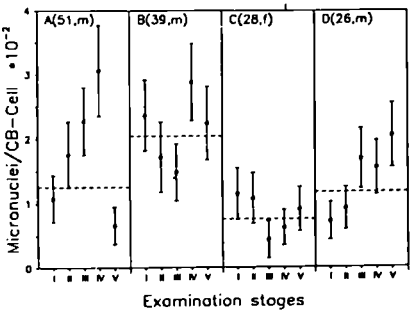


Fig.4a.

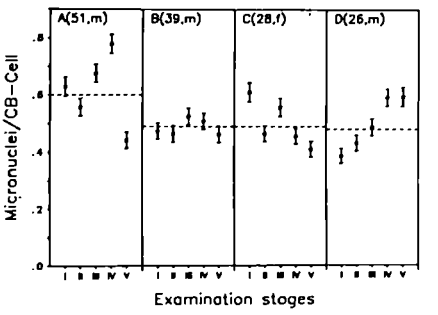


Fig.4b.

Head of Project 5: M. Aubele

II Ojectives for the reporting period

Establishment of dose-effect-curves for CTA in vitro exposed human lymphocytes. The experiment shall also elucidate the role of varying chromatin texture during the cell cycle on damage expression and compare effect efficiency with the chromosomal and micro nucleus test. In the reporting period we have concentrated on preparatory protocols and the investigation of conceptional relations of chromatin texture features on size and staining density of nuclei.

III Objectives for next period

Pilot studies on the establishment of dose-effect-curves for CTA in whole body exposed mice have already been performed. The experiments were hampered by unexpected preparatory difficulties. Harvesting of sufficiently large blood samples from living mice is nearly impossible. Animal experiments with the necessary numbers of mice to cover a large range of doses and latency times for damage expression can no longer be performed. Point 2 of the original objectives have therefore likely to be abandoned. The investigations for the next period will concentrate on the validation and refinement of CTA in in vitro exposed lymphocytes.

IV Progress achieved including publications

Introduction:

For further evaluation of the role of chromatin pattern features in cell nuclei for monitoring radiation exposure we have concentrated in the past year on in vitro irradiation of cultured and PHA-stimulated human lymphocytes.

Although the final goal of the project will be the direct analysis of nonstimulated lymphocytes from the peripheral blood of exposed species, we expect more information on the ongoing process in cycling cells.

The recent investigations demonstrated the need for carefully worked out preparation protocols. Measurements for quality control concerned primarily DNA-distributions. Fig. 1 shows typical examples.

Material and Methods:

From human blood samples mononuclear cells were separated by density gradient centrifugation and resuspended in culture medium containing PHA. After 30 min. they were irradiated by Cs-137 gamma rays with 0, 0.25, 0.5, 0.75 and 1.0 Gy. Cytological specimens were prepared after 5, 24, 48, 72, 96, 120 and 144 hours by cyto-centrifugation, fixation and Feulgen-Schiff-staining. The measurements were done by high resolution image cytometry, 100 x objective with oil

immersion (digital pixel size 0.25 micron). 200 well preserved cells were measured arbitrarily on each specimen.

Results:

The results reported were derived from one single experiment with blood from one human donor and do therefore, not claim representativity.

1) Cellular growth

The rationale is that stimulation into proliferation as well as cell cycling may be influenced by the exposure. To investigate the first effect we have pooled all cells after 5 h, when we do not yet expect G_1-cells and also all 'diploids' after 120 and 144 hours, when the vast majority should be in the G_1-phase, reclassified them by multivariate discriminant analysis and used the such trained classifier for all the diploid cells of the remaining time intervals. From this the $G_0/(G_0+G_1)$ ratios are derived as a function of time and dose. Fig. 2 shows the results for all culturing times. There seems to be a somehow enhanced G_0-G_1 transfer at intermediate times (48 h) and a retardation of the G_0-depletion at higher times.
The influence on cell cycling is derived from the total DNA-distributions (fig. 1), cleaned from G_0-cells. By conventional Gauss-fitting of G_1 and G_2-peaks we assessed G_1, S-phase, G_2 and > 5c cellular contributions. Fig. 3 shows the dose dependence of these contributions for DNA-distributions pooled from 96 to 144 hours. There seems to be a slight but significant G_2-blocking accompanied by a decrease of the S-phase fraction. All dose effects on the proliferation behaviour are not very distinct.

2) Chromatin assay in G_1-cells

Only G_1-cells have been compared as a function of time and dose by multivariate discriminant analysis. In this case all potential feature dependencies on DNA-content, stain density or nuclear size and hence on changing cell cycle contributions are avoided. The rationale is that the chromatin distribution is influenced during RNA-synthesis by the simultaneous encymatic repair processes occurring after radiation exposure. Fig. 4 shows two multivariate measures for the dose dependence after 72 hours and a multiple regression curve after 48 hours. The curves have been normalized to the same linear regression function (in arbitrary units). (M_1: First canonical variable, M_2: Sum of Mahalanobis distances in between classes in the feature space, M_3: Multiple regression)

Summary:

Human PHA-stimulated lymphocytes show changes in growth kinetics, in the occurence of highly aneuploid cell components and in the chromatin texture (CT) of G_1-cells as a function of radiation dose. Further methodological work is necessary to validate the findings and increase the sensitivity of the CT assay.

Fig. 1a: DNA distributions for 0.75 Gy from 24 to 120 hours.
Fig. 1b: DNA distributions at 72 hours for all doses (G_0 dotted distributions, proliferating cells solid distributions, each normalized to 100 %)

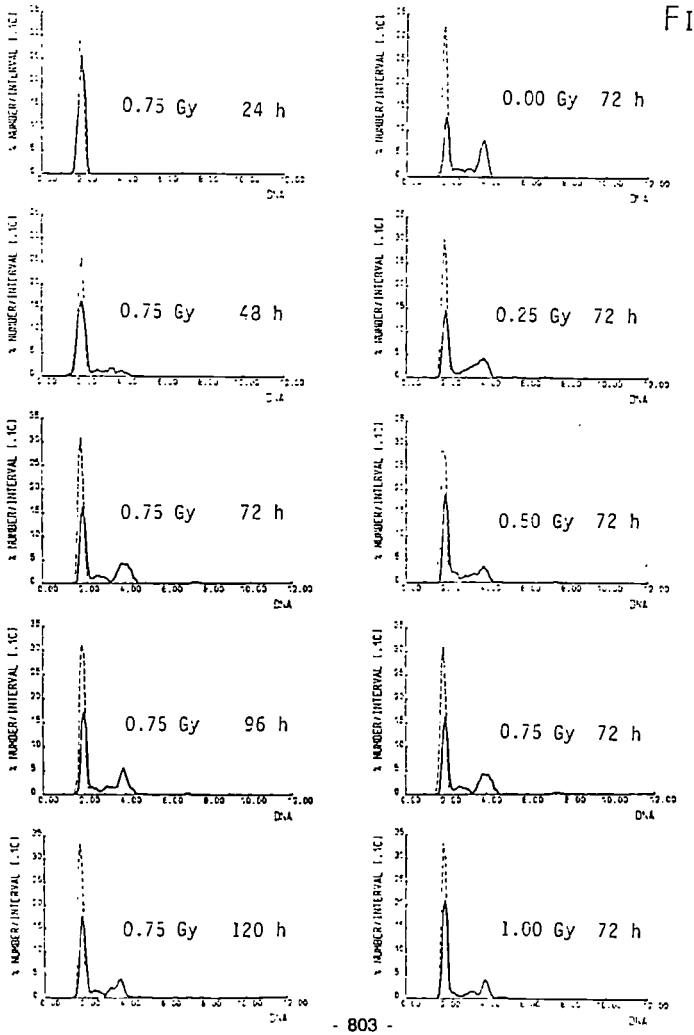
Fig. 2: $G_0/(G_0+G_1)$ fractions as a function of dose for all seven culturing times

Fig. 3: Cell cycle phase fractions as a function of dose for high culturing times (> 96 hours)

Fig. 4: Several multivariate measures derived from five class linear discriminant analysis and from multiple regression analysis for the CTA at 72 and 48 hours as a function of dose

M_1 First canonical variable (A.U.)
M_2 Sum of Mahalanobis distances in between classes in the feature space (A.U)
M_3 Multiple correlation (A.U.) after 48h incubation

FIGURE 1



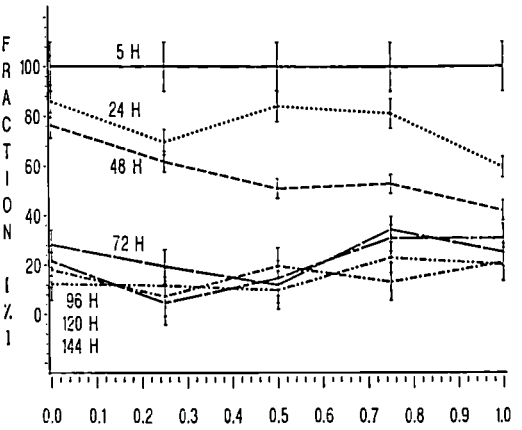


FIGURE 2

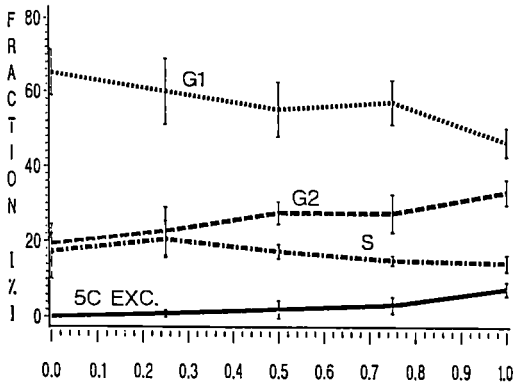


FIGURE 3

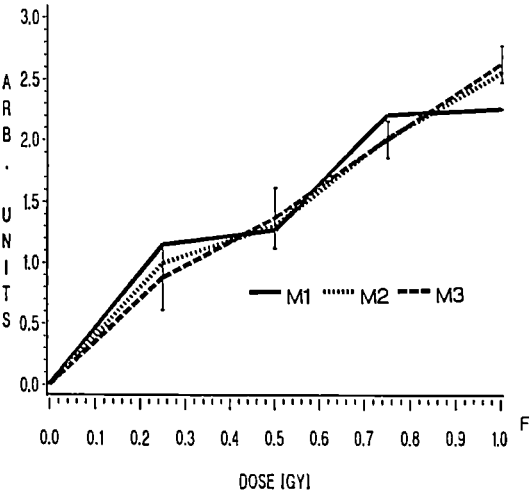


FIGURE 4

Progress Report

Contract:
Bi7-039 Sector: B13
Title: Studies on basic and applied aspects of radiation-induced chromosomal aberrations in human cells

1	Natarajan	Univ.Leiden Sylvius Lab.
2	Savage	MRC Radiobiological Unit
3	Olivieri	Università di Roma "La Sapienza"
4	Cortés-Benavides	Univ. Sevilla
5	Bryant	Univ. St. Andrews
6	Ahnström	Univ. Stockholm
7	Baverstam	Nat. Inst. of Rad. Protection
8	Feinendegen	KFA Julich GmbH
9	Johanson	Univ. Uppsala Agricult.Sciences
10	Ehrenberg	Univ.Stockholm
11	Palitti	Univ.degli Studi della Tuscia

I. Summary of Project and Global Objectives

Chromosomal aberration is considered to be as one of the most important biological effects arising as the consequence of exposure to ionizing radiation in man. The project aims at clarifying the mechanisms of chromosome aberration formation at different cell cycle stages following irradiation at different conditions in mammalian cells including man. Though DNA double strand breaks (DSBs) are considered to be the probable DNA lesion leading to radiation induced chromosomal aberrations, the factors such as the exact repair mechanisms that operate and the influence of cell cycle on the type and frequency of aberrations are not well understood. Techniques such as premature chromosome condensation (PCC) and chromosome painting (in situ hybridization with chromosome specific DNA libraries) are used in this project to study the kinetics of formation and frequencies of exchanges respectively. The mechanisms behind the phenomenon of adaptive response (the resistance of cells which have been adapted with a low dose of radiation to a further challenging dose) have to be clarified. Attempts will be made to do this using diverse biochemical and cytological approaches. The formation of radiation induced chromosomal aberrations in G_2 stage of cell cycle is being studied in mammalian cells using different classes of DNA repair inhibitors in order to understand the influence of various repair enzymes on the induced frequency of aberrations. Attention is also paid to the problem of low dose and dose rate effects by choosing appropriate in vivo and in vitro models.

I Head of Project 1: Prof. Natarajan

II Objectives for the reporting period

a. Determination of the frequencies of chromosomal translocations induced by different doses of X-rays in human peripheral blood lymphocytes using chromosome painting technique and comparison with the frequencies of dicentrics scored by conventional staining procedures.
b. Verification of the concept that increased G_2 radiosensitivity is an indicator of cancer proneness of the individual.

III Objectives for the next period

a. Determination of the frequencies of chromosomal translocations in human peripheral blood lymphocytes following low doses and dose rates of X-rays.
b. Generation of dose response curves for induction of translocations following fast neutron irradiation (1 MeV) of human lymphocytes and comparison with yield of dicentrics.
c. Standardization of electroporation technique to introduce different enzymes into irradiated human lymphocytes.

IV Progress achieved including publications

a. Human lymphocytes were irradiated with different doses of X-rays (150 kV, 6 mA) and the first division metaphases were either stained with Giemsa for scoring dicentrics or painted with chromosome specific probes. For the latter, specific DNA libraries for chromosomes # 1, 3, X, 2, 4 and 8 were biotinylated by nick translation and hybridized in situ to chromosome preparations. The hybridized chromosomes were recognized by avidin-FITC under a fluorescent microscope. Dicentric frequencies as well as translocation frequencies were determined. For calculating the frequencies of translocations for the whole genome, the frequencies obtained for the six chromosomes studied were multiplied by a factor of 2.5, this factor being based on the DNA content of these chromosomes. The frequencies of translocations were found to be about three times more than the frequencies (Fig. 1). This increased frequency of translocations in comparison to the values reported in literature (using G banding technique) appears to be due to the higher resolving power of the painting technique in detecting small translocations.
b. We have studied the G_2 radiosensitivity of skin fibroblasts derived from cancer patients with various types of malignancy as well as xeroderma pigmentosum, ataxia telangiectasia (both homo- and heterozygotes). In these experiments, the frequencies of chromatid type of aberrations induced by 0.75 Gy of X-rays given in G_2 stage of the cell cycle were determined. The results obtained so far indicate that the cells derived from all cancer patients or cancer prone individuals do not respond with increased frequencies of aberrations in comparison to controls. In our hands, this technique does not give reproducible results.

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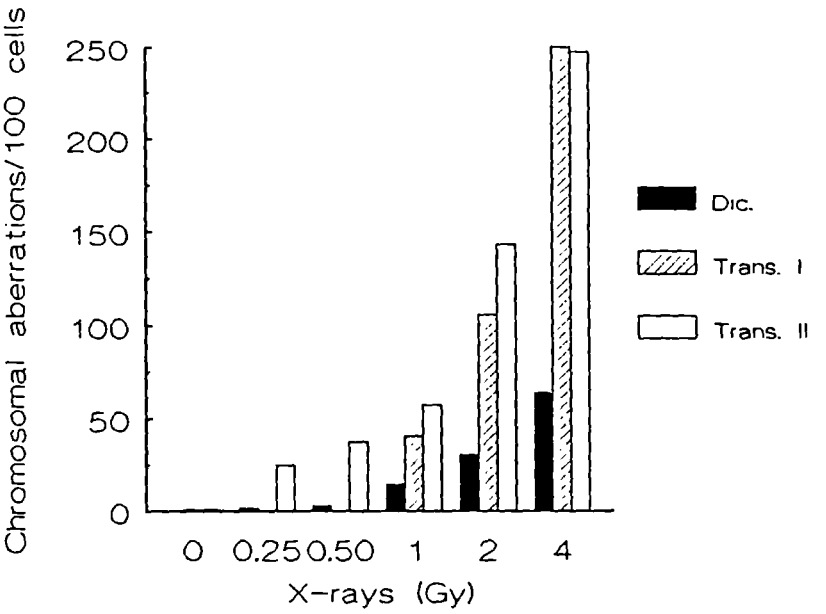

Fig. 1

Dose response for induction of dicentrics (Dic.) and translocations (Trans.) by X-rays. Dicentrics data pooled from 4 experiments, translocation data from 2 experiments presented separately (I & II).

I Head of Project 2:Dr.Savage

II Objectives for the reporting period

To develop and test BrdU-pulse cell labelling methods for applying to studies of adaptive response and other allied phenomena which rely on the scoring of chromatid-type aberrations for their demonstration and verification.

III Objectives for the next reporting period

To investigate, with the aid of the RbdU cell-marking techniques developed, the radiation sensitivity variation for the production of the various categories of chromatid-type aberrations as cells transit S and G_2 phases of the cell cycle. Also to consider the effects which mitotic delay and perturbation have upon the observed yield-time curves.
Such studies have an important bearing upon the interpretation of observed quantitative aberration frequencies which are used to establish such phenomena as adaptive response.

IV Progress achieved including publications

Introduction

Chromatid-type aberrations are a widely used end-point in radiobiological and environmental toxicology studies. They are the dominant structural change found when actively dividing cell populations are exposed to radiation and the only primary for produced by the vast majority of chemical clastogens.

However, their quantification presents considerable problems since the sensitivity to production varies with cycle transit so that no cell system provides a unique yield for a given "dose". The quantitative uncertainty is exacerbated by the fact that observed frequencies are markedly influenced by cell kinetic perturbations - perturbations that inevitably accompany any clastogenic treatment. Thus, simple relationships between dose and yield are severed and a change in frequency following a given treatment can be as much artefactual as real.

This presents very considerable uncertainties when one wishes to use this kind of aberration to verify such phenomena as, for example, adaptive response. With normal cytological procedures, there is no way of guaranteeing that two samples for comparison, obtained after different treatments, actually contain the same mixture of cells to render such comparison valid (1).

In this report, a method is described which goes some way to overcoming these problems. It allows one to identify at the time of metaphase scoring, where, in interphase, the cell was at the time of treatment. Applied to an adaptive response protocol, one can assign the location of a cell at the time of primer (adapting) dose and at the time of challenge dose. Aberrations can then be expressed "per classified cell" and the composition of any cell mixture that is used for comparison monitored, or controlled.

Rationale

If BrdU (5-10 μg/ml) is added to an actively dividing asynchronous culture, cells which are in S-phase begin to incorporate bromouracil into newly synthesized chromatin. Thus, cells from S arriving in metaphase contain two kinds of chromatin; unsubstituted TT chromatin, made early before the BrdU arrived, and substituted TB chromatin, made after. These two types are readily distinguishable by appropriate staining and the chromosomes take on a banded appearance. The programme of replication is so precise, that the presence of specific replication bands can be used to delimit borders within S and the position of a cell objectively assigned, irrespective of kinetic perturbation (2).

A similar method can be applied using late replicating bands. Here the BrdU is given as a pulse for about 6-7 h and then the medium replaced by BrdU-free (sometimes thymidine supplemented) medium. Now, late-replicating bands are TT and the changing pattern, as S-phase progresses, can be used similarly to delimit borders and assign cell positions (3).

We have combined both methods. As applied to an adaptive-response protocol, BrdU is added immediately after the primes is given and the early bands produced identify the position of a cell at that time. Immediately after the challenge dose, replacement with BrdU-free medium takes place and the cell-position at this time can now be assigned by the pattern of late-replicating bands.

Cells coming to metaphase, and stained for replication-bands, carry a variety of patterns. These, and their interpretation in terms of cell position are given in Table I. It should be emphasized that the patterns in no way interfere with the scoring of structural changes. In addition to assigning position, if a multi-sampling time regime is used, some information about cell kinetic perturbations can be obtained.

Following the completion of scoring, aberration yields can be computed for cells that were at specific developmental stages when a treatment was given and between-treatment comparisons confined to these defined cohorts.

Application

We have tested this method on a typical adaptive response protocol in stimulated human lymphocytes, using 250 kV X-rays for both primer and challenge. BrdU was added after a primer of 1 cGy and removed 6 h later following a challenge dose of 1.5 Gy. Cells were sampled 3, 6 and 9 h after challenge.

Using total aberrations and disregarding cell classification, some evidence of an adaptive response was present at 6 h but not at 9 h.

Using detailed cell classification, we found

(a) that the primer did not introduce any measurable mitotic perturbation into the population when given alone, nor did it produce any extra perturbation when given prior to challenge;
(b) that at 6 h, the observed adaptive frequency reduction was confined to cells in pre-S at the time of primer;
(c) when cells at defined stages for either primer or challenge dose were summed for all sample times, all evidence for any adaptive depression in frequency disappeared.

The method provides a large amount of information and offers several different way of analysing the data. It should help, in time, to sort

out some of the anomalies in this interesting phenomenon, some of which undoubtedly arise from the kinetic complexities of the stimulated lymphocyte population.

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Table I Replication-band staining patterns resulting from a 6 h BrdU pulse

Chromosome Staining Reaction	Interpretation: Cell Position at Start of Pulse	Interpretation: Cell Position at Finish of Pulse	Remarks
Uniform dark	pre-S or G_2	pre-S G_2	} No BrdU } uptake
Early R-zone bands	early-S	G_2	
Late R-zone (merging) bands	late-S	G_2	
Full G-zone (merging)	pre-S	early-mid S	
Late G-zone (merging)	pre-S	late-S	
Mixed pattern - early R-zone + late G-zone	early-S	late-S	BrdU pulse < S-duration
Uniform pale	pre-S	post-S	BrdU pulse > S-duration

NB The terms "early-", "mid-", "late-" S are used here for simplicity. In practice, precise, objective sub-phases are actually defined by specific band patterns (see Refs. 2 & 4).

I Head of Project 3: Olivieri

II Objectives for the reporting period

Lymphocytes exposed to low doses of radiation first followed by a challenging higher dose in G2 have been found to have lower yield of aberrations in comparison to the expected frequency. This so-called adaptive response is found to vary between individuals, and to show "cross-adaptativity" with various types of clastogenic lesions induced by subsequent exposure to chemical mutagens. The objectives of our research were to study: a) the causes of this variability and b) the characteristics of "cross-adaptivity".

III Objectives for the next period

In the next period we will focus our research on the influence of growth conditions on the expression of the adaptive response in human lymphocytes.

IV Progress achieved including publications

a) Experiments were carried out using cultures of blood from donors which, in previous experiments (Bosi and Olivieri, 1989), had displayed an adaptive response or not. In the present report AR + will be used to indicate donors having displayed an adaptive response and AR- those that have not. Whole blood (0.5 ml) was added to 4.5 ml of RPMI 1640 medium without fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2% phytohemagglutinin M (Gibco). The experiments consisted first of exposing cultured human lymphocytes to adaptating treatments and subsequently challenging the cells with high doses of X-ray. The cells were scored to see whether the prior exposure reduced the number of chromatid and isochromatid breaks induced by the challenging doses. In all the experiments the conditioning pretreatment consisted of 0.02 Gy of X-rays administered 26 or 30 h after stimulation with PHA. The cells were subsequently challenged with 0.75 or 0.40 Gy of X-rays and fixed 2 h later or 1.5 Gy of X-rays followed by fixing 6 h later. The challenge treatment was carried out after 72, 50 or 54 h. In other experiments, several compounds, whose effect on the adaptive response was to be studied, were added to some of the cultures. The following substances were used: adrenal cortex extracts (Maxicortex "2000" by "Manetti e Roberts"); insulin (Actrapid HM by Novo Farm.); thymus extracts (Leucotrofina by Ellem); hydrocortisone (Sigma); interleukin-2 and interferon.gamma (Boehringer). Irradiation was carried out with 200 kVp X-rays (Gilardoni LGL 200/8D, 0.2 mm Cu added filtration, 8 mA, 0.60 Gy/min). Two hours before fixation 0.1 ml of colcemid (final concentration 2 x 10^{-7} M) was added to each culture, and fixation was performed according to standard cytological procedures; for each point examined two parallel cultures were set up.

Overall the results of the experiments described here indicate that the variability of AR found in various donors is not linked to their genetic constitution but depends on some transient physiological parameters. Two donors, who were AR + in previous

experiments, displayed no adaptive response. On the other hand, two AR-donors displayed a clear-cut AR.

Furthermore, during the experiments, cases were observed in which, under standard culture conditions, the donor displayed no AR, and yet an AR could be evidenced by modifying the culture conditions. Therefore, our evidence seems to point to the great importance for AR of the metabolic state of cells during conditioning.

b) When cells pretreated with low doses of X-rays are challenged with the alkylating agent methyl methanesulfonate (MMS) a synergistic response of increased damage occurs. Because MMS is an alkylating agent with somewhat unusual properties that produces a different spectrum of methylated bases in DNA than do other methylating agents, attempts have been made to see if the synergism observed with MMS represents a general phenomenon or is specific to MMS. It was found that prior exposure of the cells to X-rays reduced the number of chromosomal breaks induced by N-methyl-N-nitro-N-nitrosoguanidine (MNNG) but, as observed previously, had the opposite effect on breaks induced by MMS. It appears that the repair mechanism induced by low doses of X-rays affects the chromosomal lesions induced by the two alkylating agents differently. Because cells pre-exposed to 1 cGy exhibit completely different responses to MNNG and MMS given as a challenge treatment, experiments were carried out to test whether low concentrations of these alkylating agents themselves can elicit an adaptive response that produces resistance to the induction of chromosomal damage by the same or different DNA-damaging agents. Human lymphocytes pretreated with various concentrations of MMS or MNNG were subsequently challenged with MMS, MNNG, or X-rays. When cells pretreated with MNNG were challenged with either MMS, a synergistic response of increased damage was observed. This synergism even occurred if the cells were pretreated with MMS and then challenged with various concentrations of MMS. Pretreatment of the cells with low doses of MMS, however, led to a reduction in the number of aberrations when the cells were challenged with either 1.5 Gy of X-rays or a high dose of MNNG. These experiments show that DNA damage induced by methylating agents is able to induce an adaptive response that makes the cells resistant to induction of aberrations by subsequent exposure to alkylating agents such as MNNG or to X-rays. On the other hand, irrespective of the agent used to induce the adaptive response, cells challenged with MMS show a syngergistic increase in the number of chromatid breaks, indicating that the induced repair system cannot affect certain types of lesions

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I Head of Project 4: Dr.Cortés-Benavides

II Objectives for the reporting period

Peroxides yield transient radical species that can damage DNA. Such oxygen species are also generated by ionizing radiation and are responsible of atleast part of its mutagenic and lethal effects. It is of interest to carry out experiments using human lymphocytes from different donors in order to analyze the possible protective effect of pretreatments with low doses of hydrogen peroxide on chromosome damage induced by a challenge treatment with either the true radiomimetic compound Bleomycin which, like X-rays, induces double strand breaks in DNA (Povirk et al., 1977), or acute doses of X-rays given 24 h after the conditioning treatment with hydrogen peroxide.

III Objectives for next period

Our goal for the next period will be to carry our experiments using a lower X-ray dose (1.5 Gy) after conditioning with H_2O_2 and analyze the frequency of micronuclei in CYB-treated cells from the two donors. This X-ray dose has been the more commonly employed in previous reports and adaptive response to radiation damage (Shadley and Wolff, 1987; Wolff et al., 1989) observed as chromosomal aberrations in metaphase. On the other hand, a recovery time of 12 h instead of 20 h in the presence of CYB after acute X-rays treatment will be assayed in order to analyze the adaptive response to compare the results for two fixation times.

IV Progress achieved including publications

1. Methodology

The experiments consisted of a pre-exposure of cultured human lymphocytes to "adapting" doses of H_2O_2 and a subsequent challenging of the cells with either the antibiotic Bleomycin (BLM) or X-rays.

Whole blood (0.5 ml) from three healthy donors was added to 4.5 ml of RPMI 1640 medium containing 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 2% phytohemagglutinin (PHA), to stimulate G0 lymphocytes. The peroxide treatment was always given as a single 30 min. pulse administered 24 h after setting up the cultures and the challenge with BLM or X-rays was 24 h later. In the BLM experiments cells were harvested 6 h after treatment, including the last 3 h in colcemid to analyze chromosomal aberrations in metaphase. On the other hand, cytokinesis arrest was induced by a treatment with cytochalasin B (CYB) given immediately after X-ray exposure to analyze the appearance of micronuclei in binucleate cells (Fenech and Morley, 1985).

2. Results

2.1 Effect of pretreatment with H_2O_2 on the yield of chromosomal aberrations induced by BLM.

Table 1 shows the total frequencies of chromatid and isochromatid breaks observed after conditioning treatment with three doses of H_2O_2, challenge treatment with 0.015 units/ml BLM until fixation, and consecutive conditioning and challenge treatments in 2 donors. As can be seen, no reduction in the yield of chromosomal aberrations indu_ed

by BLM was observed when the lymphocytes were "conditioned" previously with H_2O_2 (lack of adaptive response). Instead, the number of breaks observed after the combined treatments (H_2O_2 + BLM) was significantly higher than expected (one-tailed t-test). This synergism so contrast with our previous results on an "adaptive response" found when analyzing the frequency of chromosomal aberrations in lymphocytes conditioned with H_2O_2 and irradiated with X-rays later on (Cortes et al., 1990). In our opinion, these contrasting results seem to point out at the existence of different cell processes dealing with the repair or damage induced by BLM or X-rays after conditioning with H_2O_2.

2.2 Frequency of micronuclei in cells conditioned with H_2O_2 before irradiation with an acute dose of X-rays.

Human lymphocytes were first given a pulse with different doses of H_2O_2 and, 24 h later challenged with a dose of 3.0 Gy of X-rays. Immediately after X-ray exposure, 6 230g/ml CYB was added and the cells harvested 20 h later. This concentration of CYB was chosen since it has been reported that more than 90% of the cells are efficiently blocked in their cytokinesis, while for lower doses many cells escape the block (Littlefield et al., 1989).

As can be seen in Table 2, both donors showed a reduction in the frequency of micronuclei observed after the combined treatment (H_2O_2 + X-rays).

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Table 1.- Effects of a single 30 min pulse with different concentration of H_2O_2 on the frequency of chromatid aberrations induced in human lymphocytes challenged with 0.015 units/ml of Bleomycin (BLM).

Donor	Conditioning pretreatment H_2O_2	Challenge treatment BLM	Number of cells scored	Chromatid and Isochromatid breaks observed N°	observed %	expected[a] %
A	none	none	200	2	1	-
	2.5×10^{-5} M	none	200	2	1	-
	7.5×10^{-5} M	none	200	3	1.5	-
	2.5×10^{-4} M	none	200	2	1	-
	none	BLM	200	102	51	-
	2.5×10^{-5} M	BLM	200	146	73	51[b]
	7.5×10^{-5} M	BLM	200	131	65.5	51.5[b]
	2.5×10^{-4} M	BLM	182	112	61.5	51[b]
B	none	none	200	5	2.5	-
	2.5×10^{-5} M	none	200	7	3.5	-
	7.5×10^{-5} M	none	200	9	4.5	-
	2.5×10^{-4} M	none	200	12	6	-
	none	BLM	178	98	55.1	-
	2.5×10^{-5} M	BLM	200	203	101.5	56.1[b]
	7.5×10^{-5} M	BLM	200	189	94.5	57.1[b]
	2.5×10^{-4} M	BLM	Very few mitoses			

a. Sum of the two individual treatments minus the control.
b. Observed frequency significantly higher than expected ($P<0.01$) (one-tailed t-test)

Table 2.- Effects of a single pulse with H_2O_2 before irradiation with 3.0 Gy of X-rays on the frequency of micronuclei in binucleate cells induced by Cytochalasin B.

Donor	Conditioning pretreatment H_2O_2	Challenge treatment X-rays	Number of binucleate cells scored	Micronuclei observed N'	Micronuclei observed 0/00	Micronuclei expected[a] 0/00
A	none	none	2000	17	8.5	-
	2.5×10^{-5} M	none	2000	34	17	-
	7.5×10^{-5} M	none	2000	23	11.5	-
	2.5×10^{-4} M	none	2000	22	11	-
	none	3.0 Gy	2000	383	191.5	-
	2.5×10^{-5} M	3.0 Gy	2000	323	161.5	200[b]
	7.5×10^{-5} M	3.0 Gy	2000	353	176.5	194.5[c]
	2.5×10^{-4} M	3.0 Gy	2000	324	162	194[b]
C	none	none	2000	17	8.5	-
	2.5×10^{-5} M	none	2000	23	11.5	-
	7.5×10^{-5} M	none	2000	24	12	-
	2.5×10^{-4} M	none	2000	19	9.5	-
	none	3.0 Gy	2000	335	167.5	-
	2.5×10^{-5} M	3.0 Gy	2000	318	159	170.5[d]
	7.5×10^{-5} M	3.0 Gy	1453	327	162.4	171[n.s.]
	2.5×10^{-4} M	3.0 Gy	2000	297	148.5	168.5[b]

a. Sum of the two individual treatments minus the control.
b,c and d. Observed frequency significantly lower than expected (one-tailed t-test).
b. ($P < 0.01$); c. ($P < 0.05$); d. ($P < 0.1$)
n.s. Observed frequency not significantly lower than expected.

I Head of Project 5: Dr.Bryant

II Objectives for the reporting period

Manipulation of the G2 phase of Chinese hamster ovary cells by lowering temperature to lengthen the time window during which the kinetics of disappearance of chromatid breaks can be studied. To measure the kinetics of G2 chromatid aberrations in radiation sensitive mutant rodent and human lines and their normal counterparts. Use of cell and nuclear extracts of normal cells to correct defects in radiosensitive mutant cell lines that show high frequencies of X-ray induced chromatid breaks. Study effects on DNA double-strand break repair using neutral filter elution.

III Objectives for next period

Manipulation of the chromosomal response of mutant radiosensitive Chinese hamster (Xrs) and human ataxia telangiectasia and their normal counterparts using cell free extracts. Measurement of kinetics of repair of DNA double-strand breaks in radiosensitive cell mutants and their normal counterparts following X-irradiation and treatment with cell free extracts.

IV Progress achieved including publications

We have recently shown (McLeod et al., 1990a) that when Chinese hamster ovary cells are incubated at lowered temperatures (33°C or 29°C) for short periods (transient hypothermia) the G2 phase is lengthened, up to more than twofold, so extending the range of time over which repair of rejoining of chromatid breaks can be ovserved. This allows the establishment of more accurate kinetics of chromatid break rejoining than was previously possible. Using this system we showed that the rate of rejoining of chromatid breaks in CHO cells was similar at 37°C, 33°C and 29°C (MacLeod and Bryant, 199b). We found also that the rate of rejoining of chromatid breaks in xrs 5 cells (a radiosensitive dsb repair defective mutant derived by Jeggo et al. (1982, Biochemie, 64, 713-715) was similar to that for its WT parental CHO K1 cell line. These results were obtained when equiclastogenic doses of X-rays were employed to damage cells. We have recently repeated these experiments using the same X-ray dose (0.75 Gy) in both strains. Our results (Bryant et al., in preparation) show that a similar rate of disappearance of chromatid breaks occurs in both lines (xrs and CHO) following X-irradiation. These results seem paradoxical since the overall rate of rejoining of DNA double-strand breaks has been shown to be severely reduced in xrs 5 as compared to the WT CHO line. The results indicate that G2 xrs 5 cells are proficient in the repair of DNA dsb. The effect is still under investigation and further measurements of repair of dsb, following X-rays, are being made in these cell lines using the neutral filter elution technique.

Protein extracts have been prepared from both CHO and xrs 5 cells and are being assayed in porated cells treated with either X-rays or bleomycin. To date only small changes have been observed in chromosome damage in extract-treated versus non-treated samples using the micronucleus assay of Fennech and Moreley (1985) involving cyto-

kinesis block induced by cytochalsin B. This method has proved a valuable method for screening extracts. As a control, T4 ligase was used to treat electroporated and Pvu II treated cells. This ligase treatment led to a marked dose dependent decrease in frequencies of micronuclei induced by Pvu II, but experiments suggest that it leads to little or no change in the frequencies of X-ray induced micronuclei, indicating that although T4 ligase can join restriction-endonuclease cuts in cellular DNA, dsb induced by X-rays are not strongly subject to 3' hydroxyl-5' phosphoryl end ligation.

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I Head of Project 8: Prof. Feinendegen

II Objectives for the reporting period

a. Adaptive response of cell in vivo, and
b. formation of unrepaired strand breaks of cells in order to understand better the "vertical risk", meaning the propagation of radiation detriment and the resistance from lower to higher levels of biological organisation. This includes new information on the question as to the proportionality between risk and dose and the possible existence of threshold doses and threshold dose rates.

III Objectives for next period

Experiments with fractionated neutron irradiation will begin after the reconstruction of the compact cyclotron CV28 (September 1991).

IV Progress achieved including publications

Adaptive response of cells in vivo

Whole-body exposure to ^{137}Cs-gamma-rays inhibited temporarily the enzyme thymidine kinase (TdR-K) with a minimum of about 4 hours after exposure and full recovery within some 6 hours thereafter; this effect was dose dependent up to about 0.01 Gy and it was then dose-independent up to at least 1 Gy. Also a diminution in the number of hemopoietic stem cells (CFU-S-7d) in murine bone marrow was seen to be expressed maximally after 2-4 hours.

After a first exposure to D $\leq$ 0.1 Gy the reaction of the cells was altered in that TdR-K became temporarily resistant to a repeated exposure with the same dose. The number of hemopoietic stem cells seeding to the spleens of conditioned recipient mice, on the other hand, exhibited an additive effect in terms of colony formation when repeatedly exposed to the same low dose.

The investigations center on the consideration of (1) those cellular processes caused by the increase in intracellular concentration of radicals after low-dose irradiation and resulting in inhibition of TdR-K and altering function of stem cells; and (2) the consequences for risk assessment in low-radiation dose. Here, the investigation of the radical detoxification system, especially lipid peroxidation, the importance of intracellular glutathione concentration in the control of cellular processes, the regulation of TdR-K activity and cell differentiation are of pivotal interest.

Reaction of thymidine kinase after repeated irradiation

After repeated exposures to 0.01 or 0.1 Gy, the reaction of TdR-K is altered, depending upon the radiation-free time intervals between the radiation exposure events. After an interval of 0.5 hours between the first and the second dose, inhibition of enzyme activity and incorporation is accelerated, the maximal enzyme- and incorporation inhibition is observed as early as 2 hours after the last irradiation and has disappeared after 4 hours. If the second irradiation occurs 4 hours later, that is, at the time of maximal inhibition of enzyme activity after the first irradiation, then the second irradiation

raises the enzyme activity and incorporation of IUdR to normal values for at least 4 additional hours. Only when the interval between irradiations is extended to 12 hours do the cells again exhibit their normal reaction to small radiation doses (Feinendegen et al., 1988). After a threefold whole-body exposure with 0.01 or 0.1 Gy at 4-hour intervals - i.e., the third irradiation at the time after the second irradiation when the cells appear resistant - the enzyme is again inhibited.

Investigations concerning the regulation of thymidine kinase activity

Several experiments with metabolic and membrane-affecting interventions led to the working hypothesis that in the process of enzyme inhibition, the cellular systems of radical detoxification may be involved. With this in mind, experiments were undertaken to examine the control of enzyme activity and the importance of intracellular glutathione concentration.

The reduction of enzyme activity is also observed in the state of vitamin E-deficiency, 4 hours after injection of cysteamine or vitamin C as well as after the elevation of the intracellular glutathione concentration by stimulation of its synthesis. The resultant change in glutathione concentration corresponds to the observed elevated glutathione content in bone marrow cells 4 hours after irradiation with 0.1 Gy or by induced vitamin E deficiency (Elkarim-Hohn et al., 1990).

However, the change in glutathione concentration leads to reduced enzyme activity only in intact cells and does not affect the isolated enzyme. This indicates that glutathione does not affect the enzyme directly, but rather that further cellular factors must be involved.

Ongoing research on the localization of TdR-K in bone marrow cells promises more information on these factors. So far, it has been shown that the enzyme activity is regulated by one or more cellular factors which in homogenates of unirradiated cells, inhibit enzyme activity but, in the presence of ATP, lead to a rise in enzyme activity and which, in the presence of ATP, sediment upon centrifugation. As a result, enzyme activity increases after centrifugation in the presence of ATP. In irradiated cells this regulation mechanism is no longer operative and the enzyme activity after centrifugation of the homogenate, in the presence of ATP, is reduced. The TdR-K in murine bone marrow cells consists of four isozymes which are attributed to the mitochondrial and embryonal forms. Four hours after irradiation the activity of all isoenzymes are reduced to about 70% of its normal value. This radiation effect can be simulated by the induction of vitamin E deficiency.

These results therefore make it clear that cells react to low doses of radiation, and that the relation between radiation dose and observed effects is not constant. This suggests cellular adaptation mechanisms arising from a reaction of the cellular radical detoxification system following a short-lived radiation insult. The reaction of TdR-K to a change in glutathione concentration in the cell would explain the observed experimental findings after repeated radiation.

The effect of low-dose radiation on hemopoietic stem cells

After a whole-body gamma-irradiation of donor mice with 0.01-0.2 Gy, transplantation of their bone marrow into conditioned recipient mice led to a temporary reduction in the number of spleen colonies (CFU-S, colony-forming units in spleen, hemopoietic stem cells). The maximum effect of CFU-S reduction to about 70% of control was seen when bone marrow cells were transplanted 2-4 hours after irradiation;

this effect was not dose dependent.

After repeated whole-body gamma-irradiation with 2 x 0.1 Gy at an interval of 0.5 hours, the number of stem cells was significantly reduced to 43%; with an interval of 4 hours, the number of stem cells was found to be 45% of the sham-irradiated controls.

The steemingly additive effect of a second irradiation upon the stem cell count is in contrast to the reaction of the enzyme TdR-K which, at a radiation-free interval of 0.5 hours, showed an accelerated but not an increased inhibition of enzyme activity; no change in enzyme activity was observed with a 4-hour interval. Thus, enzymic response and cellular reaction are not connected to each other. Under the chosen conditions of low dose either the number, or the seeding of, stem cells to the spleens of the recipient mice could have been affected, and there was no adaptive response. This conforms to the hypothesis that increased concentrations of intracellular radicals reduce the CFU-S; CFU-S then, would not react to low dose exposure with an improved radical detoxification as was seen for the bulk of bone marrow cells, under the chosen conditions of cell handling. The radiation effect thus corresponds to results observed with higher doses or an increased number of exposures.

The reduction of the CFU-S count by 30% after a single dose of 0.1 Gy seems to indicate that cell death is not the main underlying mechanism. It is suspected that the low doses (i.e., both doses) produce a change in some stem cells which impels them either to differentiate or to go into cycle (which by itself reduces the seeding to the spleens) or to reduce seeding capability in a more general way, perhaps through a change in membrane receptors which are responsible for the colonization of the transplanted stem cells in the spleen.

In all, in a synoptic approach to interpreting the response pattern of murine bone marrow, a change in cell cycle and thus in the availability of cells for seeding appears to be the dominant cause for the reduction of CFU-S - rather than the damage in receptor function of transplanted cells.

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I Head of Project 9:Dr.Johanson

II Objectives for the reporting period

The objective has been to obtain more knowledge about cellular response to irradiation at low dose-rates. The studied effect has been the induction of micronuclei.

1. The dose-effect relationship and time dependency was studied after incorporation of ^{125}IUdR.
2. The dose-effect relationship and time dependency was studied after uptake of ^{125}I-triiodothyronine (^{125}I-T3). Contrary to a random incorporation of ^{125}IUdR into DNA, ^{125}I-T3 binds to specific DNA sequences via its nuclear receptor in a cell-type specific manner.
3. The micronuclei frequencies in erythrocytes of small rodents and trouts from areas with various ^{137}Cs contamination have been studied.

III Objectives for next period

By comparing the effects of ^{125}IUdR and ^{125}I-T3 it is possible to obtain a measure of the relative radiotoxicity. We will continue this comparison by studying the effect (micronucleifrequency) after uptake of ^{125}I-T3 and ^{3}H-T3. ^{125}I decays will give a high LET like radiation in the near region of decay and ^{3}H a low LET like.

IV Progress achieved including publications

We investigated thyroid hormone molecule (triiodothyronine T3) labelled with ^{125}I as a potential carrier of radioactive iodine isotopes to cell nucleus. For this purpose we used two cell lines, CHO and GC, originating from tissues having low and high number of T3-receptors. The cells were incubated with a constant, external ^{125}I-T3 activity concentrations for one or several doubling times (T_d). GC cells were additionally incubated in a range of ^{125}I-T3 activity concentrations for a constant time.

The uptake of ^{125}I-T3 was found to be cell specific. After incubation in the same external ^{125}I-T3 activity concentration, the uptake to GC cells was 20 times higher than to CHO cells. About 75% of the ^{125}I activity in GC cells was localized to the nuclei while no preferential nuclear accumulation was observed in CHO cells. Incubation of GC cells in various ^{125}I-T3 activity concentrations ranging from 185 Bq ml^{-1} to 74 kBq ml^{-1} resulted in a linear increase of ^{125}I-activity per cell up to 37 kBq ml^{-1} and at higher concentrations a saturation occurred.

In GC cells the frequency of micronuclei was positively correlated to the nuclear ^{125}I-T3 activity concentration and to the incubation time. The frequency of micronuclei in ^{125}I-T3 incubated CHO cells was not significantly different from the controls independent of the incubation time. Using the same cell lines we performed a comparative study with ^{125}IUdR. In both cell lines the uptake of ^{125}IUdR was linearly increased when the cells we incubated in medium containing a range of ^{125}IUdR concentrations. However, linear dose-effect curves (micronuclei frequency) were found for ^{125}IUdR concentration below 30 (GC) and 75 (CHO) decays per cell. Above these doses the number of micronuclei was almost constant. The two times higher

sensitivity in GC cells may be due to the longer doubling time for GC cells.

The efficiency of ^{125}IUdR to induce micronuclei was about 5 to 10 times higher than ^{125}I-T3 in GC cells.

In studies with ^{125}IUdR and ^{125}I-T3 the cytochalasin B (CB) micronucleus test was used. A maximum in the number of binuclear cells was observed after one t_d when incubated with 3 µg of CB ml^{-1}. The frequency of binuclear cells were negatively correlated to the ^{125}I activity concentrations and also the frequency of micronuclei. For GC cells treated with various ^{125}IUdR concentrations the dose-response curve for binuclear cell after the first t_d was similar to the survival curve obtained after the first t_d.

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3. Ludwikow, G. and K.J. Johanson (1991) Micronuclei induced by ^{125}IUdR in two cell lines, in preparation.
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I Head of Project 10: Prof. Ehrenberg

II Objectives for the reporting period

(a) Identification, in exploratory experiments, of in vitro and in vivo systems suitable for studies of the mechanism(s) of adaptation or induction of mutation-prone conditions;
(b) Development of mathematical-statistical models for deviations from linearity due to induction, at some low dose, of functions affecting dose-response relationships.

III Objectives for next period

(a) With the ultimate goal of clarifying the effect of dose-rate on mutagenic response, an adaptable cell line will be used to characterize adaptation with respect to dose-response, inhibition by MEA and persistence of the adapted condition. The result will be verified in studies at low dose rate (with persistence time between "hits" or groups of hits as a determinant of dose rates to be studied).
(b) Clarification of functions for recombination with respect to inducibility and role of large deletions as a cause of greater mutagenic effectiveness at higher doses/dose rates.

IV Progress achieved including publications

"Adaptable" cell-lines are being studied for the purpose of selecting a suitable test material (see IIa). Within the frame of this work the statement of Mendiola-Cruz and Morales-Ramirez (Radiation Res. 118, 1989, 131) that mercaptoethylamine (MEA) completely abolishes SCE induced in vivo in the mouse was subject to experimental verification (in view of the hypothesis that SCE requires induction of a recombination function of significance to dose-rate effects). It was found impossible to induce SCE by X-rays, even in the animal strain used by the authors. Mathematical models for "adaptation" and for error-prone repair with thresholds (or quasi-thresholds in consequence) have been developed and applied to published or our own previous data. Two mss. are in preparation (F. Granath et al.).

Since the inference that MEA acts by inhibition of transcription is indirect, experiments for verification of the hypothesis at the level of induction of mRNA synthesis have been started.

I Head of Project 11: Prof. Palitti

II Objectives for the reporting period

1. To irradiate with different doses 5, 11 and 15 cGy of fission neutrons (0.4 MeV average energy) human lymphocytes in vitro and post-treat with hydroxyurea (HU) arabinfuranosilcytosin (ara C) and caffeine and evaluate the frequency of chromosomal aberrations.
2. To analyze the data for:
   a) interindividual variation;
   b) effect of different classes of DNA repair inhibitors on the yield of chromosomal aberrations.

III Objectives for next period

To extend the analysis to individuals affected by ataxia telangiectasia or AT heterozygotes.

IV Progress achieved including publications

Standard whole blood cell cultures were used. The cell cultures were irradiated at 70-72 hrs after stimulation and post-treated with the inhibitors for three hours before fixation.

Table 1 shows the distribution of chromosomal aberrations among the cells; a Poisson distribution was found. Table 2 shows the effects of 3 h post-treatment with caffeine, ara C and HU on the frequency of chromatid aberrations induced in human lymphocytes by different doses of fission neutrons.

The data suggest that also for the repair of DSBs both caffeine and HU interfere with some steps of their repair processes occurring in G2 phase. In the case of ara C further data have to be collected and protocol has to be modified in order to follow the kinetic of the repair processes.

1. Antoccia, A., C. Catena, F. Palitti and C. Tanzarella (1990) Comparison between chromosomal aberrations induced by X-ray and fission neutrons in human lymphocytes treated in G2 phase, Trend in biological dosimetry, Lerici, Italy.

Table 1. Distribution of chromatid aberrations in human lymphocytes exposed to different doses of fission neutrons 3 h before harvesting

Dose (cGy)	Cells scored	N of cells with indicated number of aberrations				Aberrations per cell (Y)	Dispersion index σ^2/Y	μ
		0	1	2	3			
5	400	332	61	7	0	0.18	0.98	0.24
11	500	336	107	24	3	0.31	1.09	1.42
15	600	363	194	35	8	0.48	0.91	1.57

Table 2. The effects of 3 h post-treatments with 5 mM caffeine (caff), 5 mM hydroxyurea (HU) and 0.05 mM cytosine arabinoside (ara-C) on the frequencies of chromatid aberrations induced in human lymphocytes by different doses of fission neutrons

Treatment	Cells scored	Abn. cells (%)	Aberrations per 100 cells				Total aberrations (-gaps)
			gaps	b	b"	chr.exch.	
none	700	2.0	2.0	1.0	1.0	0.0	1.0
caff	700	6.6	2.8	3.8	0.4	0.0	4.2
HU	500	10.4	5.8	5.2	0.6	0.0	5.8
ara-C	400	13.5	3.5	11.2	1.0	0.0	12.2
5 cGy	400	20.2	3.7	14.7	4.0	0.0	18.7
5 cGy+caff	400	37.4	10.0	35.0	5.7	1.0	41.7*
5 cGy+HU	300	37.7	9.7	32.0	8.0	0.0	40.0*
5 cGy+ara-C	300	34.0	8.7	26.7	8.3	0.0	35.0*
11 cGy	500	27.8	5.2	25.6	5.9	0.6	32.2
11 cGy+caff	500	52.8	10.4	51.8	11.8	2.4	66.7*
11 cGy+HU	400	48.2	9.2	49.7	15.0	0.0	64.7*
11 cGy+ara-C	300	39.7	6.3	40.3	8.3	0.0	48.6*
15 cGy	600	43.5	10.7	40.7	6.7	0.7	48.1
15 cGy+caff	600	71.2	18.6	89.1	26.8	4.6	120.5*
15 cGy+HU	500	63.2	15.2	72.4	19.0	0.2	91.6*
15 cGy+ara-C	400	55.5	10.0	64.5	16.2	0.2	80.9*

Abn.cells: abnormal cells; b: chromatid breaks; b": iso-chromatid breaks; chr. exch.: chromatid exchanges.
Significant according to student's t test $p < 0.01$.

Progress Report

Contract: Bi7-043 Sector: B13
Title: Measurement of transformation of C3H 10T 1/2 cells by low doses of ionizing radiation.

1	Morgan	AEA Technology Harwell Laboratory
2	Mill	Nuclear Electric
3	Kellerer	GSF Neuherberg
4	Frankenberg	GSF Frankfurt
5	Tallone Lombardi	Universita degli Studi di Milano

I Summary of Project and Global Objectives

The global objectives of this project are twofold. 1) To establish a standardised experimental protocol for C3H 10T1/2 cell transformation assay between European laboratories to ensure compatibility of results, this to be completed by December 1990. 2) To establish the shape of the dose-response relationship for survival and transformation of 10T1/2 cells exposed to a range of radiation qualities down to a dose of 10 mGy. Two dose points will be completed by December 1991.

1.1: Introduction.

The principal risk from low doses of radiation is the induction of cancer. Currently the risks of developing cancer are predicted by various methods but these have not been validated at low radiation doses as routinely received during the operation of nuclear facilities and other sources of occupational exposure. Dose-response relationships for tumour induction can be studied using animal models but at low doses these become prohibitively expensive and anyway, may not be morally justifiable. An alternative is to use cell transformation in vitro for which a variety of systems are available. However only one, the C3H 10T1/2 mouse fibroblast system, provides the relatively high precision needed for work at low doses and dose-rates. This system is used in a number of laboratories in Europe and the USA.

It is clear that, if reliable data at low doses are to be obtained, a large number of transformants must be scored to reduce the statistical variation. For one laboratory this may put a large strain on resources. For such an internationally important topic, this seems an ideal area for collaboration between laboratories. With this in mind six European laboratories have spent some time during the past year on a collaborative project with the following objectives:

1) To standardise the 10T1/2 assay between participating laboratories.
2) To produce a standard code of practice for 10T1/2 cell transformation; so that other laboratories may have a standard for the comparison of results.
3) Ultimately, to establish the shape of the dose-response curve for cell transformation at low doses and dose-rates.
4) To extend these studies to more relevant cell transformation systems (eg human epithelial systems) as and when they become available.

These objectives were formalised during a meeting of five of the participating laboratories held at Harwell in January 1990.

This report is in two parts; part 1 describes the results of the intercomparison experiments carried out between November 1990 and March 1991 and involving all six participating laboratories; part 2 describes the results of experiments performed independently in the various participating laboratories.

2.0: Effect of Trypsin on Plating Efficiency (Prof. A.M. Kellerer and Dr. L. Hieber)

Evidence from experiments performed at the Berkeley laboratory suggested that trypsin concentration or batch may affect the plating efficiency of C3H 10T1/2. Since all participating laboratories obtain trypsin from

different companies it was felt important to clarify this situation. We have investigated the effect of the various trypsin, on plating efficiency, used in the different laboratories. Two different trypsin preparations from the Berkeley laboratory were dispatched, on ice, to compare with the local trypsin solution currently used in 5 out of 6 participating laboratories. As shown in Figure 1, 4 laboratories did not find significant different plating efficiency values with different trypsin. Only the group in Milan got remarkably better results with their local trypsin; this may be due to an adaptation of the local cells to local trypsin in this laboratory. One may conclude from this study that trypsin is not a critical parameter for plating efficiency, although the plating efficiencies of the cells (different cell stock in different laboratories) show significant differences in each laboratory.

2.2: Influence of Plating Efficiency on Cell Survival

Following the groups decision to use only one source of cells from the Berkeley laboratories, it was necessary to study the effect of transportation on the 10T1/2 cells. The first experiments performed at Milan with local cells and local medium showed that the decrease in plating efficiency was strongly dependant on the time on ice; the longer the time the lower the plating efficiency, (see Figure 2).

From this finding the question arises: Does the decrease in PE concommitantly change the shape of survival curves? To study this question local cells from Wurzburg have been irradiated with graded doses of Cobalt gamma rays and then either plated immediately or, placed on melting ice for 24, 48 or 72 hours, to mimic transportation, after irradiation. Although the plating efficiency decreased with time on ice, from 0.51 at zero time down to 0.09 after 72 hours, the surviving fractions of the cells held on ice for different time intervals did not differ significantly from cells plated immediately (see Figure 3). The data could be fitted very well by the same survival curve. These results make it feasible to perform intercomparison experiments with shipped cells. In a preliminary transformation experiment with cells immediately plated and cells kept on ice, performed in Wurzburg, there was also no indication that the transformation frequency may be influenced when cells were held on ice.

3.0: Antibiotic Effects (C. Roberts and S. Futter)

Before embarking on this programme careful consideration was given to the experimental protocol, and all the factors likely to influence the assay. It is well known that transformation frequency is modulated by cell density, and from data produced in this study variations in transformation frequency may be related, in part, to cell density, (see below). It is therefore important in any transformation study, to achieve a reliable plating efficiency. Many factors contribute to the plating efficiency of 10T1/2 . We have shown that as cell density is increased so the apparent plating efficiency falls. Thus to achieve reliable transformation data, rigourous control of the factors affecting the assay are required.

Bertram (1979 Cancer Letters, 7, 289-298) reported that penicillin could suppress the transformation frequency of 10T1/2 after chemical or radiation insult. However, his data suggested little effect on plating efficiency or saturation density, and the mechanism by which suppression worked was unknown. However, we decided to investigate further the effect of antibiotics on the plating efficiency and growth of C3H 10T1/2 cells.

A series of experiments, see Figure 4., showed conclusively that when cells were grown and replated with penicillin present (100 units ml^{-1} penicillin / 100 ug ml^{-1} streptomycin) in the culture media (P-P) the plating efficiency (PE) was variable with the highest PE about 30%, this is in agreement with data from other laboratories. When cultures of 10T1/2 were grown in culture medium containing gentamicin (0.5 ug ml^{-1}) and plated into culture medium containing penicillin, (G-P) again PE was poor. However, when 10T1/2 cultures grown in penicillin containing media were replated into medium containing gentamicin (P-G) then the PE improved to around 50% and when cells were cultured in media containing gentamicin and replated in media containing gentamicin (G-G) then the plating efficiency was reliable around 70%. The effect was also independent of cell density, panels 1, 2 and 3 in Figure 5 show the results of plating cells at 3, 5 and 7 cells cm^{-2} respectively. However it is clear that cell density itself affects the plating efficiency. Figure 5 shows that the higher cell densities apparently suppress the plating efficiency, (possibly through the mechanism of co-incidence), when it is high, (i.e. in gentamycin containing medium, panels 1 and 4), but not when low (in penicillin/streptomycin containing medium, panels 2 and 3).

Experiments comparing 0.05% Trypsin : 0.02 % EDTA with 0.25% Trypsin : 0.02 % EDTA were performed using growth media containing penicillin/streptomycin or gentamicin. The results shown in Figure 6 detail the effect of trypsin batch on the plating efficiency of 10T1/2.

In this experiment a single cell density was used (5 cells / cm^2). Trypsin was dispatched under code to Harwell along with cells for a transformation experiment. Using our own 10T1/2 cultures (H) and the control 10T1/2 cells sent from Berkeley (B) PE experiments were done with cells cultured in penicillin/streptomycin (P) or in gentamicin (G). Again, in this experiment greater consistency was found in the gentamicin cultures. The three panels in the figure relate to the various trypsin batches tested. Panel 1 was 0.05% Trypsin / 0.02% EDTA (Flow Labs., cat no. 16-891-49). Panel 2 was 0.25% Trypsin / 0.02% EDTA (Imperial Labs., cat no. 4-772-07). Panel 3 was 0.05% Trypsin / 0.02% EDTA (Gibco Ltd., cat no. 043-05300H).

Bertrams', experiments confirmed that penicillin appeared to be responsible for the fall in transformation frequency seen with his experiments and in our experiments increasing the concentration of penicillin from 100 to 200 units caused a drop in PE. (data not shown).

From this data it is clear that cells grown and reseeded into growth media containing gentamicin or cells grown in growth media containing penicillin /streptomycin but reseeded into gentamicin have a higher PE than cells grown in growth media containing penicillin /streptomycin or gentamicin and then reseeded into growth media containing penicillin/streptomycin.

This work has resulted in a change to our initial, agreed experimental protocol. Gentamicin produced consistently high plating efficiencies than those achieved using penicillin/streptomycin. Thus we recommended that gentamicin be used on a routine basis and that a cell density of 1-3 cells cm^2 be used in transformation experiments .

This data is currently being prepared for submission to the journal Carcinogenesis.

4.0: Scoring of Foci (Dr. D. Frankenberg)

One of the first parameters checked by the 6 collaborating groups, Berkeley Nuclear Laboratories (B), AEA Technology Harwell (H), GSF Frankfurt (F), GSF Wurzburg (W), University of Milan (M) and ENEA Rome (R), was the scoring of transformants. For this purpose, 3 groups (B,W and M) sent around a definite number of flasks or petri dishes from previous experiments for scoring by the groups B, H, F, W, and M, (R joined the collaboration after the first intercomparison). The results of this first intercomparison in scoring are given in the following.

In table 1 are shown the results of the statistical analysis. There is a considerable discrepancy between the individual scoring of B, H, F, W and M (lines 1 to 5 in table 1). Line 6 of table 1 gives the mean score totals with their standard deviations. Because of this unexpected situation all scorers, including R, met at Frankfurt for the first consensus scoring exercise. As a basis for the consensus scoring, the criteria of Reznikoff et al (Cancer Res. 33, 3239-3249, 1973) were taken. Type I foci (foci containing tightly packed cells) are not scored as malignantly transformed. Type II (massive piling up into opaque multilayers; cells only moderately polar; crisscrossing not pronounced) and type III (highly polar; fibroblastic; multilayered crisscrossed arrays of densely stained cells) are scored as malignantly transformed. The diameter of the foci was not considered as a third parameter. The consensus scoring yielded 25% less transformants (68) compared to the sum of the averages of the separate scoring (89) (last column in table 1). For support of future scoring, F provided a set of colour micrographs with 30 foci from B, W and M which were either definitely classified as type I, II or III by consensus scoring, or are still questionable.

Table 1: Result of first joint scoring exercise.

Scorer	(B)	(W)	(M)	(B)+(W)+(M)
Berkeley	58	43	17	118
Harwell	38	52	11	101
Frankfurt	32	38	16	86
Wurzburg	34	30	10	74
Milan	20	27	9	56
Mean score total+/-Stand. dev.	36+/- 38%	38+/-26%	13+/-28%	87+/-27%
Consensus	24	34	10	68
Mean total score /Consensus	1.5	1.12	1.3	1.28+/-0.19

5.0: Survival and Transformation (Dr. D. Bettega and Dr. A. Mill)

The transformation assay, like many other biological assays, is susceptible to perturbations from a variety of influences. These perturbations do not normally hinder the comparison of results when made internally within each laboratory but often preclude the direct comparison of results between laboratories. Hence intercomparison of results between laboratories is difficult unless steps are taken to minimise the effects of these factors. Probably the most important factors for transformation are the criteria used for scoring transformed foci, the growth conditions (particularly serum batch) used for the assay and the source of cells used for experimentation.

In order to standardise on scoring criteria a focus scoring intercomparison was carried out during the summer of 1990. The results of this are described above. This report describes the experiments which were carried out between the laboratories on a direct intercomparison of transformation. The basic procedures are shown in Figures 7 and 8

Cells from one participating laboratory (Berkeley) were irradiated and immediately placed on melting ice at 0°C along with a sample of unirradiated cells (earlier experiments had indicated that viability was good for times up to about 48 hours when kept on melting ice). These cells were then despatched to the other participating laboratories for subsequent culture. Samples kept at Berkeley were processed immediately after irradiation with a second set of samples kept on ice for about 48 hours before processing.

A common protocol was adhered to within the limits of each laboratory. This involved the culture of cells in both local medium and in complete growth medium as supplied by Berkeley. Since it was impractical to despatch large amounts of complete growth medium to cover all the subsequent medium changes needed for the transformation assay the medium changes at weeks 2, 3, 4 and 5 were carried out using local medium supplemented with Berkeley serum (see figure 7). It was decided that, in the first instance, the intercomparisons would be of limited size and hence an absorbed dose of 5 Gy was chosen such that an adequate number of foci would be obtained (20 were expected per laboratory). Altogether three intercomparison experiments were carried out.

5.1: Results and Discussion

Table 2a and 2b shows a summary of all the data obtained for these intercomparison experiments.

Table 2a: Results of the Survival Intercomparison (5 Gy)

Expt.	Laboratory	Time on Ice /h	Medium (Local or Berkeley)	Plating Efficiency /%	Surviving Fraction
1	Wurzburg	72	Local	22 ± 2	0.20 ± 0.03
			Berkeley	23 ± 1	0.24 ± 0.02
	Frankfurt	48	Berkeley	23	0.17
	Harwell	24	Local	40 ± 4*	0.33 ± 0.04
	Berkeley	0	Berkeley	65 ± 5	0.26 ± 0.01
		24		63 ± 4	0.32 ± 0.02
2	Harwell	48	Local	86	0.16
			Berkeley	NA	0.15
	Frankfurt	48	Local	11	0.27
			Berkeley	30	0.26
	Wurzburg	48	Local	46 ± 4	0.25 ± 0.01
			Berkeley	48 ± 2	0.28 ± 0.01
	Rome	72	Local	39	0.23
			Berkeley	44	0.25
	Milan	48	Local	26 ± 2	0.21 ± 0.03
			Berkeley	30 ± 1	0.25 ± 0.02
	Berkeley	0	Berkeley	69 ± 2	0.39 ± 0.01
		48		78 ± 4	0.32 ± 0.02
3	Harwell	48	Local	46 ± 2	0.21 ± 0.01
			Berkeley	40 ± 2	0.24 ± 0.01
	Frankfurt	48	Local	9	0.33
			Berkeley	32	0.33
	Wurzburg	52	Local	30	0.47
			Berkeley	33	0.55
	Rome	72	Local	22	0.55
			Berkeley	26	0.56
	Milan	48	Local (1)	44	0.26
			Local (2)		0.27
			Berkeley	44	0.26
	Berkeley	0	Berkeley	64 ± 1	0.62 ± 0.02
		48		58 ± 2	0.58 ± 0.01

*Assumes cells seeded at 30 cells per flask rather than the 300 per flask stated.

Table 2b: Results of Transformation Intercomparison (5 Gy)

Expt.	Laboratory	Time on Ice /h	Medium (Local or Berkeley)	Viable Cell Density /cm^{-2}		Transformation Frequency per Viable Cell x 10^4	
				Control	5 Gy	Control	5 Gy
1	Harwell	24	Local	4.8	7.9	<0.85	4.7 ± 1.3*
			Berkeley	NA	7.9	NA	8.3 ± 1.6*
	Frankfurt	72	Berkeley	7.4	5.8	0	3.1 ± 0.9
	Wurzburg	72	Local	5.2	4.8	0.33 ± 0.33	2.6 ± 1.1
			Berkeley	5.4	6.0	0.32 ± 0.32	2.6 ± 1.0
	Berkeley	0	Berkeley	6.2	9.6	0.62 ± 0.61	5.2 ± 1.0
		24		6.2	11.8	<0.58	5.0 ± 1.5
2	Harwell	48	Local	13.8	10.1	0.13 ± 0.13	3.4 ± 0.7
			Berkeley	NA	9.5	NA	2.3 ± 0.8
	Frankfurt	48	Local	1.5	2.2	0	3.6 ± 1.6
			Berkeley	4.3	5.7	0	3.3 ± 0.8
	Wurzburg	48	Local	6.7	8.4	<0.27	1.7 ± 0.6
			Berkeley	7	9.8	<0.27	1.6 ± 0.5
	Berkeley	0	Berkeley	7.3	15.2	<0.47	1.7 ± 0.4
		48		7.8	13.7	<0.46	1.4 ± 0.4
	Rome	72	Local	10.1	11.9	<0.36 ± 0.36	3.8 ± 0.9
			Berkeley	10	10.5	<0.37 ± 0.37	10.3 ± 1.5
	Milan	48	Local	4.9	3.9	<0.84	10.8 ± 2.9
			Berkeley	5.6	5.3	<0.68	12.1 ± 2.5
3	Harwell	48	Local	2.4	2.0	0.63 ± 0.62	17.5 ± 4.1
			Berkeley	2.1	2.0	2.6 ± 2.5	11.0 ± 4.0
	Frankfurt	48	Local	3.6	5.9	0	4.6 ± 1.1
			Berkeley	2.4	3.6	6.9 ± 3.5	7.2 ± 1.7
	Wurzburg	52	Local	2.2	1.9	0.78 ± 0.78	4.4 ± 1.7
			Berkeley	2.4	5.3	0.71 ± 0.71	1.5 ± 0.6
	Berkeley	0	Berkeley	2.1	5.3	1.6 ± 1.6	4.1 ± 1.2
		48		1.9	4.5	<1.8	1.9 ± 1.3
	Rome	72	Local	1.9	4.9	3.6 ± 2.6	5.8 ± 1.4
			Berkeley	2.3	5.0	1.6 ± 1.6	4.8 ± 1.3
	Milan	48	Local(1)	4.3	4.2	<0.9	8.3 ± 2.1
			Local(2)		4.5		10.7 ± 4.0
			Berkeley	4.2	4.1	0.9 ± 0.9	10.7 ± 2.3

* Calculated using the survival data from table 2a rather than on an assumed value of 3 viable cells cm^{-2}

5.2: Plating Efficiency

t was generally observed that plating efficiencies at the recipient laboratories were significantly lower than those obtained at Berkeley, even for cells kept on ice for similar times. However, apart from one laboratory, no significant differences were observed between local and Berkeley medium (figure 9).

5.3: Surviving Fraction

Survival results are reported in Figure 10 for Reference (o) and Local Serum (□) as a function of time in ice after irradiation. Survival seems not to be affected by the type of serum or by the time interval in ice. The mean values were 0.25 +/- 0.06 for Exp 1, 0.25 +/- 0.07 for Exp 2. In Exp 3 a group of three Labs (M, H, F) obtained a mean value of 0.27 +/- 0.05. the other three Labs found a mean value of 0.56 +/- 0.05. The reason for this difference is unknown at present.

5.4: Transformation

It was initially decided that each laboratory would aim to seed between 3 and 5 viable cells per square centimetre for the transformation assays. It is in this region that the transformation frequency per viable cell is least dependent on the cell density. At higher densities, particularly between 6 and 15 cells cm^{-2}, there is a large dependence on the cell density. However, higher than expected values of the plating efficiencies were obtained and for many laboratories the cell densities were not in the desired range. Combined with the large spread in both the plating efficiencies and the surviving fractions this produced a range of cell densities from 2 to 15 cells cm^{-2}. The effects of several parameters on transformation frequency were analysed.

5.5: Time on ice after irradiation.

Table 3 shows for the three experiments the ratio between transformation frequency values obtained for cells immediately plated after irradiation (TRV at 0 hrs.), and those obtained for cells plated after 24 and 48 hrs in ice (TVR at T hrs), corresponding survival ratios S(O)/S(T), are also reported.

As can be seen from the table cell density does not vary by more than 20% and so the comparison is valid.

The transformation frequency ratio is approximately 1 at 24 hours, but may be greater than 1 at 48 hrs. This difference, if significant, could be due to some repair mechanism working at ice temperature, or may be due to some loss of potentially transformed cells. Such a difference is not observed for plating efficiency (the mean value is 0.99+/- 0.15) or survival where the mean ratio is 1.15 +/- 0.11.

Table 3: Affect of Transportation on Ice on Transformation Frequency

Time in Ice (hrs)	TRV at 0 hrs/ TRV at T hrs	D at 0 hrs / D at T hrs
24 (exp 1)	1.04+/- 0.37 (S(0)/S(T)= 0.81+/- 0.6	0.81
48 (exp 2)	1.21 +/- 0.44 (S(0)/S(T)= 1.22+/- 0.08	1.11
48 (exp 3)	2.15 +/- 0.55 (S(0)/S(T)= 1.07+/- 0.04	1.18

5.6: Serum Effects

As already observed, cell density was not constant during the experiments. However, there are 9 cases where cell density with the two types of medium within the same experiment and laboratory are similar. Table 4 shows for each laboratory the ratio between the transformation frequency obtained with the reference serum and the local serum for each experiment

The corresponding cell density ratio is also shown. As can be seen, for a least 3 different sera, the variation in TRV values, in comparison with those obtained with the reference serum, are within 20%. For the two others more data are necessary to reach a definite conclusion.

As can be seen from Table 2b spontaneous transformation frequency does not seem to be affected by the serum batch.

Low values were obtained in Exp. 1 and 2. This was not the case in Exp. 3 where in 9 out or 12 cases spontaneous transformants were found.

Table 4: Effect of Serum on Transformation

Lab, Expt	Density Ratio	TRV ref/ TRV loc	Mean
H1	1.0	1.77 +/- 0.6	
H2	0.94	0.68 +/- 0.27	1.03 +/- 0.64
H3	1.0	0.63 +/- 0.27	
M2	1.36	1.12 +/- 0.48	1.21 +/- 0.12
M3	0.98	1.29 +/- 0.43	
W1	1.25	1.00 +/- 0.54	0.92 +/- 0.12
W2	1.17	0.83 +/- 0.39	
R2	0.88	2.71 +/- 0.75	1.77 +/- 1.33
R3	1.02	0.83 +/- 0.3	

5.7: Cell Density Effects

Transformation frequencies as a function of cell density are reported for the three experiments in Figure 11. As can be seen no common pattern is apparent. Figure 12 shows only the data obtained with the same serum, the same time interval in ice after irradiation (48 hrs), and comparable level of survival (SF=0.25). The data seem to indicate that transformation frequency rapidly decreases at density values higher than 4-5 cell/cm^2. The density interval between 2 and 4 cells/ cm^2 could be a region of small variation in transformation values, and so the most suitable to work in, but more data are necessary to reach a clear conclusion.

5.8: Conclusions

The viability of cells up to 72 h on ice is not a problem and is sufficient to allow transportation of cells to all laboratories.

Plating efficiencies, although high, are lower than those at Berkeley for the same time on ice. This may be due to local differences in culture conditions.

The choice of supplemented serum, (local or Berkeley) does not appear to influence the results significantly.

More experiments are necessary to reach definite conclusions about the effect of cell density on transformation before we proceed to low dose studies. This is planned for the next intercomparison experiment

Head of Project 1: Dr. Morgan

II Objectives for the reporting period

1) To participate in establishing a European standard for quantitating the C3H 10T1/2 transformation assay, with emphasis on factors effecting the plating efficiency of 10T1/2.

2) The characterisation of cell surface markers and their exploitation to enable single transformed cells to be detected.

III Objectives for next period

For the period 1991/92 we are proposing to continue our participation with the transformation experiments, extending the dose down to 1 Gy in collaboration with our European partners. During this time further modifications to the experimental protocol will be made if required. We will also continue to improve scoring of transformed foci by further collaborative scoring exercises. We also propose to sort out the problems of specificity encountered in the surface marker study. These may be due to the methods by which the proteins are isolated from the gels or their preparation for immunization and so different approaches will be used, probably involving *in vivo* immunization techniques and using whole C3H/10T1/2 cells as well as isolated proteins. Once antibodies of the desired specificity have been obtained these will be assessed for their ability to detect individual transformed cells and to determine the feasibility of a new sensitive immunological assay.

IV Progress achieved including publications

As part of a previous programme, comparison had been made between cell surface proteins from normal C3H/10T1/2 cells and those transformed by either ^{60}Co-gamma rays or the chemical carcinogen 3-methylcholanthrene (MCA). This involved growing the cells up in large scale microcarrier cultures to provide sufficient material for biochemical analysis and then extracting the membrane components with non-ionic detergent. Membrane proteins from both normal and transformed cells were then analysed and compared by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE). This led to the detection of consistent differences in membrane protein expression between the normal and transformed cells and in particular the identification of four proteins of special interest. These were of approximate molecular weight: 66, 49, 44 and 37 kD, the first of which showed greatest expression in the normal cells and was progressively lost in the transformed cells, whilst the reverse is true of the other three, ie. showing increased expression in the transformed cells.

Technical progress during this period (1990/91) has largely been concerned with the production of monoclonal antibodies against the four proteins of interest and commenced by isolating them from the SDS PAGE gels by electro-elution. They were then coupled to octyl sepharose beads, firstly to avoid problems of keeping them in solution on removal of detergent, and secondly to attempt to maintain their antigenic orientation during immunization procedures.

Monoclonal antibodies were then raised against a mixture of the four proteins using *in vitro* immunization techniques in which mouse spleen cells were cultured in flasks with the protein-coated beads in the presence of lymphokines and growth factors. These growth factors etc. simulate the immune response *in vivo* and stimulate antibody production by the spleen B lymphocytes, which were then fused with mouse myeloma cells and hybrid cells selected for by standard hybridoma methodology. Hybrid clones were tested for relevant antibody activity by screening against a mixed population of normal and gamma-transformed C3H/10T1/2 cells by both enzyme linked immunosorbent (ELISA) or immunofluorescence (IFA) assays. By this approach 15 positive clones were identified, all of which were IgM immunoglobulin isotype.

The fine specificity of these fifteen was then investigated by (i) screening against separate populations of normal and transformed cells, (ii) quantifying fluorescent staining in a flow cytometer and (iii) undertaking Western blot analysis to determine which of the proteins each antibody was specific for. Unfortunately, even by flow cytometry, only slight differences in antibody reactivity could be detected between normal and transformed cells, whilst the Western blotting indicated that the binding was non-specific as the antibodies bound to multiple protein bands.

These combined results suggest that the antibodies react with epitopes common to a variety of cell surface proteins rather than to those which are transformation-related, thus showing little difference in reactivity between the normal and transformed cells. Continuing work will investigate this problem and attempt to generate antibodies of the desired specificity.

Head of Project 2: Dr. Mill

II Objectives for the reporting period

(a) Participation in the standardisation of the C3H 10T1/2 transformation assay, Our particular role was to prepare cells for the standardisation exercises for the 5 Gy intercomparison.

(b) A study of the inverse dose-rate effect using 2.2 MeV neutrons.

(c) A direct comparison of the effect of protons and α-particles with the same LET.

III Objectives for next period

(a) Continuation of the standardisation of the transformation assay and transformation experiments at lower doses.

(b) An investigation into dose-rate effects using both X-rays and α-particles. This will be extended to include irradiation times lasting up to three weeks and will involve the use of plateau-phase as well as log-phase cell cultures.

(c) A study of possible intracellular changes in pH or ion concentrations which may be correlated to cell transformation.

IV Progress achieved including publications

Our contribution to the intercomparison experiments is detailed in part I of this report.

An intensive series of measurements involving the scoring of about 100 foci has shown the possibility of a small enhancement in the effectiveness of low dose-rates of 2.2 MeV neutrons. The dose chosen for this comparison was 0.2 Gy given either acutely or over a period of five hours. It was found that the transformation frequency at the low dose-rate was 1.3 times higher than at the high dose rate (95% confidence limits +/- 0.27). These results are shown in the accompanying figure.

In another series of collaborative experiments 10T1/2 cells were irradiated with either 0.5, 1 or 2 Gy of protons and α-particles with LET's between 20 and 23 keV μm^{-1}. No significant difference in the transformation frequencies were seen for other endpoints.

Head of Project 3: Prof. Dr. Kellerer and Dr. L. Heiber

II Objectives for the reporting period

(a) Participation in the standardization of the C3H 10T1/2 transformation assay, particularly to discover parameters that may influence plating efficiency and cell survival.

(b) Determination of the RBE of cell transformation of 5.4 keV CrK_α soft x-rays.

(c) Study of the influence of ionization density on an inversed dose-rate effect for cell tranformation.

III Objectives for next period

(a) Continuation of the standardization of the transformation assay and transformation experiments at lower doses in collaboration with the other laboratories.

(b) Study of cell transformation by low doses of low and high LET radiations; cell cycle dependence of the inversed dose-rate effect.

(c) Development of a transformation assay with C3H 10T1/2 cells and/or human cells grown in suspension.

(d) Continuation of studying cellular and molecular mechanisms of radiation-induced cell transformation in Syrian Hamster embryo cells.

IV Progress achieved including publications

Relative Biological Effectiveness (RBE) for Cell Transformation of Soft x-rays

Soft x-rays have been shown to be more effective than conventional x-rays or gamma-rays in the inactivation of mammalian cells, in production of chromosomal aberrations and in the induction of mutation, but no data on transformation efficiency has been published. We have therefore in a comparative investigation determined, the transformation rates of C3H 10T1/2 cells after exposure to CrK_α x-rays (5.4 keV) and ^{60}Co-gamma rays.

As shown in figure 13 soft x-rays are more effective than gamma also for cell transformation. The RBE value for soft x-rays versus gamma-rays was approximately 1.3 in the range of soft x-ray doses from 2 Gy to 5 Gy; the same RBE-value as it has been found for cell inactivation (data not shown). There was no recognizable dependence of the RBE on dose, which contrasts the findings with α particles. The essential result of this soft x-ray study is that electrons of low energy and of ranges less than 1 µm are more effective than fast electrons, not only for cell inactivation but also for cell transformation.

Influence of Ionisation Density on the Inversed Dose-rate Effect for Cell Transformation

Induction of transformation incidence was examined in C3H 10T1/2 cells after exposure to single and fractionated doses of intermediate and high LET particles, in order to investigate the possible LET dependence of an inversed dose-rate effect for cell transformation. An inversed dose-rate effect has been found by Hill et al (1984) with fission neutrons but not with α particles (Hieber et al., 1987). There are, in addition, a series of studies of different laboratories that are contradictory in respect to an inversed dose-rate effect.

Accelerated particles from the RARAF (Columbia University, New York) have been used to expose the cells to a single dose or 3 fractions with different time intervals (0.3, 1.5, 15, 45 and 150 min) between the fractions (Table 4). Doses were chosen leading to surviving fractions of 0.6 to 0.7.

Table 4 Range of Accelerated Particles Used

Particle	LET(keV)/ m)	Dose (Gy)
protons	2.5	0.9
deutrons	25	0.6
deutrons	40	0.3
helium-3	75	0.2
helium-3	90	0.2
helium-4	120	0.2

The transformation frequency was influenced by extended exposure times for some but not all radiation qualities. Enhancement of transformation was evident for 40, 75, and 120 keV/µm when the time between each fraction was greater than 15 minutes. As LET increases above 25 keV/µm, the differences in transformation induction between acute and extended exposures increase to a maximum at 120 keV/µm before disappearing at 200 keV/µm. Further Experiments with helium-4 ions (LET 150 keV/µm) and with Am-α-particles (LET 147 keV/µm) are underway to complete the data. The variation in enhancement with long intervals between fractionation appears to be consistent with a proposed mechanism in which a period of extra sensitivity exists in the cell cycle (Rossi and Kellerer, 1986).

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L. Hieber and A.M. Kellerer (Abstract): Transformation of C3H 10T1/2 cells by acute and fracitonated doses of 2.7 MeV α-particles. 9th International Congress of Radiation Research, Toronto (1991).

Head Of Project 4: Dr Frankenberg

II Objectives For The Reporting Period

(a) Participation in a European collaboration to evaluate parameters which affect the transformation frequency on C3H 10T1/2 cells.

(b) Development of techniques for the exposure of C3H 10T1/2 cells to characteristic ultrasoft X-rays (device of irradiation vessels, preparation of cells on 1.5 um hostaphane foils, etc). Dosimetry of C_K photons for the irradiation of C3H 10T1/2 cells. Survival of C3H 10T1/2 cells after irradiation with C_K characteristic X-rays.

III Objectives for next period

(a) For the period 1991/92 we are proposing to continue our participation with the transformation experiments, extending the dose down to 1 Gy in collaboration with our European partners. During this time further corrections to the experimental protocol will be made if required. We will also continue to improve scoring of transformed foci by further collaborative scoring excercises.

(b) Determination of transformation frequencies of C3H 10T1/2 cells after exposure to C_K photons. Dosimetry of F_K and Al_K characteristic X-rays. Evaluation of RBE- values of C_K, F_K and Al_K X-rays relative to ^{60}Co gamma rays for transformation frequencies.

IV Progress achieved including publications

Because of the extremely low range of C_K characteristic X-rays (Eph = 278 eV; intensity is decreased to e^{-1} by about 2 um of water) special glass dishes with hostaphane foils were used. The glass dishes are made from glass cylinders with an inner diameter of 30mm and a height of 20mm. Hostaphane (1.5 um thick) was stretched across one side of the glass dish and glued to the dish by araldite powder at 160° C for 3 hours. Subsequently the dishes are checked for leaks using sterile water. In order to avoid cells that may be shielded by an excess of glue or by attachment to the glass wall of the dish, a special device was constructed which produces first a ring of pure medium near the wall. Then 0.4 ml of cell suspension (1.5×10^5 cells ml^{-1}) are distributed within this ring. After 5 hours incubation, when cells are attached to the foil, 1.6 ml of medium are added and the incubation continued for another 48 hours. The cell count at this time is about 1.2×10^5 and cells are still in exponential growth phase when exposed to the ultrasoft X-rays.

The C_K photon beam is vertical and photons enter through the bottom of the glass dish, through the hostaphane foil. The dishes are placed in a holder (kept at 37° C) with the hostaphane foil facing the photon beam. Dosimetry is performed using a special ionisation chamber. The dose measurements correspond to the entrance dose i.e. the dose in "cells" just behind the 1.5 um hostaphane foil. The dose rate amounts to about 5 Gy min^{-1}

For quantitation of cell transformation it is necessary to measure cell survival as a function of dose. C3H 10T1/2 cells attached to the hostaphane foil are very flat . The mean thickness of the cell nucleus amounts to about 2 um (Schillaci et al Radiat. Res. 118, 82-83, 1989) so that the average dose to the cell nucleus relative to the entrance dose is found to be 0.55. In Figure 14 the survival curves of C3H 10T1/2 cells after exposure to ^{60}Co gamma rays (reference radiation) and C_K photons in dependence of the average dose to the cell nucleus are presented. The RBE values of the C_K photons relative to ^{60}Co gamma rays for various survival levels of C3H 10T1/2 cells are given in table 5. These RBE values are dose dependant, mainly due to the less pronounced shoulder in the survival curve obtained after C_K photon exposure.

Table 5: RBE Values of C_K photons, relative to ^{60}Cobalt gamma rays for various survival levels of C3H10T1/2 cells.

Cell Survival	RBE
0.9	4.0
0.5	3.8
0.2	2.7
0.1	2.5
0.05	2.4

Head of Project 5: Prof. Tallone Lombardi

II Objectives for the reporting period

(a)Participation in the European Collaboration project aimed at establishing a standardised experimental protocol for C3H 10T1/2 cell transformation assay;

(b)Determination of transformation frequencies for 4.3 MeV α particles in a dose range of between 0.2 and 300 cGy.

III Objectives for the next period

(a) Further standardization experiments;

(b) Experiments on dose-fractionation effect at low doses of α–particles, 4.3 MeV; LET = 101 KeV/μm 3 and 10 fractions, time interval t = 1.5, 5 and 10 h. In parallel determination of the age distribution of the cell population at the various times between fractions;

(c) Sensitivity of synchronized population to inactivation and transformation in various phases of the cell cycle.

IV Progress achieved including publications

Transformation and inactivation frequencies induced in 10T1/2 cells exposed to 4.3 MeV α particles were determined in the range interval between 0.2 and 300 cGy. Survival data are well fitted by an exponential function of the dose with a mean lethal dose value of 0.61+/-0.02 Gy. Transformation frequencies per surviving cell vs dose are reported in Figure 15. The region between 0.002 and 0.2 Gy is shown in the inset (with linear, linear coordinates). The dotted line represents the background level, corresponding to 2.5 x 10^{5} transformants per survivor in this set of experiments. The points are the results of three independent experiments, involving the use of about 6,000 samples. The initial cell density was kept to between 2 and 4 cells/cm^2. The transformation curve shows a complex behaviour. For $\mathcal{D} < .02$ Gy data are well described by a straight line with an average slope of (8.5 +/-2.) x 10^{-3}Gy^{-1}. In this dose region there is a negligible probability for a cell or its nucleus to be hit by two independent radiation tracks. Therefore a linear relationship between transformation frequency and dose was expected.

In the interval between .02 and .2 Gy there is an apparent constancy. This could be explained either by assuming an inducible repair process as suggested by Burch and Chesters (1986) (in this dose interval the contribution of multiple traversals of the cells becomes significant), or, as due to the phenomena that occur at low doses and tend towards saturation. Such phenomena could be an alteration with its own probability of leading to transformation (Ottolenghi et al. 1990), or a period in the cell cycle during which the cells are especially sensitive as suggested by Rossi and Kellerer (1986) and more recently by Brenner and Hall (1990). The analysis of the curve in the light of these models is in progress in our laboratory. The continuous line is the result of the fit to the data of the function $T(D) - K_1 (1 - e^{-\alpha D}) + K_2(1 - e^{-\alpha \cdot \beta D^2}) \cdot$ where the first term may represent the contribution of a cell population which is in a phase of the cell cycle of high sensitivity to transformation, and the second the contribution of the rest of the population.

Experiments on dose fractionation effect are underway. A total dose of 0.21 Gy was delivered either as a single fraction or as three equal fractions at time invervals of 1.5 h between the doses. In each experiment single and fractionated dose were delivered in parallel and with the same experimental conditions. Preliminary results show that transformation frequencies after fractionated exposure are higher than after single exposure by a factor of about 1.3. This is in agreement with findings by Miller et al. (1990) who reported a factor of about 2 with the same fractionation protocol for alpha particles of 40 and 120 KeV/um.

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D Bettega, P Calzolari, A Ottolenghi and L Tallone Lombardi. Criteria and techniques for analysing cell survival data, Radiation and Environmental Biophysics 30, p. 53-70 (1991).

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Although not funded by CEC contract No. B17-00430c(jr) the ENEA Casaccia Laboratory, Rome, has contributed greatly to this programme. For this reason they have been included in this section under Project 6.

Head of Project 6: Dr. Anna Saran

II Objectives for this reporting period

(a) Participation in the intercomparison of cell survival and transformation frequencies of C3H 10T1/2 cells exposed to x-rays.

(b) To study of the effect of a freeze-thaw technique on the plating efficiency and neoplastic transformation of C3H 10T1/2 cells.

(c) To study of the effect of trypsin on plating efficiency.

III Objectives for next period

(a) To study of the influence of seeding density on the transformation frequency of C3H 10T1/2 cells .

(b) To study of the inverse dose-rate effect of fission neutrons and monoenergetic neutrons of different energies.

IV Progress achieved including publications

Research on in vitro effects of ionizing radiation carried out at the ENEA Casaccia Laboratories is presently supported by contract Bi6-004 with the CEC. Therefore, the results pertaining to that contract are summarized in the appropriate section of the progress report. During 1990 it was also decided to join the group of five European Laboratories, which had received a CEC Contract for "The measurement of transformation in C3H 10T1/2 cells by low doses of ionizing radiation", in order to exchange more readily results and information. It was also practicable to assist with the attempts to standardizing the experimental methods and protocols. This participation did not require any extra financial support to our team by the CEC during the period 1990-1991.

The present activity was partly devoted to measurements of the survival and transformation frequencies of irradiated C3H 10T1/2 cell. In particular, cells were exposed to 5 Gy of x-rays at Berkeley Nuclear Laboratories, and then shipped to us on ice. The experiments were performed both with medium containing our local serum and with medium containing a reference serum supplied by Berkeley Laboratories. The preliminary results seem to indicate that the serum has a negligible influence on the survival level, and affects only moderately the transformation results of a minority of the laboratories participating in the intercomparison. In addition, no cell density effect on transformation frequency was detected.

The effect of freezing on survival and neoplastic transformation of irradiated C3H 10T1/2 cells, was evaluated using fission spectrum neutrons produced by the fast reactor RSV-TAPIRO of CRE-Casaccia at doses of 54, 108 and 162 cGy. Immediately after irradiation, cells were trypsinized, diluted, centrifuged and resuspended in 10% DMSO in culture medium at a concentration of 5 x 10^5 cells per ml. Aliquotes of this cell suspension were added to freezing vials which were put at -80°C overnight and then stored in liquid nitrogen. The survival and neoplastic transformation experiments were started a week after liquid nitrogen storage. As a general feature, no significant difference was detected in frozen and unfrozen cells with respect to survival and transformation values, and the plating efficiency was not severely affected by the freezing procedure.
A comparison of the plating efficiencies obtained after using three different batches of trypsin, two for reference and one local, showed no significant differences in all cases but one.

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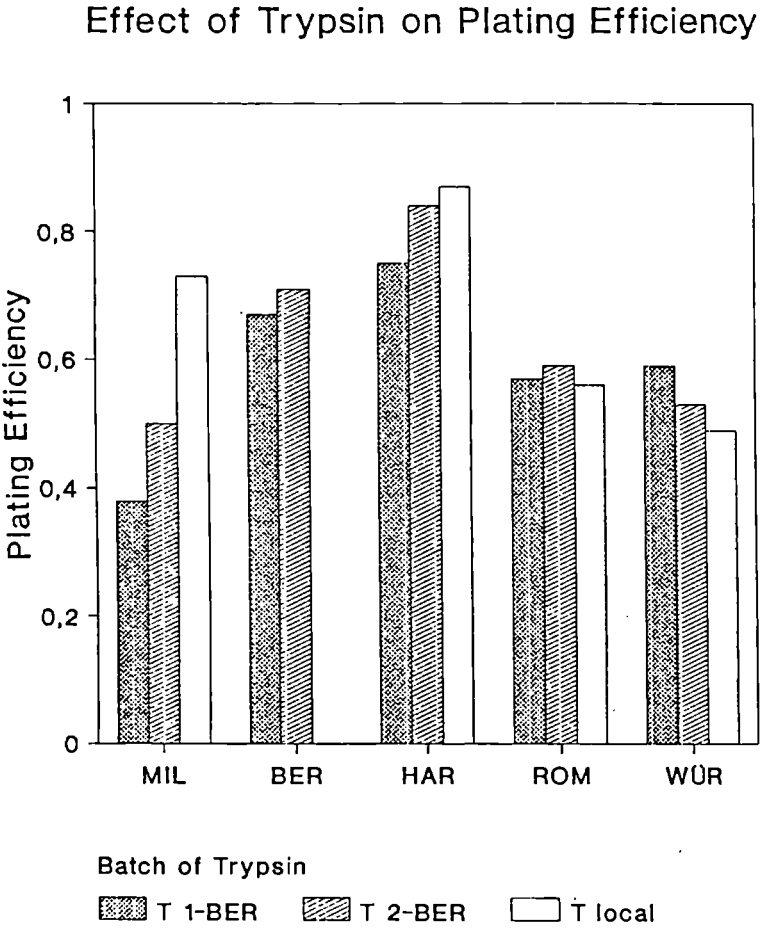
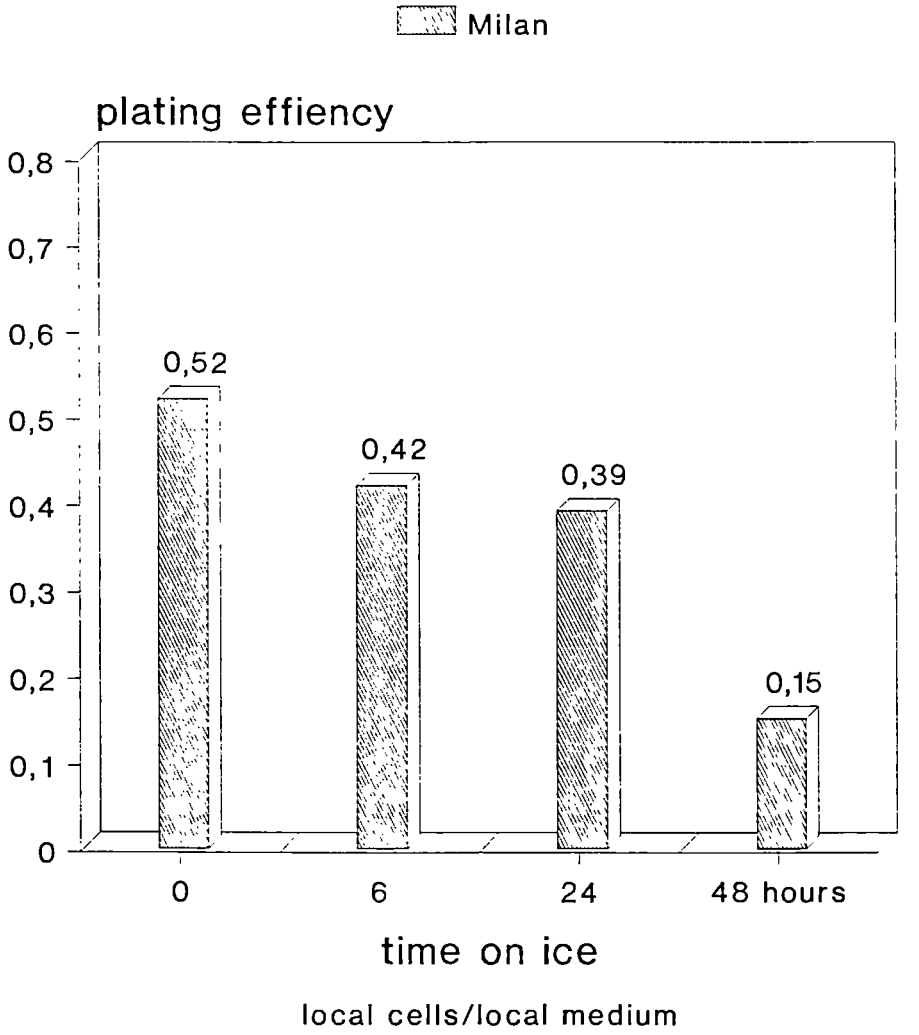

Effect of Trypsin on Plating Efficiency
Plating Efficiency
1
0,8
0,6
0,4
0,2
0
MIL
BER
HAR
ROM
WÜR
Batch of Trypsin
T 1-BER
T 2-BER
T local

Plating Efficiency

PE-experiment 1 Milan



local cells/local medium

C3H 10T1/2 Cell Inactivation

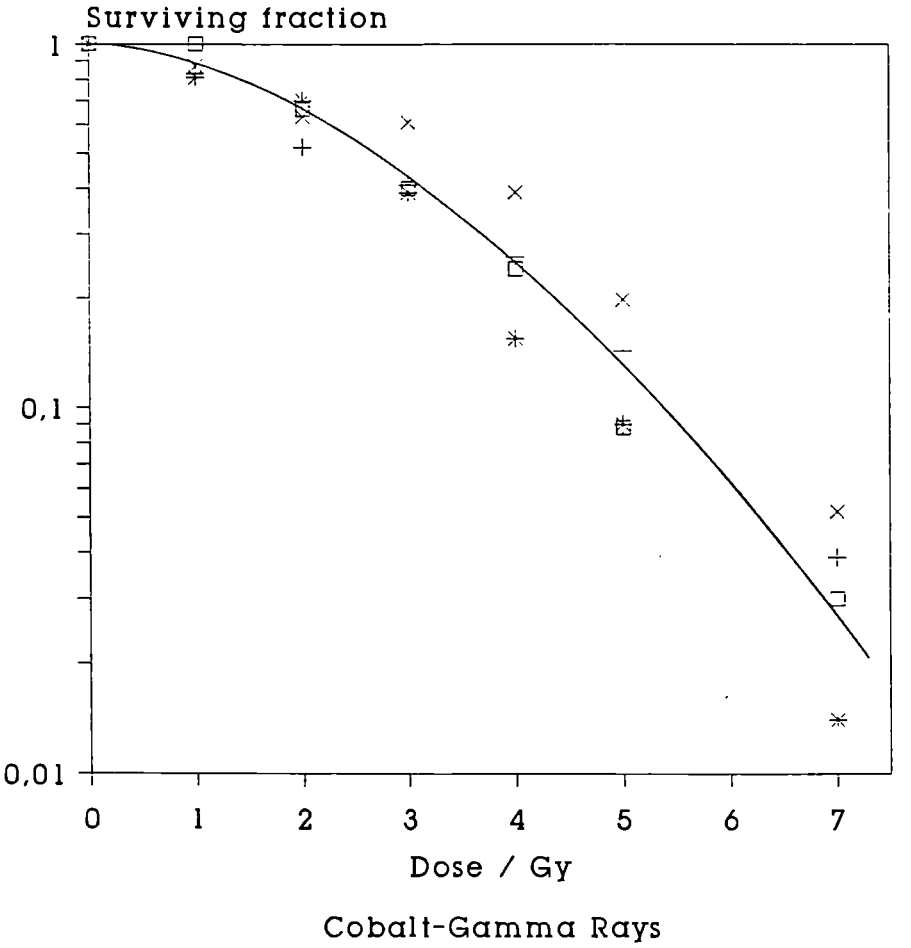


Figure 4: Effect of Antibiotic on Plating Efficiency

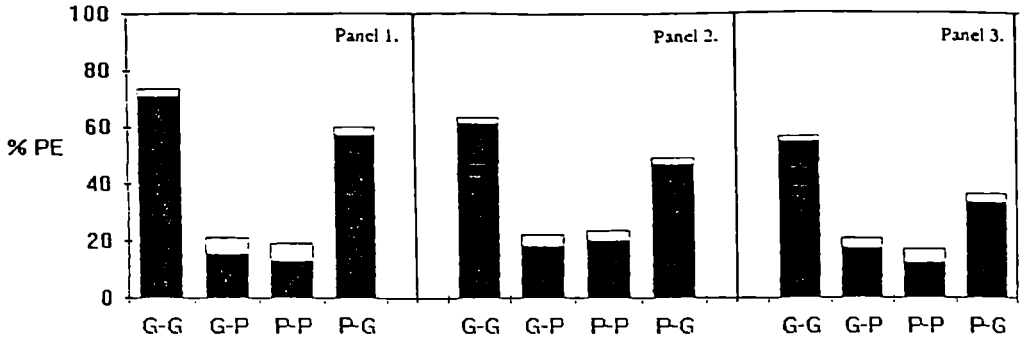


Figure 5: Effect of Cell Density on Plating Efficiency

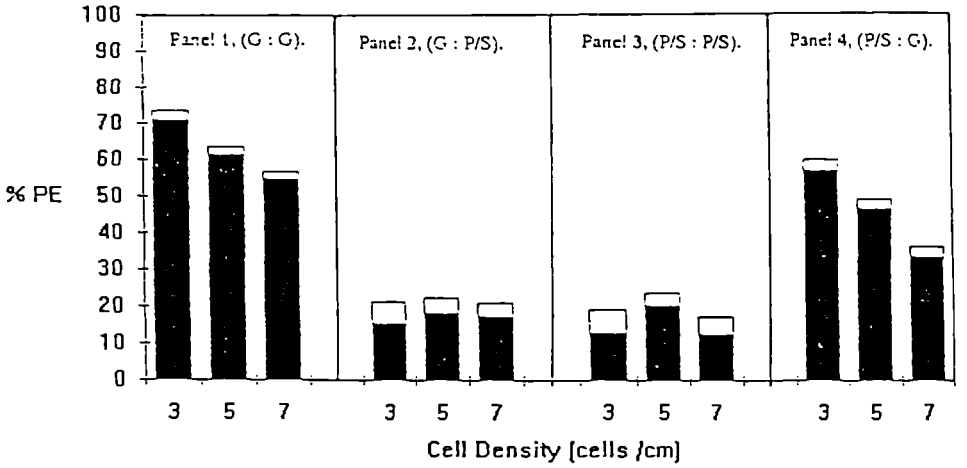
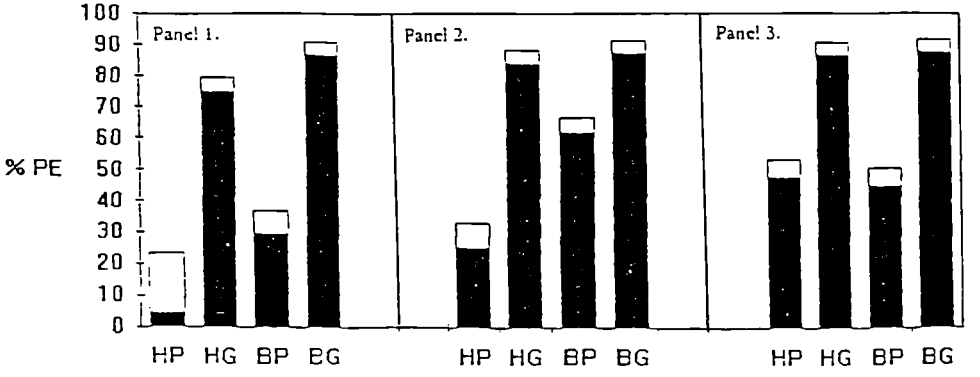


Figure 6: Effect of Trypsin on Plating Efficiency



Day -7: Cells thawed

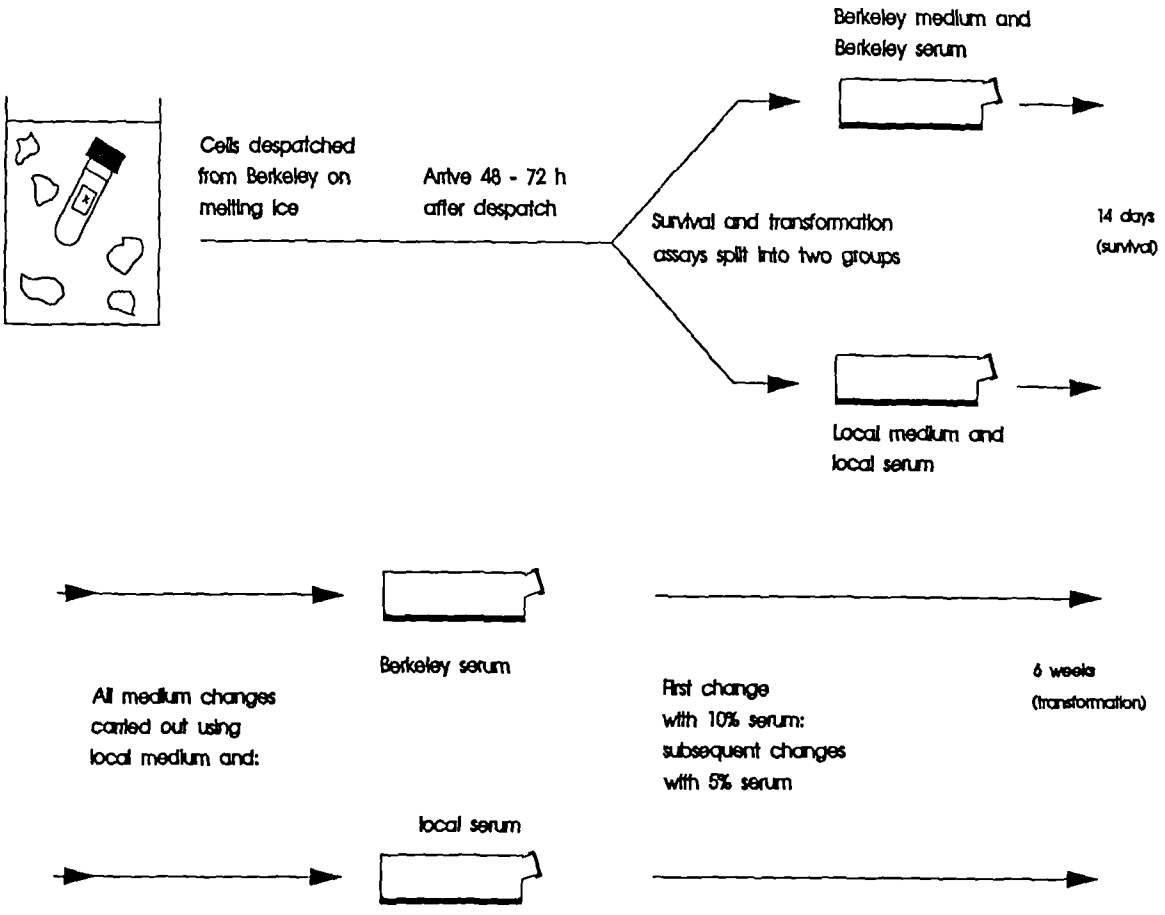
Four vials of pass 15 cells (0.75 million cells per vial) seeded into two 150 cmý flasks.

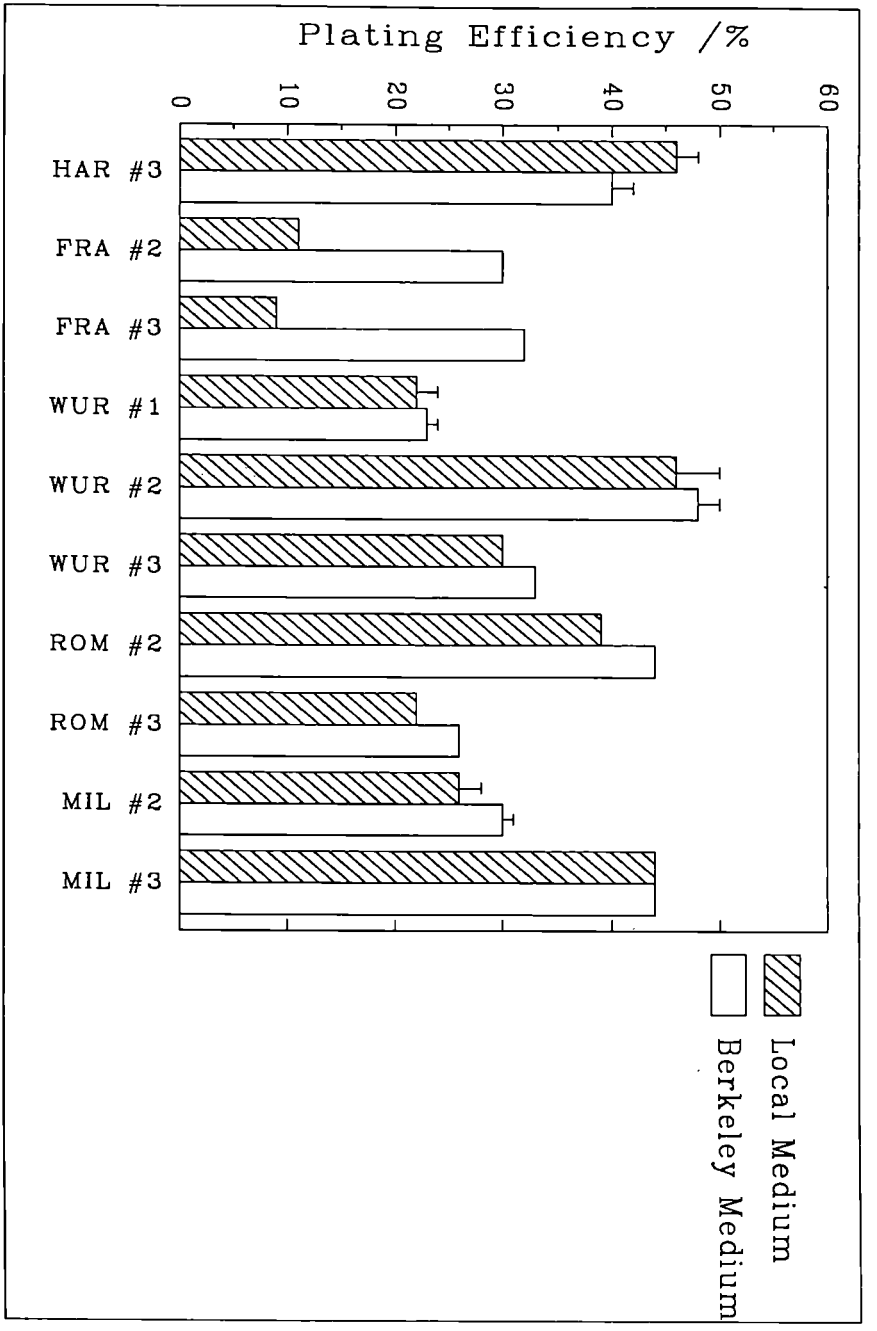
Day -5: Cells passaged

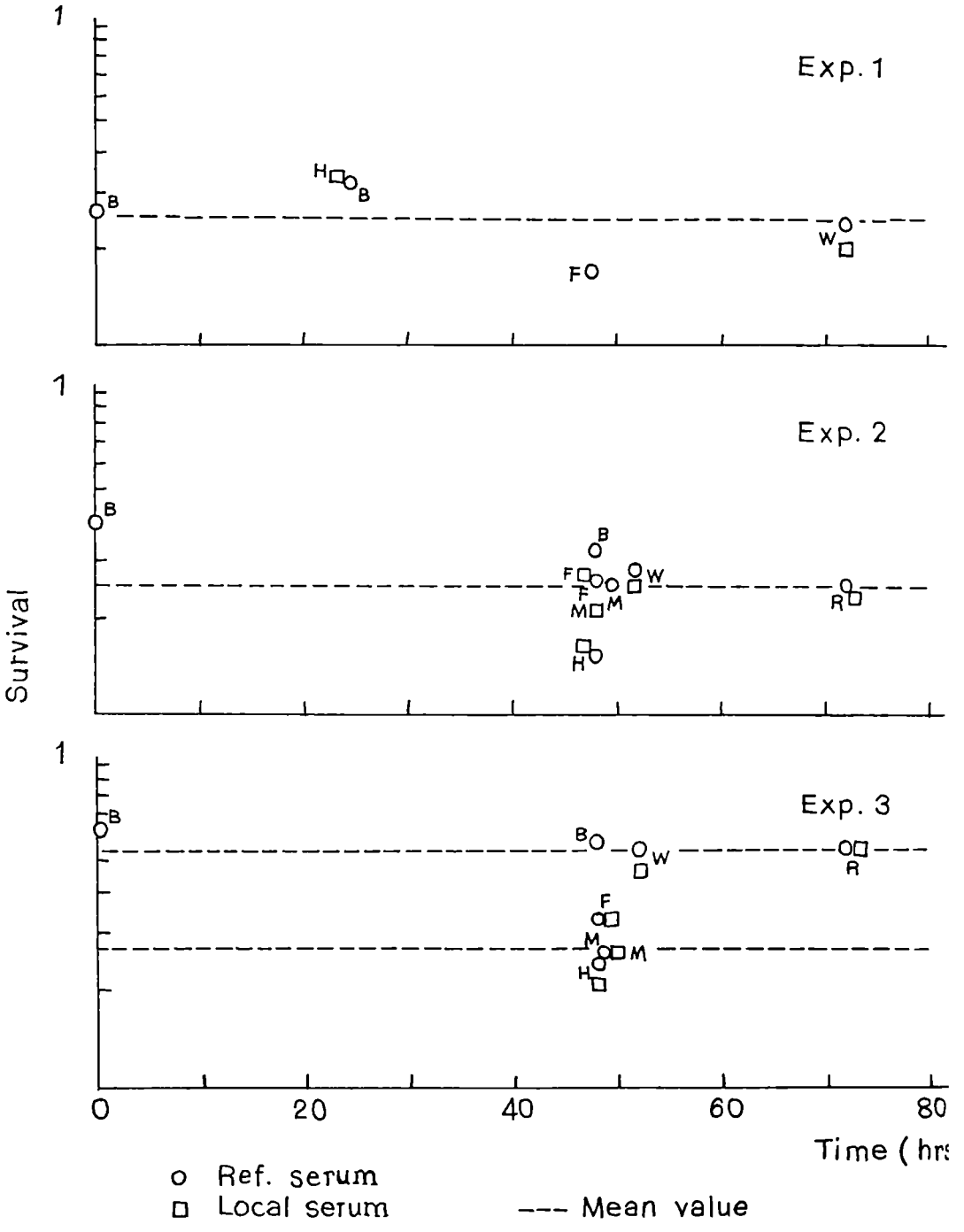
Cells subcultured into 12 flasks at approximately 50000 cells per flask.

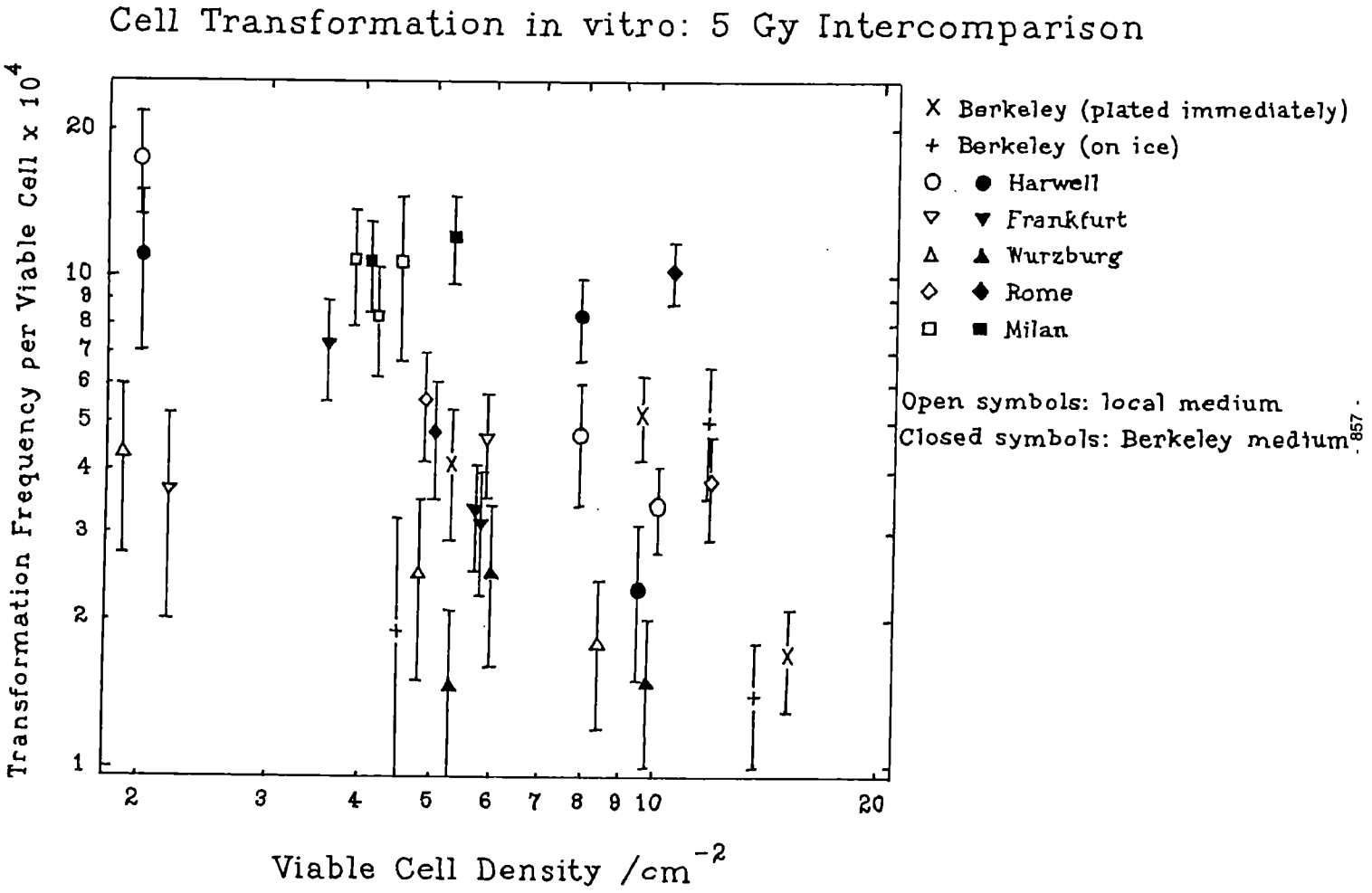
Day 0: Cells irradiated

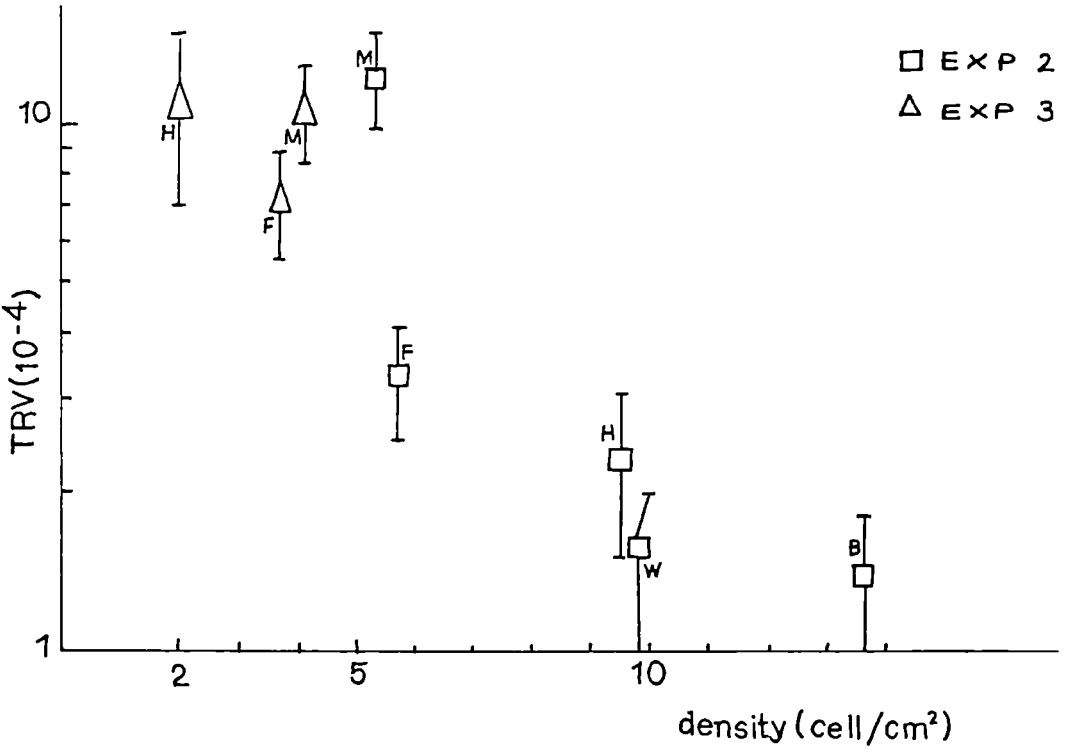
Cells trypsinised to give approximately 100 millilites of cells at a third of a million cells per millilitre. Cell suspension spun down and cells resuspended into 30 millilitres. Cell suspension split 3:1. The larger fraction irradiated with 250 kVp X-rays at 0.8 Gy/min. 1 millilitre of each suspension (control and irradiated) seeded into cryotubes and placed in melting ice at 0øC.

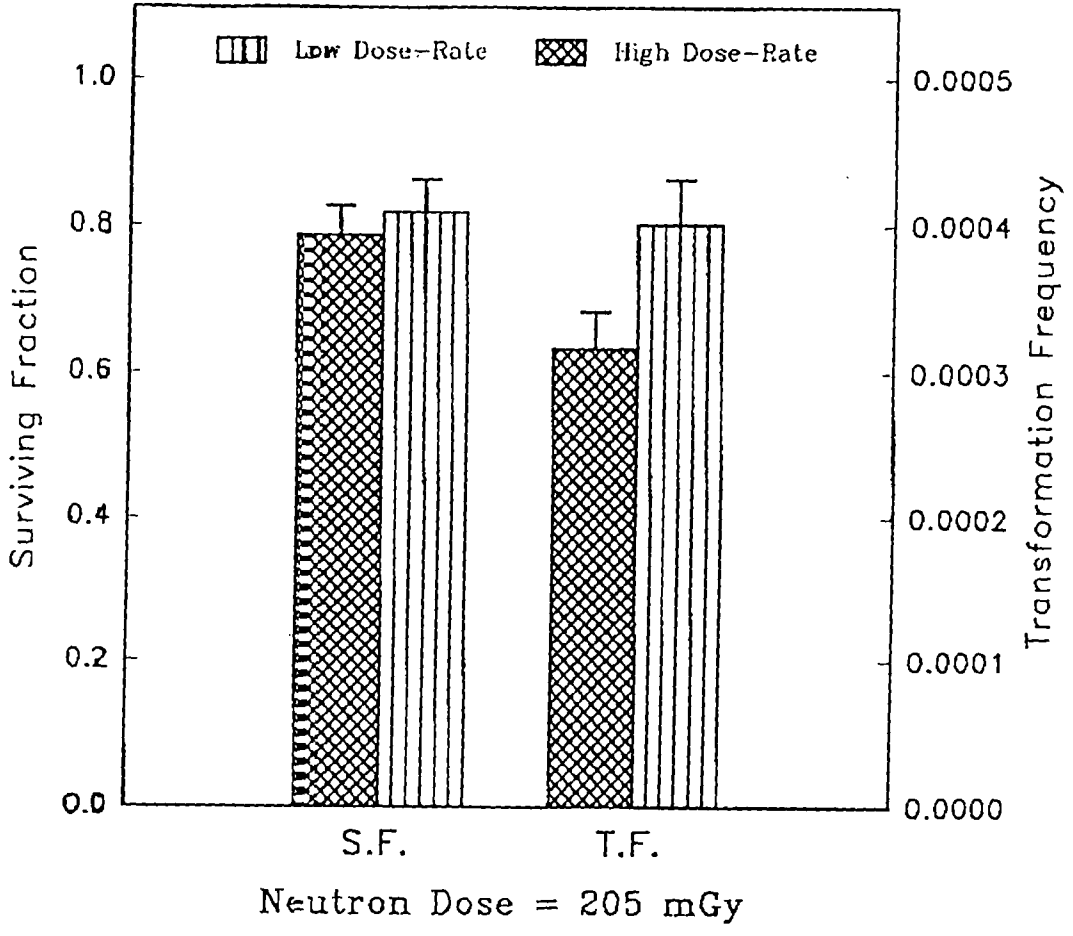

Berkeley medium and
Berkeley serum
Cells despatched
from Berkeley on
melting ice
Arrive 48 - 72 h
after despatch
Survival and transformation
assays split into two groups
14 days
(survival)
Local medium and
local serum
Berkeley serum
All medium changes
carried out using
local medium and:
First change
with 10% serum:
subsequent changes
with 5% serum
6 weeks
(transformation)
local serum

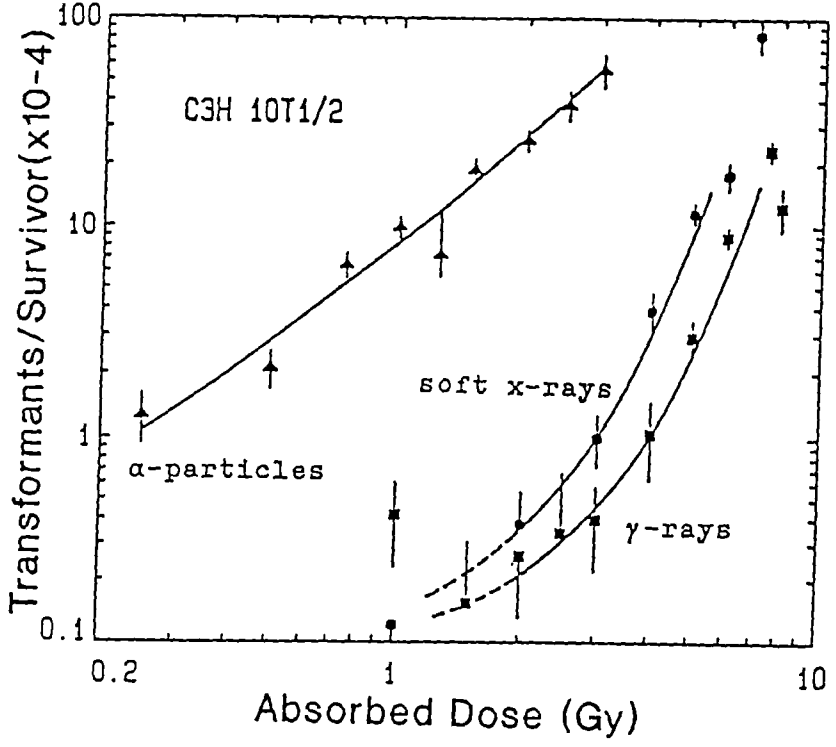

Plating Efficiency /%
0
10
20
30
40
50
60
HAR #3
FRA #2
FRA #3
WUR #1
WUR #2
WUR #3
ROM #2
ROM #3
MIL #2
MIL #3
Local Medium
Berkeley Medium

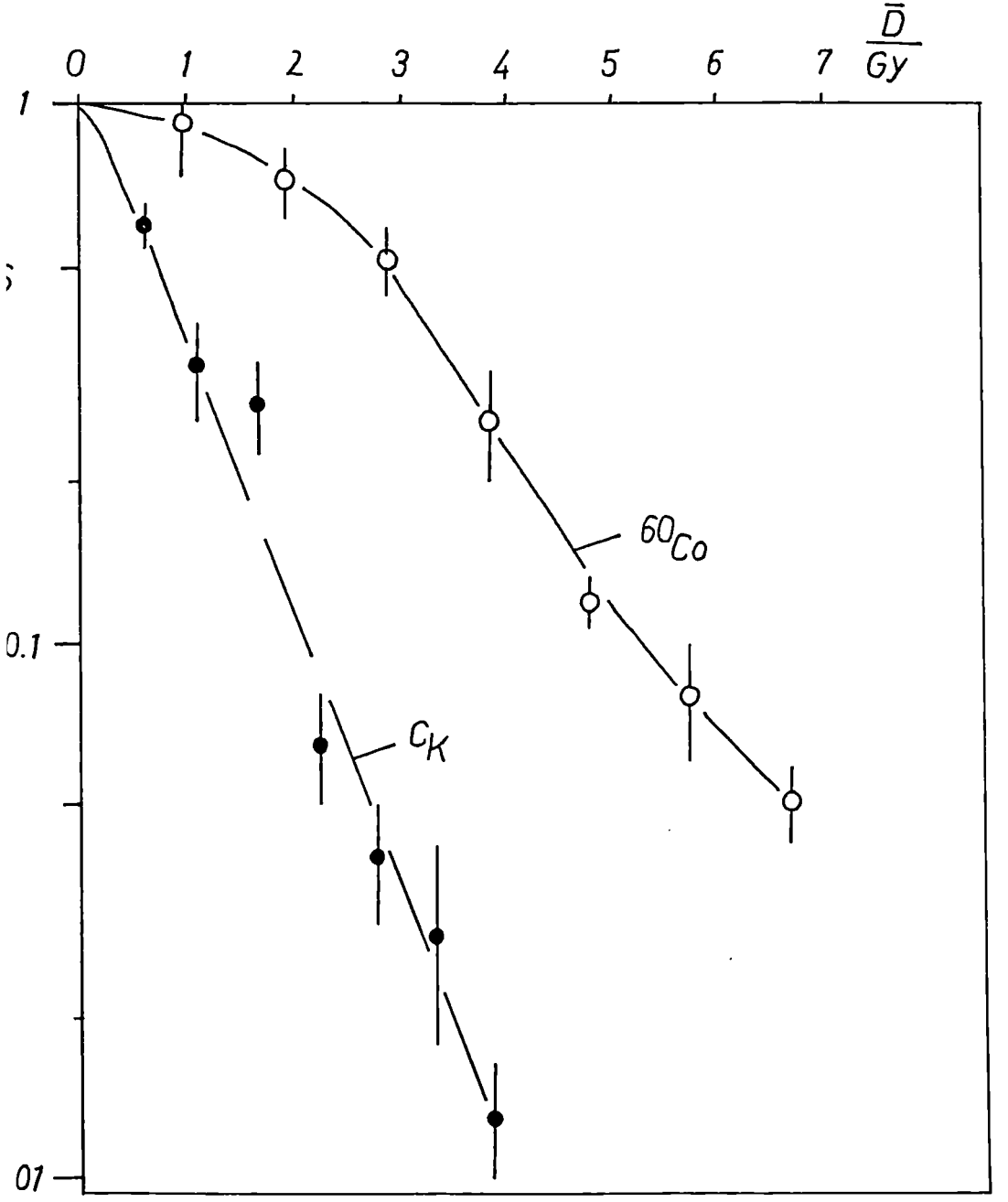

1
Exp. 1
B
H
B
W
F
O
1
Exp. 2
B
B
F
W
F
M
M
R
H
1
Exp. 3
B
B
W
R
F
M
M
H
Survival
0
20
40
60
80
Time (hrs
o Ref. serum
□ Local serum
--- Mean value

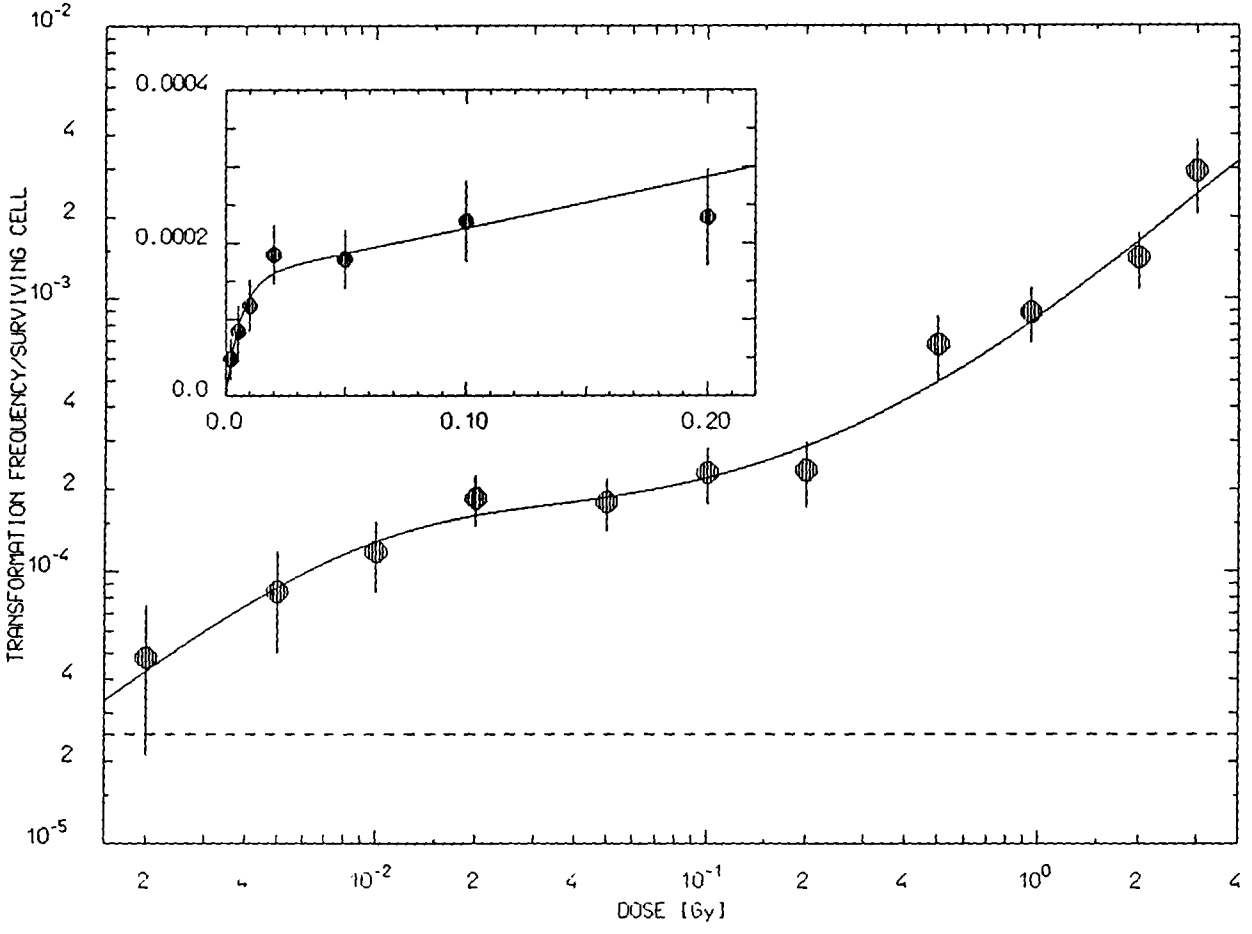

Cell Transformation in vitro: 5 Gy Intercomparison
Transformation Frequency per Viable Cell x 10^4
20
10
9
8
7
6
5
4
3
2
1
2
3
4
5
6
7
8
9
10
20
Viable Cell Density /cm^{-2}
X Berkeley (plated immediately)
+ Berkeley (on ice)
O ● Harwell
▽ ▼ Frankfurt
△ ▲ Wurzburg
◇ ◆ Rome
□ ■ Milan
Open symbols: local medium
Closed symbols: Berkeley medium
857


□ EXP 2
△ EXP 3
TRV(10-4)
10
1
2
5
10
density (cell/cm²)
H
M
F
M
F
H
W
B


Low Dose-Rate
High Dose-Rate
Surviving Fraction
Transformation Frequency
1.0
0.8
0.6
0.4
0.2
0.0
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
S.F.
T.F.
Neutron Dose = 205 mGy


C3H 10T1/2
soft x-rays
α-particles
γ-rays
Transformants/Survivor(x10-4)
Absorbed Dose (Gy)
100
10
1
0.1
0.2
1
10


0
1
2
3
4
5
6
7
$\bar{D}$
Gy
1
0.1
01
^{60}Co
C_K


TRANSFORMATION FREQUENCY/SURVIVING CELL
DOSE [Gy]
0.0004
0.0002
0.0
0.0
0.10
0.20

Progress Report

Contract: Bi6-156 **Sector: B14**
Title: Radiation-induced mutations in mammals

1 Ehling GSF Neuherberg

I. Summary of Project and Global Objectives

Estimates of genetic risk to man from exposure to ionizing radiation of necessity must be based on experimental results obtained in laboratory animals. Two sets of data are required for valid estimation of genetic risk to man from results in other species; (1) those based on a number of genetic endpoints, several of which can be applied in each species studied, and (2) those derived from a number of different species, strains or genotypes, based on one or more genetic endpoints. The key aims of the proposed project will be to provide a better basis for extrapolation of animal data to man. Genetic endpoint, strain and species comparisons will be made, which will provide critical experimental data regarding strategies in extrapolating laboratory animal data to man.

Experiments will be conducted at Neuherberg to systematically compare the spontaneous and radiation-induced mutation rates for recessive specific-locus, dominant cataract and enzyme activity alleles in the mouse as well as a comparison of the mutation rate in the mouse and hamster for dominant cataract and enzyme activity alleles. Specifically, in the mouse and hamster the dominant cataract and enzyme activity control group will be extended until mutations are recovered for both genetic endpoints in both species to provide a more accurate estimation of the spontaneous mutation rate. These data are critical in estimating the radiation doubling dose for these endpoints in germ cells of mammals. The mutation rate following irradiation of spermatogonia will be determined for dominant cataract and enzyme activity alleles in mouse and hamster as well as experiments to measure the radiation-induced mutation rate in oocytes for both genetic endpoints in both species. Finally, the comparison of the radiation-dose response for recessive specific-locus and dominant cataract mutations will be extended. Selected mutations will be characterized at the genetic, biochemical and molecular levels.

Head of Project 1: Dr. Ehling

II Objectives for the reporting period

a) Initiate experiments to extend the control sample size for dominant cataract and enzyme activity mutations in germ cells of the mouse and hamster as well as to compare the radiation-induced dominant cataract, specific-locus and enzyme activity mutation rates in germ cells of mouse and hamster.
b) Characterization of recovered mutations.

III Objectives for next period

a) Extend the control sample sizes for dominant cataract and enzyme activity mutations in germ cells of the mouse and hamster.
b) Comparison of the radiation-induced dominant cataract, specific-locus and enzyme activity mutation rate in germ cells of the mouse and hamster.

IV Progress achieved including publications

Mutation-rate experiments

During the reporting period experiments were initiated to extend the control sample sizes for mutation rate studies in the mouse and hamster as well as to determine the mutation rate following 2+2 Gy (0.75 Gy/min; 24 h fractionation interval) irradiation in male and female mice and hamsters. Sample sizes are still too small to make any meaningful comparison.

Localization of two dominant cataract loci

The dominant cataract test has been employed in the multiple endpoint test procedures to systematically compare the induced mutation rate to recessive visible, dominant visible, electrophoretic and enyzme activity alleles (Ehling et al., Mut. Res. 150, 393-401, 1985). At present, the number of dominant cataract loci in the mouse is estimated to be 30 (Ehling, Induction and manifestation of hereditary cataracts, in: A.D. Woodhead et al [Eds.], Assessment of Risk from Low-Level Exposure to Radiation and Chemicals, Plenum, New York, pp. 345-367, 1985) based upon the number of phenotypically distinct mutations known in man. In order to improve the mutation rate comparisons, the number of loci screened for the different genetic endpoints should be known so that results may be expressed on a per locus basis. Therefore, we have undertaken to identify dominant cataract genes in the mouse by mapping dominant cataract mutations recovered in radiation experiments.

A dominant cataract mutation recovered in a 3+3 Gy spermatogonial irradiation experiment (Favor et al., Mut. Res. 177, 161-169, 1987) has been shown to be X-linked in genetic comfirmation crosses. Employing the gene markers *tabby* (*Ta*) and *glucose-6-phosphate dehydrogenase* (*G6PD*) the locus was shown to be in the distal region of the X-chromosome between *jimpy* and *hypophosphatemia* (Table 1).

Table 1: Linkage analysis of the X-linked cataract mutation in the cross:

Xcat - Ta -+ / *+ - + - G6pd* x *+ - + - +* / *---*

Class	Maternal meiotic product	Offspring
Non-crossover	*Xcat - Ta - +*	176
	+ - + - G6pd	274
Single crossover I	*Xcat - Ta - G6pd*	26
	+ - + - +	47
Single crossover II	*+ - Ta - +*	68
	Xcat - + - G6pd	65
Double crossover	*Xcat - + - +*	3
	+ - Ta - G6pd	3

It is interesting to note that the human X-linked cataract-dental syndrome (Nance-Horan Syndrome) has been shown to map closely to the X-linked hypophosphatemia locus (Stambolian et al., Am. J. Hum. Genet. 47, 13-19, 1990) and would suggest possible homology between the mouse and human X-linked cataract genes. A collaborative effort has been initiated to molecularly characterize the human and mouse X-linked cataract genes.

Two independent mutations have been recovered in irradiation experiments and shown to be alleles (Kratochvilova and Favor, Genet. Res. 52, 125-134, 1988). Linkage studies locate the dominant cataract gene on mouse chromosome 10, 3-4 cM from the marker *Sl* (Table 2).

Table 2: Linkage analysis of *Cat-3* gene:

Class	Meiotic Product	Offspring
Non-crossover	*Cat-3 - +*	175
	+ - Sl	158
Crossover	*Cat-3 - Sl*	4
	+ - +	7

In the most recent list of known mutations in the mouse (Genetic Variants and Strains of the Laboratory Mouse, 2nd Edition, Lyon and Searle, eds., Oxford University Press, 1989), a total of 62 mutations have been identified which affect the eye. Of these 62, 26 have been mapped and 5 of the mapped mutations are dominantly expressed and cause cataract or abnormal lens development. With over 85 independent dominant cataract mutations recovered in mutagenicity experiments in our laboratory, there is a high likelihood that most of the mouse dominant cataract loci are represented in our group of mutations. A first estimate of the number of loci screened based upon phenotypically distinct mutations in man is useful. However, it is not certain if this is an underestimate or an overestimate since we have recently shown that both phenotypically distinct mutations may be allelic as well as that phenotypically similar alleles may be non-alleleic (Kratochvilova and Favor, Genet. Res. 52, 125-134, 1988).

Fitness effects of radiation-induced enzyme-activity mutations

Selected fitness parameters (homozygous viability, segregation ratio and relative fitness) were characterized for a total of 19 radiation-induced enzyme-activity mutations and compared with results for 33 ethylnitrosourea (ENU)-induced enzyme-activity mutations (Table 3).

Table 3: Selected fitness parameters of radiation- and ENU-induced enzyme-activity mutations

Treatment	Homozygous		Segregation ratio			Relative fitness		
	Viable	Lethal	x	SD	n	x	SD	n
Radiation	1	15	0.79	0.25	19	0.98	0.23	19
ENU	15	19	0.96	0.10	33	1.09	0.07	33
p value	<0.05		<0.01			<0.05		

The ratio of mutations which were homozygous lethal was higher following radiation mutagenic treatment than in ENU experiments. Based on specific-locus results, it has been proposed that ENU induces mainly point mutations whereas mutations recovered following radiation treatment are mainly small intergenic deletions (Ehling and Favor, Recessive and dominant mutations in mice, in: E.H.Y. Chu and W.M. Generoso [Eds.], Mutation, Cancer, and Malformation, Plenum, New York, pp. 389-428, 1984). Homozygous lethality may be associated with the involvement of a closely linked lethal factor, or the total loss of enzyme activity at a locus in homozygotes may result in lethality.

Segregation ratio expresses any differential selective pressure on the mutant allele in the heterozygous state up to the time of classification of animals. A value of 1 would be expected in the absence of deleterious effects due to the mutant allele. As mice were classified at or shortly following weaning at 3 weeks of age, effects on segregation ratio would reflect a differential probability of fertilization, implantation, embryonic development, or postnatal survival to weaning. For 12 out of 19 radiation-induced enzyme-activity mutations segregation ratio was reduced, while the segregation ratio of ENU-induced enzyme-activity mutations at the corresponding loci was normal. Kacser and Burns (Genetics, 97, 639-666, 1981) suggested that a reduction of enzyme activity *per se* does not result in deleterious effects of heterozygotes as the amount of enzyme activity is normally much greater than the physiological requirements. Therefore, a reduction by 50% in mutant heterozygotes should not result in deleterious effects. These observations would argue that the nature of the induced mutations is important for associated deleterious effects in heterozygotes: radiation-induced mutations represent multilocus deletions in which closely linked loci may affect differential fertilization or differential survival up to weaning.

Relative fitness is a measure of the probability of procreation of a mutant heterozygote relative to a standard genotype, given that the mutant heterozygote has survived to sexual maturity. Table 3 indicates that the mean relative fitness for enzyme-activity mutations recovered in radiation and ENU experiments is distributed close to the expected value of 1.

An overall measure of fitness for the induced mutations from

fertilization through reproduction would be the product of the two parameters segregation ratio and relative fitness. The mean fitness values for radiation- and ENU-induced enzyme-activity mutations are 0.77 and 1.05, respectively. Thus, the fitness of radiation-induced mutant heterozygotes is reduced and newly induced mutations would eventually be eliminated from the population through selection.

V Publications for the reporting period

Ehling, U.H.: Quantification of the radiation induced genetic risk. In: Chauhan, P.S. (Ed.) Environmental Mutagenesis and Carcinogenesis. EMSI, BARC, Bombay, 1990, pp. 57-70.

Favor, J.: Risk estimation based on germ-cell mutations in animals. Genome 31, 844-852, 1990

Favor, J.: Multiple endpoint mutational analysis in the mouse. Prog. Clin. Biol. Res. 340C, 115-124, 1990

Favor, J., and W. Pretsch: Genetic localization and phenotypic expression of X-linked cataract (*Xcat*) in *Mus musculus*. Genet. Res., Camb., 56, 157-162, 1990

Pretsch, W., and S. Merkle: Glucose phosphate isomerase enzyme-activity mutants in *Mus musculus*: Genetical and biochemical characterization. Biochemical Genetics 28, 97-110, 1990

Contract: Bi6-143 Sector: B14

Title: Mutation studies upon spermatogonial stem cells of mammals and genetic tests for non-disjunction in the mouse

1 Cattanach MRC Radiobiological Unit

I. Summary of Project and Global Objectives

1. Factors affecting the yield of mutation from spermatogonial stem cells in mammals (Cattanach)

Studies upon strain differences in genetic response to radiation may facilitate extrapolation of mouse data to man. The 101/H strain gives a different response from other mice tested. The current objective is to ellucidate the basis of this difference. Other work using fractionated X-ray and combined TEM-X-ray regimes have indicated that the genetic response of spermatogonial stem cells to radiation varies with the interval between the treatments. Studies using combined HU-treatments have also indicated that stem cells in G_1 are the most sensitive to mutation. The objective is to investigate the basis of the genetic responses obtained with different treatment regimes.

2. Experimental studies on non-disjunction (Cattanach)

We have recently developed two systems of genetic (complementation) tests using Robertsonian translocations in tester animals to detect non-disjunction and chromosome loss events in normal mice. The present aim is to evaluate the two methods for detecting chromosome 11 loss, and with one method compare the frequency of chromosomes 11 and 13 loss following X-irradiation of males and females.

3. Radiation-induced genetic damage in mouse oocytes (Tease)

An understanding of factors that influence the response of mouse oocytes to radiation-induced genetic damage may assist human risk assessment. It is not known whether a strain-dependent variability occurs with female germ cells, as found in males. A study has therefore been initiated to compare the incidence of structural chromosome anomalies following X-irradiation of oocytes in two inbred strains.

Previous work has investigated radiation-induced aneuploidy in mouse oocytes through cytogenetic analyses of unfertilised eggs and pre- and post-implantation embryos. Further experiments to detect chromosome loss using a genetic method to complement the cytogenetic studies previously carried out has been initiated.

Head of Project 1: Dr. Cattanach

II Objectives for the reporting period

1. a) To finalise an investigation of the 101/H strain mutation response to a 24h fractionated X-ray dose and initiate a new study with a single dose. b) To investigate the bases of the modified genetic responses to radiation at different intervals following stem cell population depletion. 2. To compare the effectiveness of the Rb and MBH tester methods for detecting chromosome loss in chromosomally normal mice. 3. a) To compare oocyte radiosensitivity in two inbred mouse strains, and b) to determine the rate of induced chromosome loss in mouse oocytes following irradiation of immediately preovulatory cells.

III Objectives for next period

To complete all experiments initiated under the present contract.

IV Progress achieved including publications

1. Factors affecting the yield of mutations from spermatogonial stem cells

a) Strain differences

The results of a 3 + 3 Gy study have now been finalised. The 101/H males had far longer sterile periods than the standard hybrid (median day of return to fertility 119, cf. 76) indicating higher levels of stem cell killing, but the specific locus mutation response was not significantly different (P = 0.42, 1-tailed test). Thus, 13 mutations were recovered among 7,457 offspring of the 101/H males, giving a mutation frequency of 24.91 x 10^{-5}/locus/gamete, while 30 mutations were found among 15,202 offspring of hybrid males, giving a frequency of 28.19 x 10^{-5}/locus/gamete. A follow-up experiment employing single 6 Gy X-irradiation has not yet proceeded far enough to provide meaningful results.

b) Other factors influencing genetic response

Two questions have been posed: (i) Are the stem cells remaining in the testis 24h after population depletion by X-rays or chemical agents uniformly sensitive to genetic damage and cell killing as has been postulated? (ii) Are the stem cells present in the testis 4 or more days after population depletion in the process of repopulating the germinal epithelium? Both questions have been answered by giving HU pretreatments (500 + 500 mg/kg, 3h interval) 3h prior to 6 Gy X-irradiation. Population depletion was brought about by TEM treatment (2 mg/kg). Translocations were the genetic end-

point, and cell killing was assessed by reduction in recovered

Table 1

Group	Treatments, interval	Recovered testis weight (mg)	% cells with translo-cations	Translocations/ cell
1	X-ray	101.44	12.82 ± 1.84	.148
2	TEM + X-ray 24h	78.95	23.08 ± 2.04	.285
3	TEM + (HU + HU, 3h + X-ray), 24h	52.20	25.23 ± 2.15	.297
4	TEM + X-ray, 3h	85.90	9.33 ± 1.45	.103
5	TEM + X-ray, 4d	88.94	9.60 ± 1.62	.110
6	TEM + (HU + HU, 3h + X-ray) 4d	41.10	14.78 ± 1.78	.170
7	X-ray + (HU + HU, 3h), 4d	92.5	10.56 ± 1.57	.119
8	X-ray + (HU + HU, 3h), 24h	97.20	14.24 ± 1.84	.178
9	HU + X-ray, ½h	73.65	10.9 ± 1.51	.130

testis weight. The results are shown in Table 1. Comparison of treatment groups 2 and 3 shows that the HU pretreatments enhanced cell killing 24h after population depletion suggesting that not all cells were in a sensitive non-dividing state but that some cells were in the S phase and therefore subject to killing by HU. However, no associated increase in the translocation yield was observed (P=0.47). The TEM + X-ray, 24h treatment (group 2) gave a much higher yield than obtained in previous studies. Therefore, an heterogeneity may, overall be indicated. Comparison of Groups 5 and 6 shows that the HU enhanced the level of cell killing 4 days after population depletion, again indicating that a substantial proportion of cells must be undergoing division. In this instance an enhanced translocation yield was obtained (P=0.039) consistent with enrichment of G_1 phase cells that are sensitive to translocation-induction by X-rays. Other treatments failed to illustrate any other form of HU-X-ray interactions (Groups 7, 8 and 9 vs Group 1; P=0.35, 0.59 and 0.42 respectively).

2. Experimental studies on non-disjunction in the mouse

The Rb tester method, used to detect chromosome 11 and 13 loss following X-irradiation of chromosomally normal males or females, employed the Rb(11.13)4Bnr translocation carried heterozygously in the tester animals. The chromosome 11 and 13 markers were vestigial tail (*vt*) and satin (*sa*), respectively. Following irradiation with 4 Gy X-rays the normal wild type animals were mated for one week to the tester mice and the progeny screened for the *vt* and *sa* young, these indicated chromosome 11 and 13 loss events. The same mating regime and X-ray dose was used with monobrachial homology (MBH) tester

method but here the tester animals carried both the Rb(11.13)4Bnr and RB(10.11)8Bnr translocations heterozygously. Chromosome 11 loss again was detected using *vt* as the marker.

Table 2 presents the results obtained to date. Chromosome 11 loss was detected in all 4 types of cross but the frequencies were generally lower than found with several other chromosomes previously tested. In females at least, the MBH tester system appears to be more effective than the Rb tester system for detecting chromosome 11 loss. Difficulties were encountered in breeding from Rb tester females, necessitating re-establishment of the stock on a more vigorous background. This has now been achieved and the experimental work is again in progress (Cross 4).

Table 2

Cross	Test system	Sex of parent irradiated	Chr tested	No progeny scored	No marked	% marked
1	MBH tester	♀	11	916	2	0.22
2	Rb tester	♀	11 13	3378 1429	3 0	0.09 -
3	MBH tester	♂	11	1750	2	0.11
4	Rb tester	♂	11 13	775 16	1 0	0.13 -

3. Radiation-induced genetic damage in mouse oocytes

Two experiments were initiated:

a) Female mice of the inbred strains C3H/HeH and 101/H were given either 2 or 4 Gy of acute X-rays. Fourteen days after treatment the females were induced to ovulate and metaphase I stage cells collected for assessment of induced chromosome damage. Age-matched unirradiated females were used as controls. The slides prepared from control and irradiated females were coded and randomised prior to analysis. To date, approximately 600 oocytes from control and irradiated females have been prepared for screening. As analysis is not yet complete, the experiment has not been decoded.

b) Female mice of the F_1 hybrid type C3H/HeH x 101/H were induced to ovulate and 3 hours after HCG, when the oocytes were at diakinesis of meiosis, they were given 1 Gy of acute X-rays. The females were mated overnight to males of the genotype Rb(8.19)1Ct *ru*/ Rb(9.19) *ru* and their offspring screened for the *ru* phenotype which occurs when maternal loss of chromosome 19 is complemented by paternal gain. The data obtained to date are summarised in Table 3 below. The data clearly demonstrate the ability of X-rays to induce chromosome 19 loss in immediately preovulatory oocytes. The rate of chromosome loss

is greater than that found in earlier experiments where dictyate stage oocytes were given 4 Gy of X-rays (Tease and Fisher, unpublished observations). This trend is consistent with the expectations of cytogenetic analyses which have shown

Table 3

Treatment group	Number of offspring classified	Number of *ru/ru* offspring	Dominant mutations
Control	971	0	0
1 Gy	1068	3	1

that immediately preovulatoryoocytes are considerably more sensitive to radiation-induced structural chromosome anomalies than cells at earlier stages of germ cell development.

Publications

Cattanach, B.M., Rasberry, C. and Beechey, C.V. Factors affecting mutation-induction by X-rays in the spermatogonial stem cells of mice of strain 101/H. Banbury Report, No 34: Biology of Mammalian Germ Cell Mutagenesis: Cold Spring Harbor Laboratory Press pp. 209-220 (1990).

Zsebo, K., Williams, D.A., Geissler, E.N., Broudy, V.C., Martin, F.H., Atkins, H.L., Hsu, R.Y., Birkett, N.C., Okino, K.H., Murdock, D.C., Jacobsen, F.W., Langley, K.E., Smith, K.A., Takeishi, T., Cattanach, B.M., Gall, S.J. and Suggs, S.V. Stem cell factor is encoded at the *Sl* locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell*, **63**: 213-224 (1990).

Cattanach, B.M., Rasberry, C., Evans, E.P. and Avner, P. Genetic and molecular evidence of an X-chromosome deletion spanning the tabby (*Ta*) and testicular feminization (*Tfm*) loci in the mouse. *Cytogenet. Cell Genet.* (In press).

Brockdorf, N., Kay, G., Cattanach, B.M. and Rastan, S. Molecular analysis of the Ta^{25H} deletion: evidence for additional deleted loci. *Mammalian Genome*, (In press).

Evans, E.P., Burtenshaw, M. and Cattanach, B.M. Deletions at the *Sl* locus. *Mouse Genome*, **86**: 230 (1990).

Jones, J., Peters, J., Ball, S., Cattanach, B.M. and Kwon, B.S. Molecular analysis of germline mutations at the albino locus of the mouse. *Mutagenesis*, **5**: 90 (1990).

Progress Report

Contract: Bi6-166 **Sector:** B14
Title: Radiation-induced genetic effects in germ cells of mammals

1 Van Buul Univ. Leiden Sylvius Lab.

I. Summary of Project and Global Objectives

The project is aimed at gaining information on the effects of ionizing radiation on germ cells of rodents and primates as measured by induced chromosomal translocations. Such information will facilitate a better use of animal data to estimate genetic risks due to exposure of human populations.

Head of Project 1: Dr. Van Buul

II Objectives for the reporting period

Different aspects of the very significant interspecies differences between the mouse and the rhesus monkey (Macaca mulatta) for translocation induction in spermatogonial stem cells were studied. In addition, possible mechanisms for the well established reduced transmission of induced mouse translocations were investigated.

III Objectives for next period

To gain more information in the mouse about meiotic delay of translocation carrying cells at a relative low exposure level of 2 Gy X-irradiation. Furthermore, the influence of the biology of spermatogenesis on the induction of translocations will be studied using the mouse mutants steel (Sl) and dominant spotting (W) with 'primate type' of spermatogenesis.

IV Progress achieved including publications

In the rhesus monkey the effects of combined treatments with FSH (54 I.U./ kg/week) and X-rays (1 Gy) were examined. A nonsignificant decrease of 30% in the frequency of induced translocations was recorded for follicle-stimulating hormone (FSH) pretreated animals. Comparison of these translocation data with studies on cell killing in the same monkeys show that the ratio between the probabilities that radiation induced basic lesions kill a cell or produce translocations is about 10 : 1. This value of 10 is very similar to that observed for the mouse or calculated on theoretical grounds.

All data so far obtained in the rhesus monkey (dosis-effect relationship, dose-rate effect, the effect of radiation quality, etc.) suggest that testicular repopulation after radiation damage in rhesus monkeys is mainly responsible for the observed differences between mouse and monkey. There is also evidence available that the recovery of radiation damage in the rhesus monkey is comparable to that seen in steel (Sl) and dominant spotting (W) mutations in the mouse. Preliminary data obtained by us point to a recovery of translocations from 3 Gy irradiated $W^v/+$ (viable allele of dominant spotting) and Sl^{con}/Sl^{con} (contrasted allele of steel) male mice which is comparable to the low frequencies observed in the rhesus monkey.

Using <u>in vivo</u> ^{3}H-thymidine pulse labelling of spermatocytes from mice irradiated with different doses of X-rays (6 and 7 Gy), we were able to demonstrate that cells having translocations derived from irradiated stem cells, tend to spend longer times at the meiotic prophase than normal cells. Preliminar data indicate that at the 2 Gy level this effect is much less pronounced. The recorded delay forms a good explanation for the reduced transmission of translocations to the next generation observed by others.

Publications

Buul, P.P.W. van and J.H. Goudzwaard (1990) The relation between induced reciprocal translocations and cell killing of mouse spermatogonial stem cells after combined treatments with hydroxyurea and X-rays, Mutation Res., 243, 259-266.

Buul, P.P.W. van, A. Léonard and J.H. Goudzwaard (1990) Dose-effect relationship for X-ray induced reciprocal translocations in mouse spermatogonia following pretreatment with 3-Aminobenzamide, Mutation Res., 232, 273-280.

Buul, P.P.W. van and C.M.J. Seelen (1990) Meiotic delay as possible cause for reduced transmission of radiation induced translocations to the next generation (Abstract), Int. J. Radiat. Biol., 58, 1047.

Buul, P.P.W. van and C.M.J. Seelen (1990) The relationship between induced translocations and cell killing of rhesus monkey spermatogonial stem cells after combined treatments with follicle-stimulating hormone and X-rays, Mutation Res., in press.

Rooij, D.G. de, Y. van der Meer, A.M.M. van Pelt and P.P.W. van Buul (1990) Correlation between proliferative activity of mouse spermatogonial stem cells and their sensitivity for cell killing and induction of reciprocal translocations by irradiation, In: Banbury Report 34, Biology of Mammalian Germ Cell Mutagenesis, p. 35-49.

~ Progress Report

Contract: Bi6-069 **Sector: B14**
Title: Radiation-induced genetic effects in germ cells of mammals

1 Jacquet CEN - SCK

I. Summary of Project and Global Objectives

This project is mainly concentrated on the radiosensitivity of the "resting" oocytes, which represent some 90% of the total population of oocytes in the ovary and are the most important female germ cells from the genetic point of view, since they receive the largest part of the genetically significant lifetime dose of radiation.
Up to now, most studies on radiosensitivity of the mammalian germ cells have been carried out on mice, for which inbred strains and genetic markers are available. However, the extreme sensitivity of the mouse resting oocytes to killing by radiation renders their study very difficult and has stimulated additional studies on other mammalian species, in order to obtain a better picture of the potential genetic hazard of radiation for man.
For such studies, the guinea-pig appears to be an interesting model, due to the apparently high resistance of its resting oocytes to cell killing (their LD50 could be comparable to that of humans) and to the fact that it possesses two distinct populations of resting oocytes at diplotene : an oocyte with a large nucleus, comparable to that of other mammals including man, and another with a contracted nucleus, which appears a few days after birth and which predominates as the animal ages.
With this project, we intend firstly to contribute to an evaluation of the radiosensitivity of the resting oocyte of the guinea-pig at its two different nuclear states. The study will be completed by an evaluation of the radiosensitivity of the immature oocyte at earlier stages of oogenesis, that is during embryonic life in utero.
Several parameters can be used to assess the radiosensitivity of the germ cells : our project concentrates on long-term reproductive effects and on cytogenetic effects. For the long-term reproductive effects, female guinea pigs will be exposed during intra-uterine life, early postnatal life or adult life to different doses of X-rays. 6 and 12 months after treatment, the fertility of the females will be tested by mating them with untreated males. The studied parameters will include : number of pregnant animals, pre- and post-implantation deaths, weight and normality of the live embryos, effects on oocyte killing. With regard to the cytogenetic studies, females will be X-irradiated during early postnatal or adult life and the yield of translocations induced in their two populations of resting oocytes will be analyzed at the age of one year.

Head of Project 1: Dr. Jacquet

II Objectives for the reporting period

- To perform a preliminary study in order to : 1) acquire an expertise in the basical manipulations of guinea-pigs (mating, estrus control, vaginal smears, etc...) ; 2) determine the doses of X-rays to be used in the long-term and cytogenetic experiments ; 3) adapt some techniques used in the mouse to the guinea-pig : counting of corpora lutea, fixation and staining of the ovaries, identification of the various stages of oogenesis and their chronology, culture of the oocytes to the first and second meiotic metaphases, cytogenetic preparation of their chromosomes.
- To irradiate female guinea-pigs at the chosen times and with the chosen doses of X-rays, and to observe the first effects on reproduction, 6 months after irradiation.

III Objectives for next period

- To complete our studies on the reproductive effects of radiation, 6 and 12 months after treatment of newborn animals, adult animals (pregnant + not pregnant) and fetuses in utero.
- To perform cytogenetic analysis of metaphase I oocytes irradiated during the neonatal life (resting oocytes of the "large" type) or the adult life (resting oocytes of the "contracted" type).
- To develop a method allowing in vitro fertilization of guinea-pig oocytes, for future studies on the transmission of chromosome aberrations to the preimplantation embryo.

IV Progress achieved including publications

1. Preliminary studies on the guinea-pig oocyte

1.1. Histological study of oogenesis in the guinea pig

Before starting this study, a comparison was made between various fixation and staining procedures. For our purpose, the best histological preparations were obtained when ovaries were fixed with Bouin, cut at 3 µ and stained with the "triple staining of A. Prenant" (Heidenhain's iron hematoxylin + erythrosin + light green). Ovaries which were examined, were derived from a number of control embryos and fetuses aged 26, 32 and 41 days post coitum (p.c.) [total duration of pregnancy : ± 68 days], and from control animals aged 0 - 1 days, and 4, 8 and 12 months post partum (p.p.).

The results of this study were in general agreement with those reported earlier by Ioannou. Ovaries of embryos aged 26 days contained only oogonia, either in interphase or in mitotic prophase or metaphase. Ovaries of 32 days showed the additional presence of oocytes in the first stages of meiotic prophase (leptotenes + a few zygotenes), while those of 41 days showed oogonia and all stages of meiotic prophase, with a great majority of zygotenes and pachytenes and very few diplotenes. Newborn animals (0 - 1 days) possessed only diplotene oocytes of the "large" type, plus low numbers of maturing oocytes. Some of them were already invested in Graafian follicles (five or more layers of granulosa cells surrounding an antrum). In adult animals (from 4 months p.p.), the "large" diplotenes were generally replaced by those of the "contracted" type (85-95% of the total population of resting oocytes), but one animal on the three examined at 4 months still possessed as much as 75% of resting oocytes of the "large" type.

1.2. Development of a method for obtaining chromosome preparations of metaphase I and II oocytes of the guinea-pig

In the guinea-pig, the only possibility to dispose of sufficient numbers of oocytes for cytogenetic studies is to induce their maturation in vitro. The techniques which were found to give the most satisfactory results were modified from that of Yanagimachi for the culture of guinea-pig oocytes and from those of Tarkowski and of Morrison for the fixation of the meiotic preparations.
Oocytes are obtained by puncturing the large follicles, and they are cultured in Yamada's medium, a medium which is used in our laboratory for the culture of mouse oocytes and preimplantation embryos. Like for the mouse and the rat, it was experimented that oocytes must have reached a certain critical size to be able to resume meiosis in culture. Such meiotically competent oocytes are incubated in culture medium for 6 hours, when first meiotic metaphases have to be obtained, and for 20 hours when second meiotic metaphases must be prepared. At the end of the culture, oocytes are still surrounded by the cumulus cells. Removal of these cells and of the very thick zona pellucida is difficult and represents the most critical step of the techniques, since oocytes without their zona pellucida may become very fragile and easily burst when placed on the slide for fixation, unless appropriate modifications are made to hypotonic treatment. The cytoplasm of the guinea-pig oocyte contains a great amount of yolk globules which may also considerably interfere with the spreading of the chromosomes and the quality of the preparations, if they cannot be discarded during fixation. Good chromosome preparations were obtained after repeated assays, which were performed on about 170 cultured oocytes.

An important conclusion of this study was that cytogenetic analysis of the guinea-pig oocytes must reasonably be limited to the first meiotic metaphases. Indeed, most preparations of second meiotic metaphases exhibited 64 univalent chromosomes instead of 32, resulting from the simultaneous presence of the chromosomes of the oocyte nucleus and of those of the first polar body. In some cases, these chromosomes remained separated in two sets of which the origin could not easily be ascertained, due to their very similar aspect. More often however, all chromosomes appeared mixed in a single set of 64 chromosomes. Sometimes, only one set of 32 univalents was obtained, but the proportion of such preparations was much too low to permit reasonable cytogenetic investigations.

1.3. Results of the preliminary experiments : influence of radiation on the fertility and oocyte populations in the ovaries, 4 months after treatment

Restricted numbers of female guinea-pigs were irradiated at various times of pregnancy with either 2 or 6 Gy of X-rays, and the influence of this treatment on delivery and subsequent fertility 4 months after treatment was examined. Fetuses irradiated in utero were also tested for their fertility 4 months after treatment, as well as one animal irradiated with 2 Gy on day 0.5 p.p. At the end of this short experiment, ovaries were taken from a few animals of each group, and processed for histological examination. The influence of the various treatments on the different oocyte populations was determined.

The conclusions of this experiment may be summarized as follows :
- Irradiation with 6 or 2 Gy before day 32 of pregnancy seems to be lethal for the embryos.
- After that time, irradiation of adult (pregnant) animals as well as of fetuses with a dose of 2 Gy does not seem to produce marked effects on the oocyte populations and still allows pregnancy 4 months after treatment (both groups).
- Irradiation of adult (pregnant) animals as well as of fetuses with a dose of 6 Gy strongly reduces the number of resting and maturing oocytes. However, this effect still allows pregnancy 4 months after treatment (both groups).
- Irradiation of the newborn with a dose of 2 Gy strongly reduces the number of resting oocytes and could increase the proportion of "large" resting oocytes. However, this effect still allows pregnancy 4 months after treatment (restriction : only 1 animal studied).

2. Treatment of the animals

On the basis of the results of the preliminary experiments, doses of 2 and 4 Gy were chosen for the long-term reproductive study. These doses were administered to adult (6-8 months) pregnant and not pregnant animals, fetuses (32 days p.c.) in utero, and newborn (0 - 1 days p.p.) animals. According to our preliminary histological study, the target cells were, respectively, the "contracted" resting oocytes (adult animals), the oogonia + oocytes at the beginning of meiotic prophase (fetuses in utero) and the "large" resting oocytes (newborn animals). For each of the treated groups and their corresponding controls, 25 female animals were used. It was planned to test the fertility of the animals 6 and 12 months after irradiation.

With regard to the study on the cytogenetic effects of radiation in resting oocytes, the dose of 1 Gy was chosen. Indeed, it was not sure that larger doses delivered just after birth would enable a sufficient number of oocytes to survive and be analyzed a long time after irradiation. 20 females were irradiated on the day of birth (target cells : "large" resting oocytes) and 20 others at 6 months (target cells : "contracted" resting oocytes). All animals will be killed at the age of 1 year, and their oocytes will be cultured to the first meiotic metaphase and examined for the presence of translocations.

3. First results of the long-term reproductive study

To date, results have been obtained for guinea-pigs irradiated at day 0.5 p.p. and mated at the age of 6 months. No difference could be seen between the 3 groups (0,2 and 4 Gy) for the different tested parameters (percentage of females giving youngs ; number of youngs/female ; weight of the youngs at birth). In addition, all youngs were normal. Ovaries of a few surnumerary animals were analyzed at the same time for the effects on the oocyte populations.
Irradiation with 2 Gy had apparently not inhibited the transformation of "large" oocytes into "contracted" oocytes, and the number of oocytes at the different stages was clearly not diminished 7-8 months after treatment. In animals given 4 Gy, a diminution of the number of resting oocytes was apparent in 2 of the 3 animals studied, the proportion of "large" oocytes to "contracted" oocytes being not altered.

4. Publications

P. Jacquet and S. Grinfeld (1990) Influence of some methodological factors on the radiosensitivity of the mouse zygote. Teratology 42, 453-462.

Contract: Bi6-077 **Sector: B14**
Title: Radiation-induced genetic effects in germ cells of mammals

1 Streffer Universitätsklinikum Essen

I. Summary of Project and Global Objectives

The possibility of the induction of malformations after radiation exposure during pregnancy is a serious radiation hazard. Numerous studies have shown that teratogenic effects are primarily induced, when radiation exposure has taken place during organogenesis. However, there have also been reports from animal experiments on an increased frequency of abnormal fetuses after exposure during the preimplantation stage. This result is strongly dependent on the mouse strain.

From the point of view of radiation risk, it may be even more important to look for a potential teratogenic risk after radiation exposure of germ cells, because they are at risk over the whole reproductive life time. That such a teratogenic risk actually exists has been demonstrated for mice by Nomura and by Kirk and Lyon.

Studies in our institute during recent years have shown that one of our mouse strains ("Heiligenberger", NMRI like) does respond with a higher frequency of malformations after radiation exposure during the preimplantation stage. The fact that malformations, which are observed on day 19, are inducible even in the one-cell stage suggests, that there is an effect on the genetic material and that this effect is inherited over a number of cell generations. It is conceivable that such an effect on the genetic material can also result from the irradiation of germ cells.

Thus, the global objectives of the project refer to radiation exposure of germ cell stages with different radiation qualities (X-, gamma- or beta-rays) and different dose rates (1 Gy/min and below 0.01 Gy/min in order to avoid lethal effects on the sensitive immature female germ cells). The uterine content is examined on day 19 of gestation as to early and late resorptions, dead and malformed fetuses and fetal weights are determined.

Head of Project 1: Prof. Streffer

II Objectives for the reporting period

1. Impact of X-rays (1 Gy/min; dose range 0.5-3 Gy) on fetal damage, in particular macroscopically visible malformations, after exposure of oogenesis stages (weeks 1 to 4 before copulation).
2. First experiments with gamma-rays (^{137}Cs; 3.25 mGy/min).

III Objectives for next period

1. Impact of X-rays (1 Gy/min; dose range 0.5-3 Gy) on skeletal alterations in fetuses after exposure of oogenesis stages (weeks 1 to 4 before copulation).
2. Impact of gamma-rays (dose rate below 0.01 Gy/min) on fetal damage after exposure of oogenesis stages.

IV Progress achieved including publications

INTRODUCTION

Previous experiments have shown that one of the mouse strains kept in our Institute (Heiligenberger mice; inbred strain; similar to NMRI-mice) does respond with a higher frequency of malformations after radiation exposure of preimplantation stages. From the point of view of radiation risk, exposure of germ cell stages may be even more important, because these cells are at risk over the whole reproductive life time, whereas the preimplantation period lasts only a couple of days.

METHODOLOGY

Female mice were irradiated either with X-rays (dose rate 1 Gy/min), gamma-rays (^{137}Cs; dose rate 3.25 mGy/min) or sham-irradiated. Mating started immediately after radiation exposure or, in some cases, after a delay of two weeks. Plug control was carried out every morning and those females with a vaginal plug (unequivocal sign of copulation) were singled out. 19 days after copulation (day of copulation = day 1), the mice were killed by cervical dislocation and the uterine content checked for early resorptions, late resorptions, late fetal death, surviving fetuses, and fetuses with macroscopically visible malformations.

RESULTS

1. Exposure to X-rays (high dose rate experiments)

Even after the lowest dose (0.5 Gy) used in the experiments pregnant females were obtained only during the first four weeks after radiation exposure, irrespective of whether mating started immediately after radiation exposure or with a delay of two weeks. This result is in line with previous observations reported in the literature and reflects the high radiation sensitivity of oocytes after high dose rate exposures.

Table 1 summarizes the results of the X-ray experiments. The data of the four weeks are pooled, though there is some fluctuation from week to week. This time dependence will be discussed in a future report, when more information has been collected.

Table 1: Fetal damage after radiation exposure of oocytes to X-rays (copulation within the first four weeks after radiation exposure).

Dose (Gy)	Total litter loss	Impl./Mice checked	Early[a] resorpt.	Late[a] resorpt.	Late[a] deaths	Malformations[b]
0	23.2%	864/109	6.5% (56)	1.0%(9)	0.7%(6)	4.9% (39)
0.5	22.7%	227/ 34	7.1% (16)	0.9%(2)	1.3%(3)	3.4% (7)
1	26.5%	530/ 75	9.6% (51)	1.5%(8)	0.8%(4)	6.4% (30)
1.5	29.5%	236/ 31	9.3% (22)	1.3%(3)	0.9%(2)	6.2% (13)
2	25.0%	514/ 75	18.5%*(95)	1.4%(7)	0.4%(2)	9.0%*(37)
3	55.6%*	292/ 48	29.5%*(86)	3.1%(9)	0.3%(1)	17.3%*(34)

[a] Per implantation site

[b] Per living fetus

* Significantly different from control at $P<0.01$

Table 1 shows that total litter loss is very pronounced in the controls. This fact presumably depends on the inbreeding of the strain, because in previous years, when we used colony-bred mice, the amount of total litter loss never exceeded 10%. Only after the highest dose (3 Gy) a significant increase is observed. The number of early resorptions is enhanced after doses of 2 and 3 Gy, whereas late resorptions and late fetal deaths are not affected by radiation exposure of oocytes. Similar to our experience with preimplantation stages, an increase of malformed fetuses was found and, also comparable to preimplantation exposure, gastroschises were seen almost exclusively. Radiation sensitivity of oogenesis is somewhat lower than that of 1-cell embryos, but comparable to preimplantation stages succeeding the 1-cell stage.

2. Exposure to gamma-rays (low dose rate experiments)

Up to now only a few experiments have been carried out. Therefore, only very preliminary information is available. It seems that according to the literature, the impact on prenatal death is extremely small or even absent. Malformations, however, are detectable also after this low dose rate exposure.

PUBLICATION

Müller, W.-U. and Streffer, C.
Lethal and teratogenic effects after exposure to X-rays at various times of early murine gestation.
Teratology 42 (1990) 643-650

Progress Report

Contract: Bi7-048 **Sector: B14**
Title: Radiation-induced genetic effects in germ cells of mammals

1 Van der Schans TNO-Medical Biological Lab.

I. Summary of Project and Global Objectives

The project aims at a better understanding of the fundamental principles that determine the radiation sensitivity in humans, with specific attention for the role of DNA repair in germ cells. Such knowledge is important for assessing relative radiation risk of individual persons, because people may exhibit considerable differences in their response to ionizing radiation.
In this project, the induction and repair of damage in DNA of germ cells of the Syrian golden hamster exposed to ionizing radiation is studied at biologically relevant doses.
These studies require the development of more sensitive and advanced techniques for determining radiosensitivity within the normal dose range, which approach therefore will be pursued. We shall also investigate which aspects of DNA sequence or chromosomal organisation are important with respect to their influence on the repairability of DNA damage.

The project is part of the ongoing programme of the Department of Genetic Toxicology of TNO Medical Biological Laboratory. The three main topics of this department are:
a) Assessment of the induction and repair of DNA damage *in vitro* and *in vivo* in cells exposed to genotoxic agents, in relation to genetic and/or phenotypic effects, b) Establishment of reliable procedures for biological monitoring of man potentially exposed to genotoxic agents, c) Development of methods for the dosimetry of exposure to ionizing radiation on the basis of biological effects, e.g. induction of lesions in DNA of blood cells.

This project is part of a project coordinated by Dr Favor, Neuherberg, Germany.

Head of project 1: Dr. van der Schans

II Objectives for the reporting period

i: Detection of the induction and repair of single-strand DNA breaks (including alkali-labile sites) in germ cells of the Syrian golden hamster exposed to ionizing radiation at different stages of the spermatogenesis.
ii: Development of a method for the detection of base damage in DNA in mammalian cells exposed to ionizing radiation at biologically relevant doses.

III Objectives for next period

i: Detection of the induction and repair of base damage in DNA of germ cells of the Syrian golden hamster exposed to ionizing radiation at different stages of the spermatogenesis.
ii: Development of a method to detect single-strand DNA breaks at the single-cell level by means of quantitative immunofluorescence microscopy.

IV Progress achieved, including publications

Induction and repair of damage
Exposure of cells to ionizing radiation results in damage to the DNA. This damage comprises strand breaks and base modifications. These damages may lead to mutagenesis and carcinogenesis or, when induced into germ cells, to genetic abnormalities and other hereditary effects in the offspring. It is important, therefore, to inventory and quantify the various damages to get information about their relative contribution and persistency. To this purpose we are developing sensitive immunochemical and biochemical methods to quantify single-strand breaks, alkali-labile sites and base damages. The immunochemical method is based on the binding of a monoclonal antibody to single-stranded DNA. The technique is based upon the determination of the percentage single-strandedness resulting from the partial unwinding of cellular DNA under strictly controlled alkaline conditions. Strand breaks and alkali-labile sites form initiation points for the unwinding. The extent of unwinding is a measure of the number of such sites. The results are compared with those obtained with "alkaline elution", which assays the extent of unwinding on the basis of the rate at which the DNA passes through the pores of a membrane filter in an alkaline elution fluid. The usefulness of these approaches to detect single-strand breaks was demonstrated by detection of damage and its repair in unlabelled DNA-containing cells of the human blood after *in vitro* and *in vivo* exposure to ionizing radiation. Single-strand breaks could be assayed with both techniques down to doses as low as 0.5 Gy. Subsequently, we applied this method on germ cells of the Syrian golden hamster exposed to ionizing radiation at different stages of spermatogenesis. Until now, five stages of the spermatogenesis were investigated, the mid and late spermatocytes, the early and mid spermatids and the elongated spermatids. It was found that at all stages of spermatogenesis there is a fast repair of single-strand breaks except in the latest stage, the so-called "elongated spermatids", before the differentiation to spermatozoa. Both after *in vitro* and *in vivo* irradiation, up to 90 min after exposure, no removal of single-strand breaks was observed in the elongated

spermatids.

Spermatozoa, which are easily obtainable from male morning urine, probably could provide a biological indicator of radiation damage or injury that is well suited for practical application. So far it was not possible, however, to use the immunochemical method with spermatozoa, because the DNA stays in the condensed nucleus when the cells are brought into the alkaline solution. Therefore modifications of the immunochemical method have to be introduced, in order to make these cells accessible for investigation of DNA-damage induction and repair.

Base damages can be quantified in a similar way with alkaline unwinding, when it is preceded by treatment of the DNA with damage-oriented endonucleases (i.e. a *Micrococcus luteus* extract). These enzymes recognize base damages in the DNA, upon which they will introduce a break, or remove the modified base thereby leaving an alkali labile site. This break or alkali labile site can be detected by means of "alkaline elution". In the experiments performed, up to now, base damage could be detected after *in vitro* irradiation of human blood in the dose range of 1.5 to 25 Gy. After 5 Gy, measurable base damage was still present at 1.5 h after exposure. Also leukemia patients undergoing chemo- and radiotherapy were investigated. These patients were exposed to Endoxan and total body irradiation. Base damage induced by doses of 4.5 to 8.6 Gy could be detected, even at 90 min after irradiation.

We are now trying to apply these techniques on germ cells of the Syrian golden hamster.

Female germ cells

In the TNO Medical Biological Laboratory a technique has been developed to detect DNA damages at the single-cell level. This technique uses monoclonal antibodies directed against specific DNA damages. The antibodies have a fluorescent label which can be detected by making use of a laser scan microscope. The intensity of fluorescence is a measure for the amount of damage in the cell. It was possible to detect DNA adducts in cultured cells (V79, CHO and HeLa cells) as well as in human skinbiopsies that had been exposed to genotoxic chemical agents or to UV light. Because little is known about DNA damage-processing in oocytes, we wish to collect more information about the induction of damage and the repair mechanisms operating in the oocyte. The immunofluorescence method can be used to study DNA-damage induction and repair in oocytes. The advantage of the immunofluorescence method is that only a few cells are required which is a prerequisite when a study of mammalian oocytes is intended (mice and monkey).

Publications

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Loon, A.A.W.M. van, R.H. Groenendijk, G.P. van der Schans, P.H.M. Lohman and R.A. Baan (1991) Detection of base damage in DNA in human blood exposed to ionizing radiation at biologically relevant doses. Int. J. Radiat. Biol., 59, 651-660.

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Loon, A.A.W.M. van, G.P. van der Schans, R.H. Groenendijk, P. den Boer, A.J. Grootegoed, P.H.M. Lohman and R.A. Baan (1989) Induction and repair of DNA-damage in different stages of the spermatogenesis of the Syrian goldhamster as detected with an immunochemical assay. Abstract Ned.Ver. Rad. Biol. meeting, March 1990, Utrecht, Int.J. Radiat. Biol. 55, 1040, and Book of abstracts 22nd meeting Eur. Soc. Radiation Biology, September 1989, Brussels, p. 93 (abstract).

Loon, A.A.W.M. van, F.C. Raadsheer, A.J. Timmerman, P. Muus, G.P. van der Schans, R.A. Baan, P.H.M. Lohman, 1990. Detection of single-strand breaks and base damage in DNA of human white blood cells after *in vitro* or *in vivo* exposure to ionizing radiation at biologically relevant doses. Abstract Ned.Ver. Rad. Biol. meeting, March 1990, Utrecht, Int.J. Radiat. Biol. 58, 1046.

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Schans, G.P. van der, A.A.W.M. van Loon, A.J. Timmerman, F.J.A. Kouwenberg, R.H. Groenendijk, P.H.M. Lohman and R.A. Baan, 1991, Single-strand breaks and base damage in DNA of human white blood cells in full blood; Detection after *in vitro* and *in vivo* exposure to ionizing radiation at biologically relevant doses. Book of abstracts of "Workshop on DNA repair with emphasis on eukaryoic systems. Noordwijkerhout, The Netherlands, April 1991.

Contract: Bi7-052 Sector: B14

Title: Radiobiological properties of spermatogonial stem cells in C3H/101 hybrid mice and evaluation of the model for induction of genetic damage in spermatogonial stem cells

1 De Rooij Univ. Utrecht

I. Summary of Project and Global Objectives

Although contrasting reports exist, most authors agree that there is a correlation between cell killing and induction of mutations or reciprocal translocations in spermatogonial stem cells. In 1981, Leenhouts and Chadwick published a theoretical model which fitted most of the data on the induction of translocations by ionizing radiation in mouse spermatogonial stem cells. In this model it was assumed that there are radiosensitive and radioresistant spermatogonial stem cells, for both cell killing and the induction of translocations, although no D_0 values for the different types of stem cells were known at that time.

In recent years it has become clear that both the radiosensitivity for cell killing and the proliferative activity of the spermatogonial stem cells, varies during the cycle of the seminiferous epithelium. In CBA mice it was found that when the stem cells are actively proliferating (epithelial stages IX-III), they have a D_0 value for X-rays of 2.4 Gy, and when these cells are quiescent (stages VI-VII) the D_0 is 1.0 Gy. This relation between proliferative activity and cell killing is closely similar to that found earlier in Cpb-N mice after fission neutron irradiation. Hence, quiescent spermatogonial stem cells are highly sensitive to the cell killing effect of irradiation, and they are much more resistant during active proliferation.

Unfortunately, only few data on the induction of genetic damage in the above mentioned strains of mice are available. Therefore, we now study C3H/101 (3H1) hybrid mice, which are the most widely used type of mice in radiation genetic studies. Answers to the following questions will be sought:

1. What are the D_0 values for killing of proliferating and quiescent stem cells by X-irradiation in 3H1 mice?
2. How many stem cells are present per 3H1 testis and what is the density of these cells during the epithelial cycle?
3. What are the approximate numbers of proliferating and quiescent stem cells in a 3H1 mouse testis?

This project will be continued by a cooperative project between Dr. B.M. Cattanach (Harwell), Dr. P.P.W. van Buul (Leiden) and ourselves, in which the radiosensitivity of proliferating and quiescent stem cells for translocation induction will be studied, using mice that are stage synchronized by the vitamin A deficiency/replacement method. Ultimately, all data will be available for a final evaluation of the Leenhouts/Chadwick model

Head of Project 1: Dr De Rooij

II Objectives for the reporting period

The objective was to determine the radiosensitivity of spermatogonial stem cells in 3H1 mice during the cycle of the seminiferous epithelium for X-rays. It was to be determined whether or not in this mouse too, the radiosensitivity of the stem cells changes with the varying proliferative activity of the stem cell population during the epithelial cycle.
A dose-response experiment had to be carried out in 3H1 mice receiving graded doses of X-rays. In sections of the testes of these mice taken at day 10 after irradiation, the number of undifferentiated spermatogonia had to be counted in each epithelial stage as a measure of the number of surviving stem cells. From the dose effect relationships the D_0 value for stem cell killing in each stage can be calculated.

III Objectives for next period

During the next period the absolute number of spermatogonial stem cells will be determined per mouse testis, using cell counts in whole-mounts of seminiferous tubules, image analysis equipment and testis sections.
Furthermore, a dose-response experiment has been carried out in which 3H1 mice received graded doses of 1 MeV fission neutron irradiation ranging from 0.25 to 3 Gy. D_0 values for stem cell killing and the size of the repopulating colonies will be determined as described earlier. As the dose effect relationship after fission neutron irradiation gives no shoulder these data will enable the calculation of the approximate numbers of radioresistant and radiosensitive stem cells per testis.

IV Progress achieved including publications

A dose-response experiment has been carried out in which 3H1 mice received graded doses of X-rays ranging from 0.5 to 10 Gy. In sections of the testes of these mice taken at day 10 after irradiation, the number of undifferentiated spermatogonia was counted in each epithelial stage as a measure of the number of surviving stem cells. This makes it possible to derive D_0 values for stem cell killing in the different stages of the spermatogenic cycle.

In figure 1 the testis weights of the animals are given at ten days after irradiation. The very low dose of 0.5 Gy has very little influence on the testis weight. After 1 Gy of X-rays there is a marked drop in testis weight, which is most likely caused by the depletion of differentiating spermatogonia, which form the most radiosensitive cell population in the testis. Above 1 Gy there is a more or less gradual decrease in testis weight.

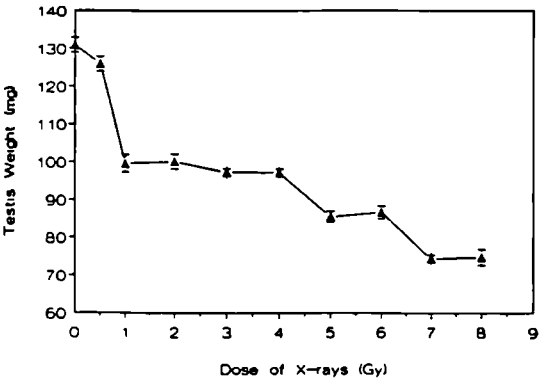

Figure 1. Testis weight of 3H1 mice ten days after graded doses of X-irradiation.

So far 4 animals per dose have been counted for the doses 1.0, 2.0, and 3.0 Gy, and 2 animals per dose for the doses 0.5 and 5.0 Gy. In figure 2 the means of the numbers of undifferentiated spermatogonia present at ten days after irradiation are indicated, with the stages on the x-axis corresponding with the stages at the time of irradiation. The length of the spermatogenic cycle was assumed to be 207 hours, and the lengths of the stages was calculated from the frequency of appearance.

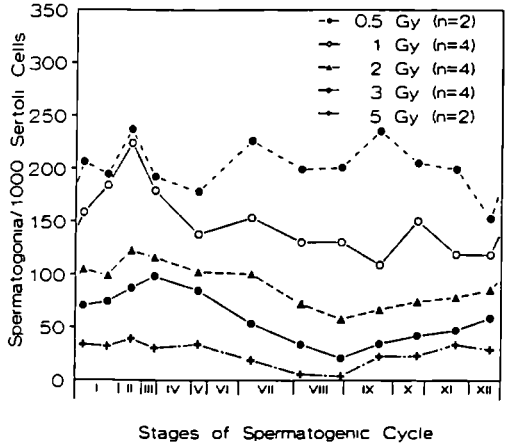

Figure 2. The survival of undifferentiated spermatogonia after graded doses of X-rays. The numbers of undifferentiated spermatogonia per 1000 Sertoli cells present at ten days after irradiation were determined throughout the spermatogenic cycle. The stages indicated are the stages at the time of irradiation.

The data of the cell counts have also been placed in separate dose response graphs, examples of which are given in Figure 3. From these curves a first indication of the radiosensitivity of the different stages of the spermatogenic cycle was obtained by means of an unweighed linear regression analysis (the final analysis of the data will consist of a weighted linear and a linear-quadratic regression analysis). There does not seem to be an indication of a shoulder region in most of the response curves, except perhaps in those of the stages mid/late I through late III. This can be seen in figure 2, where the data points for 0.5 Gy and 1.0 Gy are very near to each other for these stages.

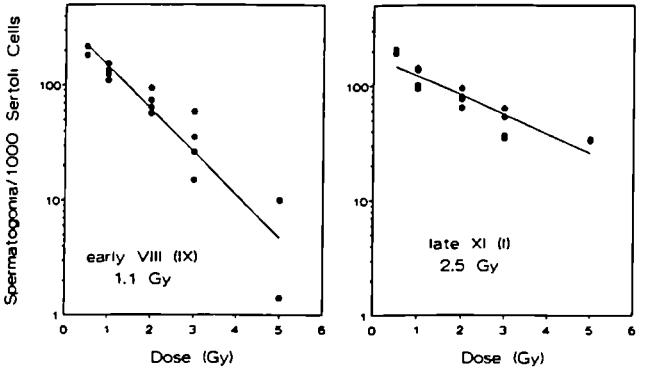

Figure 3. Dose response curves for spermatogonial stem cells in two epithelial stages. The stages indicated are the stages at the time of irradiation. The corresponding stages that were counted are in parentheses.

In figure 4 the D_0's obtained from the dose response graphs are combined to give an indication of the radiosensitivity of the spermatogonial stem cells throughout the cycle of the seminiferous epithelium. The radiosensitivity seems to be highest when the spermatogonial stem cells are in a non-proliferating state (stage VIII). This has also been found for the CBA mouse after X- or neutron irradiation. The highest radioresistance seems to occur in the stages XII and V. However, it should be remembered that these D_0's are deduced from an unweighed regression analysis, and that the cell counts have not been completed yet.

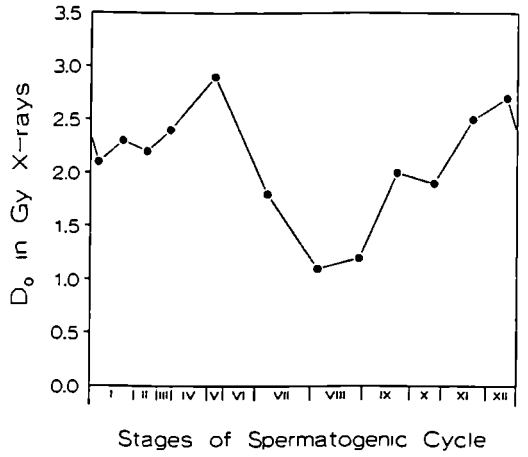

Figure 4. The D_0 of the spermatogonial stem cells throughout the cycle of the spermatogenic epithelium in 3H1 mice. The stages indicated are the stages at the time of irradiation.

When the cell counts of the separate stages are taken together, an overall picture of the radiosensitivity of the total population of spermatogonial stem cells can be obtained. In figure 5 the dose response for the total population of stem cells indicates a radiosensitivity with a D_0 of 2.1 Gy X-rays, which is somewhat higher than the D_0 of 1.8 Gy X-rays that has been found for the CBA mouse.

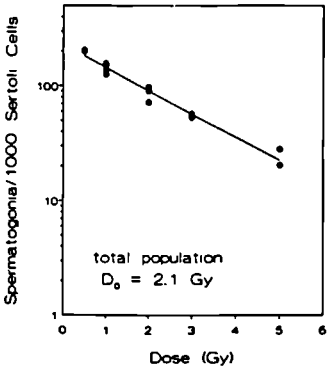

Figure 5. Dose response curve for the total population of spermatogonial stem cells.

Contract: Bi6-226 Sector: B14

Title: Studies on spontaneously-arising genetic and partially genetic disorders in man within the framework of the evaluation of genetic radiation hazards.

1 Lohman/Sankaranarayanan Univ. Leiden, Sylvius Labor.

I. Summary of Project and Global Objectives

This project is aimed at (i) making detailed analyses of the population prevalence of naturally-occurring multifactorial, Mendelian and chromosomal diseases in order to assess the validity of the estimates of prevalence currently used in the context of the evaluation of genetic radiation hazards in man; (ii) making use of these data and those that bear on the severity of these diseases to arrive at estimates of detriment and (iii) exploring and devising methods that can be used for making quantitative estimates of risk of multifactorial diseases.

The work completed during 1986-1989 includes (i) a systematic analysis of the prevalence of naturally-occurring multifactorial diseases in the population of Hungary and comparison of these estimates with those published in the literature and (ii) estimation of detriment associated with these diseases, on the basis of the above information and data that bear on mortality and other aspects of these diseases, the latter extracted from the records of the Central Statistical Office in Budapest. The data that have been collected in this project have already been used in the Reports of the United Nations Scientific Committee on the Effects of Atomic Radiation (1988) and of the BEIR Committee of the U. S. National Academy of Sciences (1990).

Head of Project 1: Prof. Dr. Lohman and Prof.Dr. K. Sankaranarayanan

II Objectives for the reporting period

For the period from January 1990 to February 1991 the main objectives have been (i) a re-examination of the concepts and assumptions used in genetic risk estimation and a critical analysis of the impact of knowledge on the nature of mutations (spontaneous and radiation-induced) on the estimation of the risk of Mendelian disease due to radiation exposures and (ii) an analysis of the data (epidemiological and aetiological aspects) on severe visual handicaps in Hungary and their use in estimating detriment associated with these conditions; this part of the work is carried out in collaboration with Dr. A. Czeizel, National Institute of Hygiene, Budapest, Hungary.

III Objectives for next period

The next period will be devoted to a compilation and analysis of data on (epidemiological and aetiological) and estimation of detriment associated with (i) hearing defects in Hungary; (ii) cancers in Hungary and (iii) diseases of old age (65+ age groups). Additionally, an attempt will be made to devise methods for the estimation of the risk of multifactorial diseases in human populations exposed to radiation. Item (iii) will also include computer-simulation studies to examine the effects of changes (e.g., doubling) in mutation rate (as a result of radiation exposures) on the prevalence of multifactorial diseases.

IV Progress achieved including publications

1. Re-examination of concepts and assumptions used in genetic risk estimation and analysis of the impact of knowledge on the nature of mutations to the estimation of the risk of Mendelian disease. Some of the principal conclusions from this part of the work arew the following: (i) about 50% of naturally-occurring Mendelian diseases are due to point mutations and the remainder due to DNA deletions; (ii) point mutations do not appear to be distributed at random throughout the gene; likewise, the breakpoints of deletions are also non-randomly distributed; (ii) in mouse germ cells, most radiation-induced mutations are DNA deletions and this is true in general of radiation-induced mutations in mammalian somatic cells; (iii) On the basis of chromatin and DNA organization in cells and the biophysical and microdosimetric properties of ionizing radiation, one can qualitatively explain the predominance of deletions; for spontaneously-arising and radiation-induced point mutations, there may be common elements in mechanisms, but for spontaneously-arising and induced deletions, the extent of overlap in mechanisms is difficult to discern at present.

On the basis of these and other findings, arguments are advanced to support the thesis that (i) ionizing radiation is probably not efficient in inducing the very specific molecular changes that are known to underlie spontaneous mutations which cause naturally-occurring dominant genetic diseases; (ii) the doubling dose estimate of 1 Gy that is used to estimate risk for autosomal dominant and X-linked diseases is conservative; (iii) the 1% prevalence figure for these diseases that is used for this purpose may be too high; (iv) the current estimate of risk of dominant and X-linked diseases may need to be revised downwards; (iv) the choice of an overall doubling dose for genetic risk estimation (which take into account the numerically very large class of multifactorial disorders and for which there is no simple relationship between mutation and disease) depends on what indicators are perceived to be relevant in the human context and is

largely judgemental and (v) since, among radiation-induced mutations, recessives predominate, adverse genetic effects of radiation exposure are primarily those associated with induced recessive mutations in the heterozygous condition.

2. Severe visual handicaps in Hungary. The recorded prevalence of severe visual handicaps (blindness + severe vision defects) is about $0.42/10^3$ school-age children in Hungary; this estimate is based on 20 territorial regions the data for which was extracted from the Records of the Central Statistical Office, Budapest. If the five highest figures are considered to reflect the "true" prevalence, then the figure is $0.6/10^3$.

For ascertaining the relative proportions of the different aetiological categories, school-age children from two schools (one in Budapest and an other in Debrecen; both for children with "low" vision; n = 169 and 132 respectively) and from the Institute for blind children in Budapest (n = 190) were evaluated. The aetiologial groups and their respective percentages are: (a) choroido-retinal degenerations [10.0%]; (b) retinoblastoma [1.8%]; (c) optic atrophy [6.7%]; (d) high myopia plus retinal detachment [13.4%]; (e) cataracts [16.7%]; (f) congenital abnormalities of the eye leading to with vision defects such as an- and microphthalmus, buphthalmus and coloboma [15.1%]; (g) syndromes associated with vision defects such as Marfan syndrome and albinism [9.6%]; (h) nystagmus and/or hypermetropia [9.0%]; (i) vision defects resulting from prenatal causes [1.8%]; (j) vision defects resulting from perinatal damage [11.0%] and (k) vision defects due to postnatal causes [4.9%].

On the basis of data on onset, mortality and other epidemiological parameters, it is estimated that for those with these vision defects (i) the average years of LL is of the order of 12 y and (ii) the average life expectancy is about 58 y. At the population level, the total number of years of LL is about 100 per 10^4 livebirths and the total number of years of impaired life is about 500 per 10^4 livebirths. The material is being written up for publication.

Publications

Czeizel, A., K. Sankaranarayanan, M. Szondy (1990). The load of genetic and partially genetic diseases in man. III. Mental retardation. Mutation Research 232, 291-303.

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Sankaranarayanan, K. (1990). How healthy or sick are we, genetically speaking? Proc. of a Workshop on Genetic Effects of Ionizing radiation (D. J. TerMarsch and N. E. Gentner, Eds), AECL Res, Chalk River Laboratories, Chalk River, Canada, AECL -10230, pp 11-12.

Sankaranarayanan, K. (1990) Genetic risks of ionizing radiation: 1988 UNSCEAR estimates and future perspectives. Proc. Workshop on Genetic Effects of Ionizing Radiation (D. J. Termarsch and N. E. Gentner, Eds), AECL Research, Chalk River Laboratories, Chalk River, Canada, AECL-10230, p 21-22.

Sankaranarayanan, K. (1991a) Ionizing radiation and genetic risks. I. Epidemiological, population genetic, biochemical and molecular aspects of Mendelian diseases. Mutation Res, in press.

Sankaranarayanan, K. (1991b) Ionizing radiation and genetic risks. II. Nature of radiation-induced mutations in experimental mammalian in vivo

systems. Mutation Res, in press.
Sankaranarayanan, K. (1991c) Ionizing radiation and genetic risks. III. Nature of spontaneous and radiation-induced mutations in mammalian in vitro systems and mechanisms of induction of mutations by radiation. Mutation Res, in press.
Sankaranarayanan, K. (1991d). Ionizing radiation and genetic risks. IV. Current methods, estimates of risk of Mendelian disease, human data and lessons from biochemical and molecular studies of mutations. Mutation Res, in press.
Sankaranarayanan, K., and A. Czeizel (1991e) Disease spectrum. In Genetics of the Hungarian populations. Akademiai Kiado, Budapest and Springer-Verlag, Berlin, in press.

Progress Report

Contract: Bi7-002 **Sector: B15**
Title: Osteosarcoma and tumours of the haemopoietic systemm by low-dose irradiation.

1	Höfler	GSF Neuherberg
2	Höfler	Univ. München - Technische
3	Erfle	GSF Neuherberg
4	Skou Pedersen	Aarhus Universitet
5	Schoeters	CEN - SCK
6	Bentvelzen	TNO-ITRI

I. Summary of Project and Global Objectives

Under normal circumstances osteosarcoma is a rare tumour, but in both animal models and in humans contaminated with bone-seeking radionuclides the osteosarcoma incidence is extremely high. The difference between the normal and induced incidence rates makes osteosarcoma an ideal model for the study of radiation-induced events in isolation. *In vivo* analysis of radiation-induced osteosarcomagenesis has already yielded considerable information regarding the dose-effect relationship between irradiation and tumorigenesis. At the same time, the advent of molecular biological technology has made possible great progress in the unravelling of the genetic events ultimately responsible for the malignant transformation of cells. The present project has been designed to bring together these two areas of expertise, and thereby define the molecular mechanisms responsible for the osteosarcoma-inducing action of irradiation. Our current goals are to refine the dose-response analysis to include risk-estimates at low-dose irradiation, to identify the nature of the radiation-transformed target cells, and to define the molecular events taking place following irradiation. The ultimate objective of the project is to use analysis of molecular events *in vitro* and *in vivo* to provide a new approach for the assessment and detection of radiation-induced tumour risk.

Head of Project 1: Prof. Dr. H. Höfler

II Objectives for the reporting period

Radiation-induced osteosarcoma is initiated by disruption of the genetic programme controlling bone cell development. In conjunction with other participants in the programme we are investigating the molecular and cellular basis of osteosarcomagenesis at low doses of radiation.
The primary objectives at this stage of the contract are:

1) To quantify biological factors influencing estimations of the risk of osteosarcoma induction at low doses of incorporated radionuclides.
2) Establishing methodology for the identification of early genetic events in radiation-induced osteosarcoma formation.

III Objectives for next period

It is our long-term goal to investigate the concordance of defined genetic events with the biological risk of carcinogenesis in bone tissue during exposure to low-dose radiation. *In vitro* experiments are to be used to develop a predictive model for risk assessment in radiation-induced tumour formation. These require molecular analysis of the genetic alterations occurring at an early stage in radiation-induced osteosarcomagenesis. Thus we will search for novel gene mutations and characterize alterations to gene loci encoding known tumour suppressor proteins. In collaboration with GSF-MZP Neuherberg and SCK-CEN Mol we will develop *in vitro* models containing clearly defined mutations in tumour suppressor genes to allow quantification of other early molecular events of osteosarcomagenesis.

IV Progress achieved including publications

Part I: Long-term *in vivo* analysis of dose-dependency

A series of experiments have been concluded that were designed to determine the oncogenic risk following incorporation of a short-lived bone-seeking radionuclide (^{227}Thorium) in conjunction with a background exposure to low levels of a long-lived isotope.
Low-level long-term exposure was modelled by injecting three month old female NMRI mice with 1.85 kBq/kg of ^{227}Actinium (mother-nuclide of ^{227}Th), giving a mean skeletal dose of 100 cGy per 600 days. At the age of 4 or 12 months cohorts received an additional treatment with a relatively low activity of the short-lived α-emitter ^{227}Th (mean skeletal α-dose burden of 50 cGy and 200 cGy at doses of 9.25 and 37 kBq/kg ^{227}Th respectively).
The rates of osteosarcoma development under the different experimental conditions are summarized in Table 1. In the lower dose range (^{227}Th 9.25 kBq/kg or ^{227}Ac plus ^{227}Th 9.25 kBq/kg) a similar (i.e. not significantly different) osteosarcoma rate was observed in animals treated at either 4 or 12 months of age. The older age of the animals presumably compensates for the later incorporation of the ^{227}Th. At the higher dose (^{227}Th 37 kBq/kg or ^{227}Ac plus ^{227}Th 37 kBq/kg) the osteosarcoma rate in the older age groups was significantly lower than in those groups receiving ^{227}Th at a young age. Despite this fact, low level background irradiation by ^{227}Ac seems to unmask the promoting

efficiency of the older age. An increase of the osteosarcoma risk by incorporation of the additional activity of ^{227}Th in animals with a low activity background burden of ^{227}Ac was significant only for the groups older at the time of ^{227}Th incorporation and is seen at both levels of ^{227}Th activity.

Table 1: Osteosarcoma incidence in female NMRI mice treated with ^{227}Thorium and ^{227}Actinium

Treatment*	Age at ^{227}Th incorp.	Age 201-600 days	Age 601-1000 days
untreated	-	0% (0/ 48)	5% (2/ 40)
A	-	2% (1/ 45)	10% (3/ 31)
T 9.25	4 M	0% (0/ 47)	10% (4/ 42)
T 9.25	12 M	2% (2/114)	7% (7/ 95) c
A T 9.25	4 M	9% (4/ 47)	21% (6/ 29)
A T 9.25	12 M	5% (6/110)	27% (23/ 84) c
T 37	4 M	9% (4/ 46)	44% (14/ 32) a
T 37	12 M	2% (2/112)	17% (16/ 94) a d
A T 37	4 M	13% (6/ 45)	57% (16/ 28) b
A T 37	12 M	7% (8/109)	35% (28/ 81) b d

* A = 1.85 kBq/kg ^{227}Actinium T = ^{227}Thorium, numbers mean kBq/kg
a: p = 0.003 b: p = 0.03 c: p = 0.0003 d: p = 0.006

Part II: Analysis of the early molecular events in osteosarcomagenesis

Whilst the entire genome is subjected to damage from irradiation, alteration in the function of particular sets of genes has far reaching consequences for carcinogenesis. These include the protooncogene and tumour suppressor gene groupings, where a series of unrelated genes encode proteins regulating cell proliferation and differentiation. Disruption of the normal function of these genes by external noxae, including irradiation, can predispose the affected cell to tumorigenicity. We have chosen initially to analyze the two best characterized tumour suppressor loci, encoding the p53 and RB1 (retinoblastoma) proteins. These genes are subjected to loss of function mutation in many, if not all, induced and inherited tumours, and thus are prime candidates for early molecular targets of osteosarcoma-inducing radiation damage.
To analyze these alterations in tumour-suppressor genes in osteoarcoma a number of cell lines were established from radiation-induced mouse osteosarcomas in collaboration with MZP-Neuherberg. DNA and RNA were extracted, and the structural integrity of the p53 tumour-suppressor gene locus analyzed by genomic Southern blotting. DNA was digested with several different restriction enzymes and the restriction fragments analyzed by hybridization with radiolabelled p53 cDNA. Digestion with the restriction enzymes EcoRI, BamHI and TaqI revealed that in 5 of the 9 cell lines gross rearrangements of the p53 gene could be demonstrated. In each of these cases the normal allele was missing, such a loss of heterozygosity removes the unmutated allele and results in a characteristic total loss of function at the tumour suppressor locus.
The sensitivity of the Southern blotting analysis is restricted to detection of major rearrangements in the p53 locus. To determine if in other tumours there

have been minor changes to the DNA we have chosen to analyze directly the p53 cDNA present in each tumour.
Tumour cell mRNA was converted into cDNA by reverse transcription, creating a DNA template for PCR-directed amplification. This is necessary as p53 sequences normally represent only a very small portion of cellular RNA. The complete translated region of the cDNA (comprising exon 2-11), as well as smaller regions containing selected exons were amplified, and the length of the product compared to wild type p53 amplificates using high resolution polyacrylamide gel electrophoresis developed in participation with the project of TU Munich (see adjoining report of H. Höfler, TU Munich). This analysis allows fine mapping of alterations in the DNA sequence and has a resolving power sufficient for the detection of length alterations as small as 1 base. If the target exon is missing from the cDNA there will be no product when that particular exon is amplified, and the product of the entire coding region will be smaller. The results of this analysis are summarized in Table 2.

Table 2: **Result of the length analysis of the PCR amplification products obtained from different regions of the p53 gene**

cell line	OS 43	OS 46	OS 47	OS 48	OS 49	OS 50	OS 51	OS 52	OS 56
IHC	pos.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	pos.
exon 1-11	+	s	+	-	-	+	l	+	+
exon 4-11	nd	s	+	-	-	nd	l	nd	nd
exon 7-11	nd	s	+	-	-	nd	+	+	nd
exon 8-11	+	-	+	-	-	+	+	+	+
exon 4-6	+	+	+	+	-	+	-	+	+
exon 7	+	+	-	+	-	+	+	+	+
exon 8	+	-	+	+	-	+	+	+	+

IHC immunohistochemistry, + product of normal length, s shorter product, l longer product, - no amplification seen, nd not done

This analysis indicated that, in addition to gross alterations in the genomic organization, there have been a series of small deletions within the p53 gene in radiation-induced osteosarcoma. These can be summarized as follows: In the cell line OS 46 a mutation has deleted parts of exon 8 and 9, in OS 47 exon 7 has been lost, and in OS 51 exon 5 is missing.
Whilst the two techniques described above can identify insertion or deletion of bases in the p53 gene, mutations that do not affect the length of the cDNA are undetectable. These include point mutations changing the sequence of the cDNA. It has been established that detectable levels of p53 protein in the cell are frequently associated with point mutation in the coding sequence. The

alteration of a single amino acid residue results in accumulation of p53 protein to a detectable level. We have analyzed expression of p53 by immunohistochemical analysis. Cells were stained with an anti-p53 specific monoclonal antibody (PAb 421) and an FITC-bound second antibody and visualized by fluorescence microscopy. p53 immunostaining was detected only in the cell lines OS 43 and OS 56. These two lines did not shown genomic alterations by either Southern blotting or PCR analysis. These are potential candidates for loss-of function point mutations, and the entire coding region will be sequenced to determine the location of any point-mutations in these tumours.

In summary, during this first reporting period we have continued the long-term *in vivo* dose-dependence studies and established the methodology for molecular analysis of specific gene alterations accompanying osteosarcomagenesis. Our initial findings have established that the tumour-suppressor locus p53 is subject to mutation at a very high frequency in radiation-induced osteosarcoma. This observation will be further investigated and the information used in joint experiments with our collaborators in SCK-CEN Mol, MZP Neuherberg and MRC, England.

Publications:

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Müller, W. A., Luz, A., Murray, A. B., Linzner, U.: Induction of lymphoma and osteosarcoma in mice by single and protracted low alpha doses. Health Physics 59, 305-310 (1990)

Head of Project 2: Prof. Dr. H. Höfler

II Objectives for the reporting period

Osteosarcoma induction in man following irradiation has assisted in generating dose-response and risk-assessment data for radiation-induced carcinogenesis. Using a murine model of α-emitter induced osteosarcoma in the mouse our collaboration with Pathology-Neuherberg has implicated alterations in the tumour-suppressor locus encoding p53. Our contribution to the overall programme is to compare the data obtained using the murine model with that in human osteosarcoma. As human osteosarcoma tissue is very scarce we have chosen to establish the molecular biological analysis using non-osteosarcoma tissue. Our goal during this period of the contract has been to establish methods for the detection and analysis of mutations in nucleic acid obtained from osteosarcoma.

III Objectives for next period

We will analyse the p53 and RB1 tumour suppressor loci in human osteosarcoma material. To date material from a total of 11 cases has been collected. Simultaneous PCR-amplification of sets of different exons of the RB1 gene will be used to rapidly screen for loss of retinoblastoma gene activity. Exon specific amplification of the p53 locus in collaboration with GSF-Neuherberg will also be used to analyze alterations in p53 by direct sequencing of the PCR amplificates. The nature of the alterations in the human tumours will then be compared with that in the radiation-induced murine osteosarcomas (Project 1).

IV Progress achieved including publications

Loss of function mutations to the retinoblastoma gene (RB1) and p53 tumour suppressor gene has been implicated already in the development of osteosarcoma (see accompanying report Pathology-GSF Neuherberg). Southern blot analysis is effective in detecting large deletions or rearrangements to these genes. However, the pathogenetic mechanism of tumour suppressor gene inactivation does not require large deletions in the genes, inactivating point mutations may also result in loss of the locus. In the p53 gene we have already established that PCR-amplification is adequate for the detection and characterization of small gene alterations. In the case of the RB1 locus tremendous difficulties arise with this methodology because of the size of the gene (some 200 kb and 27 exons). However, by amplifying and sequencing numerous small regions of the gene it may be possible to detect point mutations with a high degree of sensitivity. The simultaneous amplification of different exons is necessary to contain costs for the programme, and we have developed the methodology for this by using formalin fixed, paraffin embedded human retinoblastoma tissue as a test target. This choice of material was made as the frequency of RB1 mutation will approach 100%. To date we have established reaction conditions for analysis of 26% of the coding region of the RB1 gene. Using this technique we have identified and sequenced 3 different mutations from the 24 cases tested so far.

The collection of human osteosarcoma material has been continuing, and we now have access to 11 cases. From five of these we have already extracted RNA and DNA for use at a later date in the PCR-based analytical process.

Head of Project 3: Prof. Dr. V. Erfle

II Objectives for the reporting period

a) Characterization of cooperating effects of α-irradiation and retrovirus infection on cell proliferation in mandibular condyles.

b) Pathogenicity studies of treated tissues and cell lines established therefrom.

c) Expression of transcription factors and growth control genes in treated tissues.

III Objectives for next period

a) Characterization of cooperating effects of α-irradiation and retrovirus infection on cell proliferation in mandibular condyles.

b) Pathogenicity studies of treated tissues and cell lines established therefrom.

c) Expression of protooncogenes, structural genes typifying osteogenic differentiation, and genes associated with cell proliferation.

IV Progress achieved including publications

Endogenous retroviruses are activated both in the early latent period of radiation-induced osteosarcomas and in the radiation-induced tumours. *In vitro* and *in vivo* studies have shown that activated endogenous retroviruses induce significant effects on skeletal cells of the mouse, including induction of osteogenic differentiation and new bone formation, as well as induction of benign and malignant bone tumours in infected newborn mice.
In order to study a cooperative effect between α-irradiation (^{224}Ra) and retroviral infection on neoplastic transformation of skeletal cells and tissue, mandibular condyles of newborn mice were irradiated with doses ranging between 0.007 kBq/ml and 7.4 kBq/ml ^{224}Ra for 3.5, 7, 10.5, 14, and 21 days in the presence or absence of infectious RFB MLV retrovirus. It has previously been shown that continuous irradiation of mice in this dose range, over a period of 36 weeks, induces osteosarcomas in 10 to 95% of animals. Control tissues were either infected with RFB MLV or untreated. After the end of the treatment period the tissues were transplanted into syngeneic mice (1 to 4 condyles/mouse). Within an observation period of 3 months after transplantation no tumour development was observed, suggesting that under these conditions either the dose or the irradiation period are not sufficient to induce malignant transformation of mandibular condyles.
To study the effect of irradiation and retrovirus infection on cell growth, the cell number in mandibular condyles was determined after various treatments: irradiation (7 days) was followed by RFB MLV infection (14 days) and vice versa; controls were either irradiated (21 days) or infected with RFB MLV.
The highest cell number was observed in tissues which were irradiated for 7 days and infected thereafter with RFB MLV for 14 days. These data suggest that irradiation increases the susceptibility of skeletal cells to the proliferation-inducing effects of RFB MLV.

Irradiated and/or retrovirus infected mandibular condyles were dissociated by collagenase and single cell suspensions were used to establish monolayer cell lines. The highest cell proliferation was found in cell lines established from tissue which had been irradiated and infected, indicating a cooperative effect of α-irradiation and RFB MLV infection on growth of skeletal cells.
The c-fos protooncogene plays a significant role in osteogenic differentiation, in transdifferentiation of cartilage cells to bone cells, and in terminal differentiation processes of bone cells in osteosarcomas. The v-fos oncogene, on the other hand, is a potent inducer of osteosarcomas. Further, *in vivo* studies with c-fos transgenic mice have indicated that endogenous retroviruses can cooperate with cellular oncogenes in bone tumour development.
Northern blot analysis of irradiated mandibular condyles showed a high transient expression of the c-fos protooncogene between 30 min and 2 hours after start of the culture and down regulation of c-fos after 6 hours, similar to that observed in non-irradiated control condyles. In contrast to published reports on fibroblast cell lines using toxic doses of γ-irradiation, increased expression of c-jun or c-myc was not found in ^{224}Ra-irradiated condyles. These data suggest that the observed stimulation of cell proliferation is not a result of enhanced transcriptional activity of the growth control genes c-fos, c-jun, or c-myc.
Irradiated condyles, however, showed an enhanced RNA level of the T1 gene after treatment with ^{223}Ra. T1 is a gene stimulated by the oncogene Ha-ras and was isolated from Ha-ras transformed NIH3T3 cells. T1 expression was analyzed by reverse transcription of total cellular RNA from irradiated condyles and sequence-specific amplification by PCR of a 400 bp sequence. In contrast to non-irradiated tissues, irradiated tissues showed expression of T1 at 2 hours after start of the culture, suggesting that T1 is activated by ^{223}Ra. Further *in situ* hybridization analysis will be carried out to study the cell specific expression of genes, which are sensitive to ^{223}Ra in irradiated mandibular condyles.
During the reporting period ^{224}Ra became no longer commercially available. As an alternative, ^{223}Ra was obtained from the GSF-Institute of Pathology, Neuherberg, and subsequent experiments were carried out with ^{223}Ra. For future experiments this radionuclide will be available upon request.

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Head of Project 4: Prof. Dr. Skou Pedersen

II Objectives for the reporting period

To perform a molecular analysis of target genes for radiation carcinogenesis

1) To study provirus activation through the cellular stress response

2) To analyze proviral integration sites in tumour tissues

III Objectives for next period

To perform a molecular analysis of target genes for radiation carcinogenesis

1) To study provirus activation through the cellular stress response

2) To analyze proviral integration sites in tumour tissues

IV Progress achieved including publications

Endogenous retroviruses are frequently activated in association with radiation-induced osteosarcomagenesis in laboratory strains of mice. Our work addresses the following questions of interest for understanding of immediate and late effects of ionizing radiation: How are endogenous proviruses activated? Are the activated viruses pathogenic? What features in a retroviral genome may affect its pathogenicity for bone tissues? What molecular mechanisms may contribute to the effect of a retrovirus on bone cells?
All viruses included in the studies are available as molecular clones and all are closely related to the prototype endogenous ecotropic murine leukemia virus, Akv. The viruses induce lymphomas, osteopetrosis, and osteomas in NMRI mice, but differ in potency of disease induction. In CBA mice, on the other hand, the virus effects are frequent osteomas and only rare lymphomas. Interesting differences in organization of the tandem repeat sequences in U3 of the LTR are found among the viruses. Studies in a number of cell lines show that the LTR differences affect the potency and specificity of virus-directed transcription. These expression assays reveal that the most potent bone-pathogenic isolate in our collection, RFB MLV, may represent a novel transcriptional phenotype. Recombinant mapping will be undertaken to study the significance of such differences for viral pathogenicity.
Our long-term goal is to locate nucleotide positions in the virus genome that may affect bone-pathogenicity. In a parallel study (Hallberg et al., 1991) of the T-lymphoma inducing murine leukemia virus SL3-3, we have identified single nucleotide positions in the U3 region of the viral genome that are crucial both for expression in cultured T-lymphoma cells and for lymphomagenicity in the animals, thus directly linking *in vivo* and *in vitro* results.
The protooncogene c-fos may be activated as part of the stress response that is an immediate consequence of irradiation. c-fos also plays a role in normal osteogenic differentiation, and potential interactions between this protooncogene and a virus are therefore of major interest for understanding the role of activated viruses in the effects of radiation on bone tissues. Our recent transient expression studies indicate that the c-fos product can specifically *trans*-regulate expression from virus

LTRs in an osteogenic cell line. The effect is stimulatory at lower levels of c-fos expression and inhibitory at higher levels. Mapping of the *cis* responsive elements in the LTR is currently under way. Such regulatory interactions may be involved in both early and late effects of radiation on bone tissue.
Our studies of proviral integration sites in DNA from virus-induced and radiation-induced tumours use both Southern hybridization techniques and polymerase chain reaction techniques. These studies are progressing well but no clear pattern of immediate biological significance has emerged during the reporting period.
All studies are carried out in close collaboration with GSF-Abteilung für Molekulare Zellpathologie, München (FRG).

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Head of Project 5: Dr. G. Schoeters

II Objectives for the reporting period

For radiation protection purposes it is important to identify cells at risk for tumour development after incorporation of low doses of bone seeking radionuclides. This may help to estimate osteosarcomogenic doses and allows tracing radiation-induced events which may be related to subsequent development of bone cancers.
Our first year's work in this frame was focused on 1) the identification of precursor cells for bone cells among bone marrow and assessment of their radiosensitivity; 2) the development of an efficient model for osteosarcomas in our Balb/c mice. In these animals osteogenic cell populations will be studied during the latency period for bone tumour development.

III Objectives for next period

We will study *in vitro* phenotype changes which may be related to radiation-induced bone tumour transformation. Our *in vitro* assay for osteogenic differentiation of bone marrow cells will be used. Cultures will be obtained from non-contaminated mice, from mice contaminated *in vivo* with ^{241}Am and which are at high risk for bone tumour development, and ^{223}Ra will be added *in vitro* to differentiating marrow cultures (collaboration J. Schmidt, GSF).
Cell proliferation and differentiation will be quantitatively evaluated using autoradiography and immunocytochemistry. The focus will be on differentiation markers such as collagen type I, alkaline phosphatase, osteocalcin, mineralization.

IV Progress achieved including publications

Methods

1. Bone marrow cells involved in osteogenic differentiation

In the conventional osteogenic culture assay for bone marrow cells, bone marrow is flushed as an organ fragment from the femur diaphysis of adult mice. It is incubated *in vitro* on a collagen sponge and its three-dimensional structure remains undisrupted. After a lag time of 13 days, mineralization can be followed quantitatively via measurement of ^{85}Sr uptake which has been added to the tissue culture medium (^{85}Sr is used as a tracer for Ca) (Schoeters et al., Cell Tissue Kinet., 21:363-374, 1988).
1.1. To select further which cell populations among the heterogeneous marrow cells are involved in osteogenic differentiation, stromal marrow cells were selectively cultured via the long-term bone marrow culture (LTBC) technique where the stromal cells form an adherent layer. Subsequently these LTBC were irradiated with 10 Gy of X-rays to kill haemopoietic stem cells residing in the adherent layer.
1.2. Involvement of proliferating cells in osteogenic differentiation was determined in marrow fragment cultures after *in vitro* incorporation of $^{3}HTdr$ with high specific activity, i.e. killing of proliferating cells. The osteogenic capacity of these various cultures was assessed by measuring the mineralization rate via uptake of ^{85}Sr.

2. Radiation sensitivity of osteogenic differentiation of bone marrow cells *in vitro*

2.1. Sensitivity to X-irradiation
Bone marrow was irradiated by X-rays (1, 2, 4, 8, 16 Gy), either *in vivo* or *in vitro* prior to onset of the cultures. Femoral marrow was then incubated *in vitro* as an

organ fragment and ^{85}Sr uptake was measured to evaluate the osteogenic capacity.

2.2. Sensitivity to α-radiation
Male Balb/c mice were injected intravenously with ^{241}Am. The doses ranged between 40 to 500 Bq/g mouse, non-contaminated mice were included as controls. Cultures were initiated one day, one week and several weeks (from 4 to 14) after ^{241}Am injection. Marrow cells were cultured either as intact fragments (plugs) or the stroma was cultured as an adherent layer and subsequently, when loaded on a sponge, tested for its osteogenic capacity (^{85}Sr uptake).

3. Bone tumours in Balb/c mice after a single i.v. injection with ^{241}Am

More than two years ago, male and female mice were injected, at 10 weeks of age, with ^{241}Am doses ranging between 45 and 250 Bq/g. All mice have now died and were autopsied, samples were taken for histopathology and carcasses were radiographed for diagnoses of bone tumours (preliminary results have been obtained).

Results and Discussion

1. Bone marrow cells involved in osteogenic differentiation

1.1. Stromal cell involvement
Similar to the intact marrow fragments, the stromal cells cultured from bone marrow according to the long-term bone marrow culture technique, display osteogenic differentiation characteristics *in vitro*. After the stromal cells reach confluency, the cells are scraped from the bottom of the tissue culture flasks and loaded on a collagen matrix. The prerequisites to get calcification are a high cell density and three-dimensional (3D) configuration. The stromal cells are still an heterogeneous cell population of mainly adherent fibroblast-like cells, macrophages and haemopoietic stem cells which home in the adherent layer. Killing the haemopoietic stem cells by irradiation of the confluent LTBC with 10 Gy of X-rays did not impair osteogenic differentiation after the irradiated cells were brought in 3D configuration. Haemopoietic stem cells are not further needed for the osteogenic differentiation.

1.2. Involvement of proliferating cells
Next to differentiation we found that also proliferation of cells is needed for bone differentiation of marrow cells. This has implications for the sensitivity to irradiation and suggests a possible regulatory role of cell growth factors. Incorporation of $^{3}HTdr$ in DNA synthesizing cells of marrow plug cultures showed that the osteogenic capacity was more reduced if $^{3}HTdr$ was added between day 6 and 9 of *in vitro* incubation than between day 3 and day 6. Cell proliferation related to osteogenic differentiation was lowest during the first 3 days of culture.

2. Radiation sensitivity of osteogenic differentiation of bone marrow cells *in vitro*

2.1. Sensitivity to X-irradiation
In vivo and *in vitro* irradiation of adult femoral bone marrow damaged the bone formation capacity *in vitro*. After doses higher than 4 Gy the mineralization capacity of marrow plugs was significantly reduced as shown by the measurement of ^{85}Sr uptake.

2.2. Sensitivity to α-irradiation
Four weeks after ^{241}Am injection of adult mice with doses higher than 150 Bq/mouse, the marrow showed a reduced mineralization capacity when cultured as a

fragment. This contamination corresponds with a skeletal dose rate of 25 mGy/day and an accumulated skeletal dose of 0.75 Gy.
If stromal cells were cultured from the bone marrow and subsequently brought in 3D conditions allowing osteogenic differentiation, a reduced mineralization capacity *in vitro* was noticed 4 weeks and 13 weeks after Am injection and this from the lowest dose tested (40 Bq/g mouse, skeletal dose rate : 7 mGy/day, accumulated dose after 1 month : 0.2 Gy).
The osteogenic capacity of marrow *in vitro* showed a high radioresistance for acute X-irradiation. In contrast, relatively low doses of α-irradiation from bone-seeking radionuclides had effects on the osteogenic differentiation of marrow cells *in vitro*.

3. Bone tumours in Balb/c mice after a single i.v. injection with ^{241}Am

43% of female Balb/c mice injected with 124 Bq/g developed bone tumours, while only 23% of male mice developed bone tumours after injection of 103 Bq/g. At lower doses of 45 and 90 Bq/g respectively, 71% and 64% of female mice developed bone tumours. These diagnoses are obtained from X-ray pictures and need further histopathological confirmation, but it is obvious that in female Balb/c mice a single injection with a relatively low dose of ^{241}Am induces a substantial proportion of bone tumours.

Conclusion

Selective culture techniques showed that 1) the osteogenic cells among adult bone marrow belong to the adherent bone marrow cell population, 2) a three-dimensional configuration and high cell densities are needed for osteogenic differentiation *in vitro*, 3) besides differentiation of cells in these cultures, cell proliferation takes place, 4) haemopoietic stem cells are not needed further for osteogenic differentiation of stromal cells in 3D cultures.
Despite high resistance for X-irradiation, the osteogenic capacity of marrow cells is impaired after low α-irradiation *in vivo*. The *in vitro* osteogenic assay allows to detect radiation-related changes at dose levels which were shown to induce a high number of bone tumours (> 50%) in the same mouse strain.

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Head of Project 6 : Dr. P. Bentvelzen

II Objectives for the reporting period

The oncogenic transforming principle in irradiated BALB/c mouse DNA has proven to be homologous to the transregulator (TR) gene of the mouse mammary tumour virus. Thus a baculovirus expression vector was constructed to allow the large-scale production of the TR gene product. This protein would then be used to screen a mouse genomic DNA library in order to identify genes reacting with the TR protein, potential candidates for irradiation-induced genes via TR activation.

III Objectives for next period

To purify the TR gene product from insect cells expressing the TR-baculovirus construct, and to label this protein with biotin. The labelled protein will then be used to screen a mouse genomic DNA library constructed in a bacteriophage vector. Sequences showing a positive reaction with the biotinylated TR protein will be further characterized. The final aim is to assess whether the detected sequences (candidate radiation-activated TR responsive genes) are overexpressed in different kinds of radiation-induced tumours in mice and rats.

IV Progress achieved including publications

A baculovirus expression vector containing the mouse mammary tumour virus transactivator gene was constructed as follows: Firstly, a plasmid was created placing the retroviral transactivator gene under the control of the baculovirus polyhedrin promotor. This plasmid was electroporated into host Sf9 insect cells together with DNA containing the wild type baculovirus "Autographa californica multiple nuclear polyhydrosis virus". By means of homologous recombination the MMTV TR gene was introduced into the baculovirus vector within the polyhedrin gene. By means of repeated limiting dilution the recombinant virus was purified to homogeneity. Upon infection of large scale Sf9 cultures with the recombinant virus a 36 kD protein was seen to be present in the nuclei of the infected cells. This material is presumed to represent the TR protein produced by the vector

In NIH/3T3 cells transformed by retroviral transactivator genes (from the mouse mammary tumour virus, the bovine leukemia virus or the human immunodeficiency virus) multiple copies of these genes proved to be integrated into chromosomal DNA. Expression of these sequences was not detectable, however, at either the RNA or protein level. Prokaryotic genes, which were present in the plasmid vectors carrying the retroviral TR genes, also proved to be integrated along with the TR genes, but were also not expressed. This lack of active expression is possibly due to hypermethylation of the integrated sequences. Presumably, oncogenic transformation by these TR genes is caused by a "hit and step aside" phenomenon.

Progress Report

Contract: Bi6-089 Sector: B15
Title: The dosimetry and metabolism of incorporated radionuclides

1 Harrison NRPB

I. Summary of Project and Global Objectives

The objectives of this project are to understand the behaviour of bone-seeking radionuclides in the skeleton and to provide biokinetic data that can be used to improve assessments of doses to radiation-sensitive cells in the skeleton. To assess risks from incorporated radionuclides and to calculate dose per unit intake for workers and members of the public.

Results of animal experiments, supported by human data for radium contamination, have shown that osteosarcoma is the major late effect of internal contamination with bone-seeking alpha-emitters. It has also been shown that myeloid leukaemia is induced preferentially in mice given alpha-emitters in amounts that are less than optimal for osteosarcoma induction.

A comparative study to examine the differences in distribution and retention of the three bone-seeking radionuclides ^{239}Pu, ^{241}Am and ^{233}U is in progress with the objective of relating differences in the distribution of dose within the skeleton and the extent of irradiation of the different cell types, with the observed incidence and distribution of osteosarcoma and myeloid leukaemia.

A study to determine the distribution and retention of ^{210}Po in the skeleton of rats after intraperitoneal injection of either ^{210}Po or ^{210}Pb and a comparative study of the distribution of ^{238}Pu and ^{210}Po in marmoset bone are currently in progress.

Head of Project 1: Dr Harrison

II Objectives for the reporting period

To continue with studies of the distribution of ^{239}Pu, ^{241}Am and ^{233}U in mice, their retention in individual bones of the skeleton, and micro-distribution within bone. To continue with studies of the induction of osteosarcoma and myeloid leukaemia by these radionuclides.

To begin studies to determine the distribution and retention of ^{210}Po in the skeleton of rats after intraperitoneal injection of ^{210}Po or ^{210}Pb and comparisons of the distribution of ^{238}Pu and ^{210}Po in marmoset bones.

III Objectives for next period

To continue with studies of the micro-distribution and quantification of dose following the intraperitoneal injection of ^{239}Pu, ^{241}Am and ^{233}U in mice and with long-term studies of the induction of osteosarcoma and myeloid leukaemia by these radionuclides.

To continue with studies to determine the distribution and retention of ^{210}Po in the skeleton of rats after intraperitoneal injection of ^{210}Po and ^{210}Pb and continue with comparisons of the distribution of ^{238}Pu and ^{210}Po in marmoset bones.

IV Progress achieved including publications

The study of the comparative toxicity of ^{239}Pu, ^{241}Am and ^{233}U in mice can be considered as consisting in four parts: radiochemical measurements of the distribution of the nuclides between the skeleton and soft tissues at times up to 448 days after intraperitoneal injection as their citrate complexes; radiochemical measurements of the retention of the nuclides in individual bones of the skeleton over the same period; autoradiographic studies of the distribution of the nuclides within bone; and comparisons of osteosarcoma induction in groups of mice given intraperitoneal injections of ^{239}Pu, ^{241}Am and ^{233}U activity to deliver equivalent average skeletal doses.

The initial study of the distribution and retention of the nuclides in the skeleton and soft tissues after systemic injection of 40 kBq kg^{-1} at times up to 448 days is complete. The results obtained were used to calculate average bone doses over the period of 0.52 Gy for ^{239}Pu, 0.45 Gy for ^{241}Am and 0.10 Gy for ^{233}U.

A second study examining the retention and distribution of ^{239}Pu, ^{241}Am and ^{233}U in the individual bones of the skeleton with time has also been completed. The nuclides deposited preferentially in the main body of the spine, limb girdles and ribs with lower concentrations in the lower limbs, paws and caudal vertebrae. The distribution pattern was similar to that observed in rats by other workers. The differences in the relative concentrations of the nuclides in the individual bones was reduced as time progressed and remodelling occurred, leading to a more homogeneous distribution. The inhomogeneity function, which describes the deviation of the relative concentration of the individual bones from the concentration of the whole skeleton, was calculated. Inhomogeneity was greatest for plutonium and least for americium. The decrease in inhomogeneity with time was more pronounced for plutonium and uranium than americium.

Autoradiographic studies using the femur, lumbar vertebrae and mandibular condyle are in progress. Alpha-track autoradiographs have been examined to qualitatively determine the gross distribution of each radionuclide at 1, 7, 28, 112, 224 and 448 days after intraperitoneal injection. The autoradiographs showed that plutonium was deposited fairly evenly on endosteal bone surfaces and to a lesser extent on periosteal surfaces. At later times, some burial in the form of lines of activity was apparent as well as areas with more diffuse activity indicative of redeposition during bone remodelling. Progressive accumulation of plutonium in marrow was also observed. Americium was deposited more evenly on all bone surfaces including those of vascular canals within bone mineral. Again, some burial occurred in the form of lines of activity and some accumulation of americium in marrow macrophages was also seen but less than for plutonium. Uranium was deposited on all bone surfaces but not evenly, concentrating preferentially on some parts of the surface, with burial at later times after injection. Little uranium was seen in the marrow.

To quantify the distribution of alpha activity within the bone, fission track autoradiographs of femur sections have been produced. Track counts on random areas of sections have been used to make preliminary estimates of dose. The initial calculations show that while the doses to endosteal surfaces from ^{239}Pu and ^{241}Am were greater than average bone doses at both 1 day and 224 days after administration, the dose from ^{233}U was greater than the average bone dose initially but lower at 224 days. The bone marrow dose from ^{239}Pu was about the same as the average bone dose at 1 day and slightly higher at 224 days. Marrow doses from ^{241}Am and ^{233}U were lower than those for ^{239}Pu. Further calculations are being undertaken to take account of doses to bone surfaces and marrow from activity near but not on bone surfaces.

To compare osteosarcoma incidence, groups of mice (50 - 100 per group) were injected with either ^{239}Pu, ^{241}Am or ^{233}U at one of three dose levels. For ^{239}Pu the dose levels were 5, 15 or 25 kBq kg^{-1}. The corresponding amounts for ^{241}Am and ^{233}U, to give equivalent average bone doses, were 6, 17 and 29 kBq for ^{241}Am and 40, 118, and 197 kBq kg^{-1} for ^{233}U. To date, approximately 70% of the animals have died. Eighteen bone tumours have been observed to date, 10 in animals given plutonium. Eight myeloid leukaemias have been identified and several additional suspected myeloid leukaemias are currently being histopathologically evaluated.

The alpha particle induction of acute myeloid leukaemia (AML) in the CBA/H mouse is characterised by deletion and rearrangement at specific sites of chromosome 2. It is now known that these chromosomal changes are induced directly by ionising radiation in mouse haemopoietic cells and probably represent initiating events for AML. Two of the myeloid leukaemias induced in this studies have been karyotyped using conventional methods and both of these have shown the chromosome 2 rearrangements characteristic of AML. Cells from future suspected leukaemias will be karyotyped in the same way.

A study of the distribution and retention of ^{210}Po in the skeleton of rats is in progress. Tissue distribution results show a skeletal retention of about 10% at 7 days. Autoradiographs of femurs and vertebrae show a fairly uniform distribution of activity throughout the marrow at 7 and 200 days after administration; the values for tissue retention at later times are not yet available. Studies of the distribution and retention of ^{210} in the rat skeleton after administration of ^{210}Pb are planned.

A study of the distribution and retention of ^{238}Pu and ^{210}Po in the marmoset has been carried out. Four male marmosets were given injections of ^{239}Pu and ^{210}Po in citrate solution; 2 animals were killed at 1 week and 2 at 1 month. The results obtained for the distribution of plutonium in liver and bone were reasonably consistent with the current ICRP dosimetric model. Results for the tissue distribution of polonium at 1 week were similar to the values obtained for rats, with about 10% of the total activity retained in the skeleton. Autoradiographs prepared from the femurs of marmosets showed activity on bone surfaces, attributable to ^{239}Pu, and throughout the marrow, attributable to ^{210}Po (see figure 1).

The current ICRP biokinetic model for polonium assumes that for polonium entering the blood stream, 10% is deposited in each of the liver, kidneys and spleen and the remaining 70% is uniformly distributed throughout the rest of the body. The results obtained in these studies, together with other available data, suggest that appropriate values for the initial distribution of systemic polonium might be 30% in liver, 10% in kidneys and 5% in spleen. The results for uptake and distribution of the ^{210}Po in the skeleton suggest that it would be appropriate to make specific allowance for the uptake of 10% of systemic ^{210}Po in red bone marrow; this would increase the dose to this tissue by a factor of about six.

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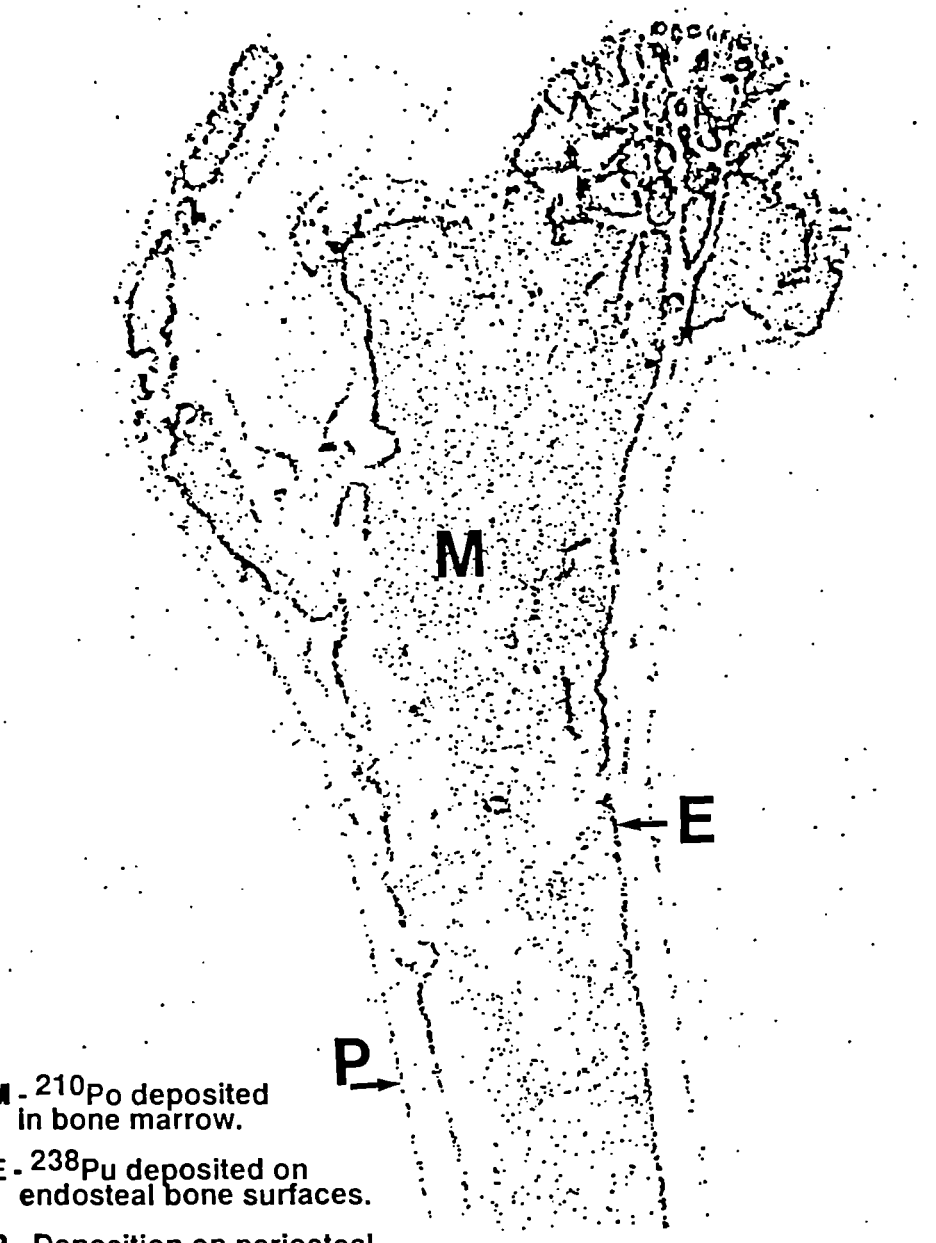
Fig.1. A CR-39 autoradiograph of ^{210}Po and ^{238}Pu in marmoset bone.



Progress Report

Contract: Bi6-064 **Sector: B15**
Title: Studies on myeloid leukaemia and osteosarcoma induced in mice by Ra-224

1 Humphreys MRC Radiobiological Unit

I. Summary of Project and Global Objectives

The potential contamination of the environment by the nuclear power industry and the heightened public awareness of the threat of radiation, maintains the need for the experimental investigation of the effects of α-particle emitters. Leukaemia is perceived to be the greatest threat, yet the risk of its induction from α-particle emitters is not yet convincing; this project is concerned principally with the investigation of this risk in mice.

Firstly, the continuing long-term study of the effects of a single injection of ^{224}Ra on male CBA/H mice is confirming the earlier indication (Humphreys *et al.* 1985) that there is a range of administered amounts of ^{224}Ra (below that which induces a maximum yield of osteosarcoma) in which more myeloid leukaemia than osteosarcoma is induced (Humphreys *et al* 1989). A second phase of the long-term studies was begun as a result of evidence that the induction of osteosarcoma by ^{224}Ra can be increased as much as ten-fold by protracting the administration of activity in time (Müller *et al* 1978) and vindicated, more recently, by results showing that the protracted administration of much smaller total amounts of ^{224}Ra has a very significant effect on the induction of leukaemia (Müller *et al* 1988, Müller *et al* 1989). Clearly, if α-particle emitters are more leukaemogenic when administered in many small fractions than when given in a single amount, then existing radiological protection standards for these radionuclides will need to be re-examined. Our main current aim, therefore, is to extend our studies to investigate fully the potential for increasing the leukaemogenic effects of α-particle-emitting bone-seeking radionuclides by protraction of administration.

Head of Project 1: Dr Humphreys

II Objectives for the reporting period

1. To continue the investigation of the ratio of induced myeloid leukaemia to that of osteosarcoma as a result of single injections of different amounts of ^{224}Ra into adult male CBA/H mice.

2. To continue the study of late effects arising from:

a. The administration of ^{224}Ra to adult and four week old male CBA/H mice protracted over eight weeks.

b. The continuous administration of ^{224}Ra from paratibially injected ^{228}Th.

III Objectives for next period

1. To continue the investigation of the late effects of a single administration of ^{224}Ra with particular emphasis on haemoblastoses other than myeloid leukaemia.

2. To assess the late effects of the administration of ^{224}Ra protracted over eight week periods and to begin to assess the late effects of the continuous administration of ^{224}Ra arising from paratibial injections of ^{228}Th.

IV Progress achieved including publications

Table 1 shows the current status of the long-term study following single intraperitoneal injections of ^{224}Ra (or of diluting solution only) into male CBA/H mice (84 ± 5 days old at injection).

Table 1
Single injection experiment
Twelve week old male CBA/H mice
Status May 1991

Injected ^{224}Ra (Bq g^{-1})	0	69	139	280	550
No. of mice entered	400	400	400	400	400
Myeloid leukaemia	0	5	11	16	17
Osteosarcoma	0	0	3	3	2
Mouse-days exposure	283216	274178	267029	269272	263396
Myeloid leukaemia (mouse days 10^{-5})	0	1.82	4.12	5.94	6.45

All of these mice are now dead and the results so far show an overall six-fold greater incidence of myeloid leukaemia than of osteosarcoma, in the range 69 to 550 Bq g^{-1} ^{224}Ra administered and that the yield of myeloid leukaemia increases with the amount of ^{224}Ra injected. Histopathological analyses, however, are not yet complete and the final ratio of myeloid leukaemia to osteosarcoma may still change.

The present status of the experiment in which sixteen intraperitoneal injections of ^{224}Ra (or of diluting solution) were made over an eight week period into 12 week old mice is shown in table 2.

Table 2
Multiple injection experiment
Male CBA/H mice
Twelve weeks old at first injection
Status May 1991

Total ^{224}Ra (Bq g^{-1})	0	32	64	128
No. of mice entered	200	200	200	200
No. of mice dead	148	198	199	200
Myeloid leukaemia	0	1	0	6
Osteosarcoma	1	0	1	1
Mouse-days exposure	136019	132888	133535	133262
Myeloid leukaemia (mouse days 10^{-5})	0	0.75	0	4.50

The difference which is apparent between the yield of myeloid leukaemia in the group given 128 Bq g^{-1} ^{224}Ra and an interpolated value from the single injection experiment has been shown to be statistically insignificant. A more complete analysis of the results will be possible as more histopathology becomes available.

Effects of protraction have been demonstrated on four week old NMRI mice which are greater than is suggested by these present results (Müller *et al* 1989). In an effort to investigate the possible influence of age, three groups of four week old male CBA/H mice have been injected intraperitoneally with ^{224}Ra in sixteen injections spaced at approximately equal intervals over an eight week period. The present status of this experiment is shown in table 3.

Table 3
Multiple injection experiment
Male CBA/H mice
Four weeks old at first injection
Status May 1991

Total ^{224}Ra (Bq g^{-1})	64	128	256
No. of mice entered	100	80	81
No. of mice dead	34	27	21
Myeloid leukaemia	0	0	0
Osteosarcoma	0	0	0
Mouse-days exposure	63122	50820	50931

Thirty five mice are now dead in a single group of 200 four week old male CBA/H mice each given 64 Bq g^{-1} ^{224}Ra in a single injection.

The prohibitively time-consuming nature of multiple injections of large numbers of mice over long periods prompted the investigation of paratibial injection of ^{228}Th as a source of ^{224}Ra. This method involves the injection of ^{228}Th in a form which allows the bulk of the parent thorium to remain at the site of injection (near to the tibiofibula in the left leg) while the radioactive daughters are distributed by the circulation. Effectively therefore, since ^{228}Th has a very much longer half life than any of its daughters and since ^{224}Ra is the first daughter, a paratibial injection of ^{228}Th is equivalent to a continuous contamination by ^{224}Ra. In addition to eliminating the labour-intensive need for repeated injection this method has the obvious advantage of simulating the exposure conditions which might result from an environment contaminated by α-particle emitters. Preliminary mesurements have confirmed the tissue distribution of ^{224}Ra already observed in NMRI mice. These measurements have also shown that ^{212}Pb accumulates continuously in blood, tentatively confirming that ^{220}Rn was being lost from bone (Humphreys *et al* 1984). Groups of 12 week old male CBA/H mice have, therefore, been injected with amounts of ^{228}Th calculated to be leukaemogenic from the amount of ^{224}Ra liberated into the circulation (table 4). These experiments are being carried out in collaboration with GSF, Neuherberg.

Table 4
Paratibial injection of ^{228}Th
Twelve week old male CBA/H mice
Status May 1991

Injected ^{228}Th (Bq g^{-1})	3.4	6.8	13.6	27.2
No. of mice entered	200	200	200	200
No. of mice dead	1	3	4	0
Mouse-days exposure	31824	40501	47601	31970

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Humphreys, E.R., Loutit, J.F., Major, I.R., Stones, V.A. (1985). The induction by ^{224}Ra of myeloid leukaemia and osteosarcoma in male CBA mice. International Journal of Radiation Biology, 47, 239-247.

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Contract: Bi6-061 Sector: B21

Title: Impairment of the hemo-lymphopoietic cell system and its microenvironment by ionizing radiation. Pathogenesis of non-stochastic and neoplastic effects and conditions for a long term restoration

1 Fliedner Univ. Ulm

I. Summary of Project and Global Objectives

It is the purpose of this project to extend the knowledge base necessary to improve our understanding of the possibilities and limitations to recognize the type of response of human radiation exposure and to establish therapeutic strategies that can improve the survival of persons accidentally exposed to ionizing radiation. On this basis, it is the purpose of this project to obtain answers to 3 key questions: What are relevant biological indicators to predict the consequences of accidental radiation exposure as a basis for appropriate therapeutic strategies? In what way can one improve the pathophysiological knowledge necessary to cope with radiation accident consequences? In which way can one improve therapeutic options for the management of hematopoietic injury by stem cell transplantation and by administration of recombinant regulatory factors?

To answer the first question, our group is well on its way to establish - in close cooperation with the Institute of Biophysics of the Ministry of Health in Moscow - a "radiation accident case history data base". The purpose is to associate biological indicators determined in radiation accident victims especially early after radiation exposure with the actual clinical course and outcome. In the forefront are indicators such as granulocyte-, platelet-, lymphocyte-, reticulocyte- and erythrocyte numbers per mm^3 blood as a function of time after accidental exposure. The data base contains now more than 300 radiation accident case studies. 10% have so far been analysed in detail and many more will be added. It is of particular importance to know, that by now an agreement has been reached with the Institute of Biophysics in Moscow as to which clinical data should be analysed to be available for computer retrieval. It became obvious that one can predict (on the basis of blood cell changes and of subjective signs and symptoms) within 3-5 days after accidental whole body exposure whether or not a person will require "substitutional" or "replacement" therapy.

Studies are in progress to find answers to the second question: pathophysiological knowledge necessary to improve radiation accident management. We used 2 approaches: an simulation model approach and an experimental approach. In order to be able to interpret blood cell changes after accidental exposure it is necessary to develop further biomathematical simulation models for granulopoiesis and for the megakaryocyte/platelet system. It is only by the simulation of the cell changes that one can calculate the remaining stem cells in the body as a basis for estimating the extend of the radiation strain to the blood cell forming tissues. Further improvements were made with respect to the granulocyte system model and a new approach was used to develop a megakaryocyte-platelet system model. These studies are being complemented by clinical as well as animal experimental studies. Clinical studies are being performed in patients receiving therapeutic whole body irradiation, followed by stem cell transfusions using bone marrow or blood as sources for stem cells. The data obtained - blood cell recovery curves - are of tremendous value to correlate the hemopoietic recovery with stem cell numbers transfused (measured as CFU-GM or CFU-GEMM). It is on this basis, that recombinant regulation factors can then be tested as to their pathophysiological mechanisms. In dogs, partial body irradiation studies have been performed to improve our understanding as far as stem cell migration is concerned as a means to allow survival after inhomogeneous radiation exposure. It is in this context that we have studied the influcence of recombinant hemopoietic regulation factors on hemopoietic recovery and have found that it is possible, indeed, to enhance recovery if given at appropriate time intervals using appropriate dose schedules. On the basis of work carried out in 1990, we are confident, that by the end of 1991 we will have made significant advances in the attempts to improve clinical radiation accident management.

II. Objectives for the reporting period (1990)

1. Improvement of existing and development of new knowledge with respect to the key biological indicators of the blood cell forming tissues to predict the clinical course and outcome after accidental whole body irradiation.

2. Improvement of existing and develop new knowledge with respect to the pathophysiological mechanisms of blood cell changes using biomathematical simulation models and clinical as well as experimental studies using whole and partial body irradiation.

3. Improvement of existing and development of new knowledge to treat hemopoietic failure as a consequence of whole and partial body exposure using stem cell transfusion technics as well as recombinant regulation factors.

III. Objectives for next period (1991)

In 1991 (7. year of contractural period) it is our objective to continue to work on the objectives established for 1990 with emphasis on the following details:

1. Expansion of the computer data base of radiation accident clinical case histories. It will be possible by the end of 1991 to have more than 400 to 500 case studies in order to analyse the relationship of early blood cell changes to the clinical course and outcome.

2. Improvement of the granulocyte system simulation model in order to analyse the pattern of changes after accidental irradiation in relation to stem cell changes with and without stem cell transfusion and recombinant factor treatment. Development of a megakaryocyte-platelet simulation model as a basis to understand better the pathophysiological mechanisms of platelet changes after accidental irradiation.

3. Performance of stem cell transfusion in patients (in collaboration with stem cell transplantation units) with and without the use of recombinant regulation factors to establish appropriate protocols for clinical radiation accident management.

IV. Progress achieved including publications

1. Progress achieved

As far as the radiation accident clinical data base is concerned, progress was made through the fact, that intensive efforts were made to establish an internationally acceptable computer compatible case history form in collaboration with experts in Moscow. It is now possible to extend our data base to include several hundred radiation accident victims in order to correlate blood cell changes with clinical course and outcome. It is the first time in radiation accident research that such an effort is being made which is indispensible for establishing an knowledge based expert system for radiation accident management.

Significant progress has been possible in the further development of biomathematical computer assisted blood cell simulation models. In collaboration with the Department of Systems Engineering, it became possible to improve the granulocyte response model and to pave the way for an entirely new model for the megakaryocyte-platelet system in order to understand platelet changes and to use them as biological indicators for radiation exposure.

Cytogenetic studies were performed in leukemic patients after total body irradiation and bone marrow transfusion to

study the type of chromosomal aberrations seen in host lymphocyte metaphases as a function of time after radiation exposure. These studies will serve to analyse the radiation dose to the lymphocyte systems and the extend and severity of graft-versus-host disease and relapse rate of leukemia. This information is of importance in the improvement of the management of radiation accident victims.

Experimental studies were carried out in dogs using partial body irradiation to improve our understanding in the role of migratory stem cells and the possibilities of the use of recombinant regulation factors to enhance hematopoietic regeneration after extensive radiation exposure.

2. Publications

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2. Fliedner, T.M.: Hematological Indicators to Predict Patient Recovery after Whole Body Irradiation as a Basis for Clinical Management. In: Ricks, R.C. and Fry, S.A.: The Medical Basis for Radiation Accident Preparedness II. Elsevier, New York, Amsterdam, London, 1990

3. Nothdurft, W.: Use of Peripheral Blood Stem Cells for Transplantation. Experimental Protocols performed by the Ulm Group. In: H.J. Seidel (Edit.): The Hemopoietic Stem Cell. Universitätsverlag Ulm GmbH, 1990 (ISBN 3-927402-19-2)

4. Szepesi, T., J. Naudé and B. Schneider: Blood cell Changes as Indicators of Reversible and Irreversible Hemopoietic Damage to the Stem Cell Pool. In: H.J. Seidel (Edit.): The Hemopoietic Stem Cell. Universitätsverlag Ulm GmbH, 1990

5. Maiwald, M. et al.: Further Analysis of Radiation Accident Cases based on Lymphocyte Counts. In: H.J. Siedel (Edit.): The Hemopoietic Stem Cell. Universitätsverlag Ulm GmbH, 1990

6. T.M. Fliedner: Cell System Physiology and Pathophysiology: A Challenge for Biomathematical Modeling. International Seminar on Modeling and Signal Processing of Cellular Systems. Internationale Institute Schloß Reisensburg, Günzburg, October 28-30, 1990 (accepted for publication 1991)

7. Nothdurft, W., K. Baltschukat, L. Kreja, D. Krumwieh, F.R. Seiler, and W. Weinsheimer: Effects of recombinant human granulocyte colonystimulating factor (rhG-CSF) and granulocyte-macrophage colony-stimulating factor (rhGM-CSF) on canine bone marrow cells in vitro. (submitted to Intern. J. of Cell Cloning, April 1991)

8. Nothdurft, W., T.M. Fliedner, L. Kreja, D. Krumwieh, F.R. Seiler, C. Selig und W. Weinsheimer: Untersuchungen zur therapeutischen Wirksamkeit des Zytokins "granulocyte-macrophage colony stimulating factors" (GM-CSF) nach starker Strahlenschädigung des Knochenmarks. 4. Symposium "Molekulare und zelluläre Mechanismen der biologischen Strahlenwirkung", 27.2.-1.3.1991, Essen, Abstract Book, p.58

9. Nothdurft, W., C. Selig, T.M. Fliedner, A. Hintz-Obertrais, L. Kreja, D. Krumwieh, B. Kurrle, F.R. Seiler and W. Weinsheimer: Hematological effects of rhGM-CSF in normal dogs and in dogs exposed to total body irradiation with a radiation dose of 2.4 Gy (submitted to Intern. J. of Radiation Biology, March 1991)

10. Baltschukat, K., and W. Nothdurft: Hematological effects of unilateral and bilateral exposures of dogs to 300-KVp X rays. Radiat. Res. 123, 7-16, 1990

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12. Ziegler, B., Kreja, L., Bunjes, D., Spiess, S., Seidel, H.J., Fliedner, T.M.: Positive selection of CD34-/HLA-DR-Positive bone marrow (BM) cells by immunomagnetic particles: Their response to hemopoietic growth factors. Blut 61, p.120, 1990

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14. Nothdurft, W., Baltschukat, K., Selig, C.: Differences in radiation response between cells in S-phase and Non-S-phase cells of the granulocyte/macrophage progenitor (GM-CFC) compartment. submitted to Int. J. Rad. Biol., Mai 1990

15. Fliedner, T.M.: Das Stammzellsystem der Hämatopoese: physiologische und pathophysiologische Grundlagen. Verh. Dt. Ges. Path. 74, S. 1-18, 1990

16. Weinsheimer, W., Arnold, R., Heinze, B., Tibken, B., Hofer, E., Frickhofen, N., Fliedner, T.M.: Relationship between the number of intact stem cells and kinetic of hemopoietic reconstitution in humans after total body irradiation. ESRB, 23. Ann. Meeting, Dublin, 23.-26.9.1990, Abstract Book

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19. Kindler, H., Densow, D., Fliedner, T.M.: RADES - Medical assistance system for the management of irradiated persons. AIME-Conference, Maasstricht 1991

CONTRACT : BIO-065 Sector : B21

Title : Non-stochastic effects of irradiation in man : diagnosis, prognosis and treatment of acute radiation injury.

Prof. JAMMET **CIR Paris**

I. Summary of project and Global Objectives.

Title of the project : Experimental and Clinical Research on management of Radiation Accident Casualties.

The global objective of the project is to develop and validate the laboratory methodologies, the paraclinical investigations and the clinical protocols which specifically apply to the clinical management of global and partial accidental overexposure to ionizing radiations.

The two main areas of research concern : i) the assessement of damage (diagnosis, prognosis) and ii) the modalities of the immediate treatment of the life threatening effects of acute overexposures and the modalities of prevention and treatment of late sequelae.

The project relies on both experimental research and clinical investigations.

Experimental research is designed to improve the physiopathological knowledge relevant to irradiation accidents. It includes the physiopathology of irradiated lymphocytes (mainly the mechanisms of radiation induced apoptosis), the physiopathology of the hematopoietic systems, including bone marrow stromal cells, of the central nervous system and of the connective and vascular tissues after irradiation.

Clinical laboratory and paraclinical investigations comprise the evaluation and validation of biological parameters for diagnosis, biological dosimetry and prognosis (chromosomal aberrations, chromatine structure, apoptosis, HLA typing, blood cell kinetics in human and animal models).

Clinical research comprises the evaluation of different treatments for the regeneration of hematopoietic, connective and vascular tissues (cytokins, growth factors, drugs) with particular attention to the indications of allogenic bone marrow transplantation (cord blood, specific T Cell depletion, etc...)

Prevention and treatment of late radiation effects (such as fibrosis and sclerosis) are also considered experimentally (animal model) or in clinical trials.

II. Objectives for the reporting period (1990).

1. Implementation and evaluation of methods for the analysis of the chromatine structure of irradiated lymphocytes (image analysis, fluorescence video-microscopy and molecular biology of apoptosis).

2. Evaluation of biological indicators of total body irradiation at the serum level.

3. Protocols for treatment of myeloid aplasia.

4. Evaluation of treatment of radiation induced fibrosis by superoxyde dismutase (SOD).

III. Objectives for next period (1991).

1. Further evaluation of plasma parameters as indicators of total or partial irradiation, and in the monitoring of hematopoietic recovery.

2. Comparison of fluorescence image analysis, quantitative video-microscopy, flow cytometry and molecular biology for the assessement of apoptosis in irradiated blood cells.

3. Clinical evaluation of treatment of radiation induced myeloid aplasia with recombinant hematopoietic growth factors.

4. Further evaluation of SOD efficacy in the prevention and treatment of fibrosis. New routes for SOD administration.

IV. Progress achieved including publications.

1. Experimental research.

1.1. Quantitative image analysis of intensified fluorescence video-microscopy has been developped at the Institut Curie, allowing the observation of chromatine structure alterations and apoptosis on live cells, in experimental irradiation conditions. It has been shown that the DNA intercalation of fluorescent probes (acridine orange or ethidium bromide), which does not occur in quiescent cells, reflects local DNA-protein interactions in the chromatine of live cells and provides a new tool to study subtle changes in the physiology of irradiated cells (gene activation) or the

mechanisms of radiation induced apoptosis.

1.2. The electron microscopy analysis (Pr Said - Kremlin Bicêtre) of irradiated nerves (rat model) has shown specified ultrastructural alterations.

1.3. Radiation induced dementia has been studied in a rat model (Dr J.Y. Delattre - Pitié Salpétrière).

2. Clinical laboratory and paraclinical investigations

2.1. Total body irradiation (TBI)

The variations of hematological and biological parameters have been studied in serial blood samples from patients during and after several modalities of total body irradiation (Pr Cosset - Institut Gustave Roussy).

These parameters include blood cell counts, HLA typing, chromosome aberrations, cortisol, ACTH, adrenaline and noradrenaline, thyroid hormones and TSH, amylase, etc...

2.1.1. Blood cell counts

- Granulocytes have been analyzed during and after a single dose (10 Gy - 4 hours) total body irradiation (TBI) in 25 patients, and during and after a fractionated (11 x 1.35 Gy - 4 days or 6 x 2 Gy - 3 days) TBI in 24 patients. A granulocyte "peak" was found to be a constant feature 8 hours after the start of the 10 Gy single dose TBI. The mean amplification factor is 3,5, but with large variations from patient to patient. The same peak is observed after the first fraction (1.35 Gy - 2 Gy) of fractionated TBI. It is then significantly lower than after 10 Gy.

This "peak" could only provide the physicians with a supplementary parameter able to give a rough idea of the delivered dose. The large interindividual variations would not allow a very precise retrospective evaluation of the dose given.

In the same patients, the kinetics of disappearance of lymphocytes in the blood stream has been analyzed. The rate of lymphocytes still present is 50%, 8 hours after the beginning of a 10 Gy - 4 hours TBI, and 25%, 24 hours after the

start of this same irradiation.

This disappearance is clearly dose-related; however, here again, the interindividual variations preclude any reliable retrospective evaluation of the dose. It could only represent an additional clue.

2.1.2. HLA typing

The expression of class I HLA antigens has been studied before and after a 10 Gy - 4 hours TBI in 8 patients.

Twenty four hours after the beginning of this 10 Gy - 4 hours irradiation, the mean expression of the class I HLA antigens is 38% : that is clearly too low to permit a reliable HLA subtyping.

The same studies performed at 6 hours show a mean expression of 74,9% : this would allow subtyping in most cases.

Therefore, for class I HLA antigens, it should be recommended to take blood samples as soon as possible.

Preliminary results indicate that class II antigens expression could be even more sensitive to irradiation.

2.1.3. Chromosome aberrations

Precise cytogenetic analysis have been performed for 6 patients during and after single dose TBI, and for 3 patients during and after fractionated TBI. Preliminary results show that the retrospective evaluation of the dose which is allowed by this method is extremely precise in the 2-8 Gy range. We detected a trend for overestimating the dose below 2 Gy, and for underestimating the dose above 8 Gy.

2.1.4. Cortisol and ACTH

A constant rise of blood cortisol have been detected in 13 patients after 10 Gy - 4 hours TBI. (mean amplification factor : 6.5). This peak is lower (amplification factor x 4-5) after a 1.35 - 2 Gy TBI.

We could also detect an initial transitory increase in blood ACTH. The kinetics of the peaks ACTH - CORTISOL - GRANULOCYTES is consistent with the lymphocytes of an initial rise of ACTH inducing the cortisol rate, inducing itself a release of granulocytes by the bone marrow.

Although somewhat by the interindividual variations, cortisolemia could represent a supplementary parameter to retrospectively estimate the dose.

2.1.5. Adrenaline, noradrenaline, thyroid hormones, TSH

No significant variation of these parameters could be detected after either single dose or fractionated TBI in 19 patients.

2.1.6. Amylase

A constant and dose dependant increase in amylasemia after irradiation of the salivary glands could be confirmed from an extensive analysis of 93 irradiated patients. A comparison with a previous series of 73 italian patients (BECCIOLONI et al) and a biomathematical analysis of all these data are in progress.

2.1.7. EGF

Assays of epidermal growth factor (EGF) in saliva and serum have not shown reproductible patterns of variation, partly because of large variations in saliva secretion after irradiation.

2.2. Partial body irradiation

2.2.1. Hemicorporeal irradiation

The study of chromosomal aberrations and blood cell counts in patients treated by sequential hemicorporeal irradiation (Pr Laugier, Hôpital Tenon) has provided precise and unique dose-related data concerning the biological effects of heterogenous body irradiation.

2.2.2. Local irradiation

The genotoxic effects of protracted loco-regional irradiation (50-60 Gy in 5-6 weeks for breast cancer radiotherapy) or peripheral blood lymphocytes have been measured at the level of chromosomal aberrations, DNA repair efficiency and HGPRT mutations in 14 patients. Irradiation leads to a chromosomal aberration frequency in peripheral lymphocytes equivalent to 2 Gy of acute total body irradiation. DNA repair capacity is slightly but significantly altered.

3. Treatment of myeloid aplasia

Clinical research concerned the evaluation of different treatments for the restauration of hematopoiesis. Protocols including the use of recombinant growth factors (GM-CSF, G-CSF) have been pursued.

New modalities to improve or extend the indications of allogenic bone marrow transplantations such as ombilical cord blood, specific T cell depletion have been studied.

4. Treatment of radiation induced fibrosis

Thirty patients with invalidating radiation induced fibrosis were treated topically with liposome encapsulated bovine superoxide dismutase (SOD). A clinical and paraclinical score, including telethermography, pH, plethysmography, etc..., was used to evaluate the extent of fibrosis. It was significantly improved by SOD treatment.

Publications.

O. RIGAUD, G. GUEDENEY, I. DURANTON, A. LEROY, M.T. DOLOY, and H. MAGDELENAT.

- Genotoxic effects of radiotherapy and chemotherapy on the circulating lymphocytes of breast cancer patients.

I. Chromosomes aberrations induced in vivo.

Mutation Research, 242(1990) 17-23, Elsevier

Key-words : chromosome aberrations, radiotherapy, chemotherapy, breast cancer

- Genotoxic effects of radiotherapy and chemotherapy on the circulating lymphocytes of breast cancer patients.

II. Alteration of DNA repair and chromosome radiosensitivity.

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Key-words : DNA repair, chromosomal radiosensitivity, radiotherapy, chemotherapy, breast cancer.

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Bull. Cancer, 1991, 78, 6, p. 525

Progress Report

Contract: Bi6-079 **Sector:** B21

Title: Development of conditions allowing restoration of hemopoiesis by allogeneic purified and in vitro multiplied pluripotent hemopoietic stem cells

Head (s) of project: D.W. van Bekkum and G. Wagemaker, TNO-ITRI

I. Summary of Project and Global Objectives

The project aims at the following research objectives:
1/ to study the effect of haemopoietic growth factors in vivo and in vitro;
2 / to investigate non-toxic conditioning regimens for bone marrow transplantation;
3 / to develop methods to identify and isolate hemopoietic stem cells.

Briefly, preclinical studies will be carried out in total body exposed rhesus monkeys to study whether treatment with haemopoietic growth factors can shorten the period of profound pancytopenia and/or immunodeficiency after transplantation of a limited number of bone marrow cells, or without bone marrow transplantation. In the latter situation, it is also investigated as to whether the response to administration of growth factors can be used as a prognostic indicator of haemopoietic damage.

Following a radiation accident resulting in exposure to high, but inhomogeneous doses of ionizing radiation, non-toxic conditioning treatment by immunosuppression could be decisive when optimal bone marrow transplants are not available and allogeneic transplants would be considered, which, although resulting ultimately in partial chimeras or rejection, could be of temporary benefit to ensure survival. Earlier work in mice showed that treatment with monoclonal antibodies against T lymphocytes may be useful. A large spectrum of antibodies is therefore currently tested in mice ultimately selecting such antibodies for which equivalents in humans are available and which could be tested by preclinical studies in rhesus monkeys.

Concentration of haemopoietic stem cells and removal of T lymphocytes which are responsible for graft vs host disease would be of considerable benefit for imperfectly matched bone marrow transplantation. Considerable stem cell enrichment (40-140-fold) with less than 1% contamination of T lymphocytes has already been achieved using a method by which stem cells are selected on the basis of binding to the CD34 monoclonal antibody ICH3, which is in turn conjugated to Protein-A coated immunomagnetic beads. The stem cells can be safely eluted from the beads by competition with excess immunoglobulin and recovered. This method will be improved and used for allogeneic MHC matched, sex-mismatched grafts in rhesus monkeys with sustained chimerism (recognized from karyotyping) as an endpoint parameter.Chimerism will be studied as a function of the radiation dose and the number of T lymphocytes present. The method will be adapted to human treatment modalities.

Murine stem cells can be expanded in vitro 3-5 times by a short incubation with IL-3. This will be extended to preclinical studies in rhesus monkeys.

II. Objectives for the reporting period

In the reporting period, much emphasis has been laid on the preparative method used to isolate stem cells by positive selection based on binding to CD34 antibodies and the use of the resulting fractions for preclinical autologous as well as allogeneic bone marrow transplantation in rhesus monkeys, for the purposes outlined before.

III. Objectives for next period

In the next reporting period, we aim to have completed the studies on non-toxic conditioning regimens that are currently underway in mice and to report on the ongoing studies on mitigation of radiation induced pancytopenia in rhesus monkeys by treatment with hemopoietic growth factors as well as on the in vitro effects of haemopoietic growth factors on stem cells.

IV. Progress achieved including publications

We proposed to develop a simple, rapid, safe and generally applicable method to positively select for and purify hemopoietic stem cells for routine use in autologous as well as allogeneic BMT. The method is based on recognition of the stem cells by the anti-human CD34 monoclonal antibody ICH3. ICH3 is a high avidity mouse IgG2a that neither modulates nor has any effector functions such as cytotoxicity. In pilot experiments, sorted ICH3 positive cells were shown to effectively reconstitute hemopoiesis in autologous lethally irradiated rhesus monkeys. To develop a method suitable for the large-scale preparation of BM stem cells, Protein A was covalently bound to immunomagnetic beads and ICH3 was conjugated to Protein A. Cells bound to the antibodies can then be eluted from the protein A beads by competitive elution using excess soluble IgG. The present project envisages the further development and tests for the practical feasibility of this method, using standardized experimental conditions for autologous as well as allogeneic BMT in rhesus monkeys, thereby focussing on the immunosuppression required to prevent rejection of allogeneic highly purified stem cell concentrates and the use of cytokines to accelerate immunohemopoietic reconstitution in vivo.

In the reporting period, we aimed at quality control and reproducibility of the ICH3/Protein A/immunomagnetic beads method and on allogeneic transplantation in MHC-matched, sex mismatched rhesus monkey donor/recipient pairs to assess the potential of these stem cell concentrates, to establish the radiation dose required for acceptance of such highly purified cells and to establish the number of T-lymphocytes that can be allowed in a bone marrow graft without causing unacceptable GvHD.

A large number of experiments was done to ascertain reproducibility and quality control of the CD34-positive bone marrow fractions to be used for transplantation purposes. Using an optimal cells/beads ratio, the method appeared to be equally efficient for prefractionated stem cell concentrates, obtained by density centrifugation and E-rosette sedimentation to remove residual T-lymphocytes, and for unfractionated bone marrow subjected to Ficoll-centrifugation to remove granulocytes and red cells. In both cases, about 1% of CD34-positive cells were obtained, which contained on the average 60 - 70 % of progenitor cells as measured by colony-formation in response to GM-CSF. Since we established earlier by an assay based on measuring regeneration rate after autologous transplantation, that the number of regenerating stem cells in vivo in FACS-sorted CD34-positive cell fractions

runs closely parallel to the content of in vitro colony-forming cells, it may be safely assumed that this result is also indicative for the number of regenerating stem cells in the isolated CD34-positive cells. The CD34-positive cell fractions can be easily depleted of a small fractions of monocytes/ macrophages by a similar procedure using a CD11b monoclonal. The quality control of the fractions obtained was further done by measuring the forward and perpendicular light scatter, showing that the majority of the cells have light scatter properties compatible with those of stem cells as identified in the window set in the light scatter plot of unfractionated bone marrow. In addition we routinely measured content of T-lymphocytes by CD4/CD8 monoclonal antibodies and showed that in any case the CD34-positive cell fractions are depleted for 2 to 3 logs of T-lymphocytes. By these experiments we set a standard for CD34-positive fractions suitable for allogeneic bone marrow transplantation, using a method that equally well handles 10^6 as well as 10^{10} bone marrow cells.

Because of the availability of suitable MHC-matched, sex-mismatched sibling donors for monkeys selected for allogeneic bone marrow transplantation, we started with pilot experiments on the use of these fractions for allogeneic transplantation. For these purposes, we studied regeneration rate of peripheral blood cells in comparison to conventional T-lymphocyte depleted bone marrow grafts and monitored chimerism as a function of time after transplantation. Chimerism was determined in bone marrow by measuring the number of donor-type karyotypes in IL-3 and GM-CSF stimulated bone marrow samples, as well as in T-lympnocytes stimulated with IL-2 and PHA. In addition, in 2 monkeys, we deliberately added 5 x 10^5 per kg body weight peripheral blood T-lymphocytes to the bone marrow grafts in an attempt to establish an upper limit for the number of T-lymphocytes.

Conventional T-lymphocyte depleted control grafts following a relative low TBI dose of 7.4 Gy (orthovolt X-rays) will result in sustained partial bone marrow and peripheral blood T-lymphocyte chimerism without causing GvHD. The 2 monkeys treated in this way were used as standards. The CD34 positive cell engrafted as rapidly as the controls. Karyotyping 3 weeks after transplantation showed that this rapid engraftment was donor derived. However, after more prolonged periods of time, monkeys conditioned with 8.3 Gy TBI showed only 2-5 % donor type karyotypes in their reconstituted bone marrow, suggesting that either the endogenous residual stem cells had taken over, or that the graft had been partially rejected. Surprisingly, however, donor type peripheral blood T-lymphocytes in these monkeys have been very high during the entire observation period, which is a result comparable to a successful bone marrow transplantation for SCID in humans. To date, a suitable explanation for this peculiar type of split chimerism is not available. To exclude that this result was attributable to a limited capacity of CD34 positive cells to supply sustained bone marrow progeny, we raised the conditioning TBI dose to 9 Gy. In 3 of 3 evaluable monkeys transplanted following 9 Gy TBI, bone marrow chimerism remained as high as in the conventional controls. These results demonstrate that CD34-positive cells are very well capable to produce myeloid as well as T-lymphocyte progeny for sustained periods of time, a feature that was hitherto unexplored. In addition, the CD34-positive grafts apparently require a more intensive conditioning regimen than the conventional control grafts.

The same data demonstrated that graft-versus-host disease did not occur from grafts that contained 10^5 T-lymphocytes 3per kg or less, but did occur, and lethally so, in 1 of 2 recipients of grafts to which 5 x 10^5 peripheral blood T lymphocytes were deliberately added. This result indicates that the upper limit of T lymphocytes allowable in an MHC-matched bone marrow graft, a situation in which GvHD is entirely determined by minor histocompatibility33 disparities, is in between 1 and 5 x 10^5 per kg body weight.

So, in conclusion, these experiments demonstrated the validity and reproducibility of the proposed stem cell purification method, established the usefulness of the CD34 positive cells for allogeneic bone marrow transplantation, showed that CD34 positive cells can be used to achieve bone marrow as well as T-lymphocytes chimerism, indicated that CD34 positive cells require more intensive conditioning of the recipients than conventional grafts and showed in a prospective way the number of T-lymphocytes that is acceptable in an MHC-identical bone marrow graft.

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Contract: Bi6-059 **Sector: B21**
Title: Radiation damage and recovery of the immune system

1 Doria ENEA

I. Summary of Project and Global Objectives

Research is concentrated on the problems related to damage and recovery of the immune system after radiation exposure. These aspects are of particular importance for the treatment of radiation accidents because they result in a decreased resistance to pathogens and in development of autoimmune diseases and tumours. The adopted experimental approach, using genetically homogeneous animals and standardized irradiation and treatment conditions, is likely to provide reproducible data to evaluate the consequences of radiation accidents and to design appropriate strategies for effective medical intervention.

Mouse populations will be studied after whole-body exposure to acute X-ray doses ranging from 1 to 10 Gy. Irradiated mice will be left untreated or, starting immediately after irradiation, will be treated by one or more injections of immunoregulatory molecules, such as recombinant cytokines (IL-3, IL-4, IL-5, IL-6, IL-7). Untreated and treated survivors will be studied to assess the number and activity of blood leukocytes and to evaluate the immune functions of spleen lymphocytes (mitotic responses to Con A and LPS, antibody production, T helper cell activity, IL-2 production and IL-2R expression. Comparison of the effects of various treatments after different radiation doses should indicate the conditions for optimal intervention to accelerate recovery of the immune system and to prevent radiation death.

Cell typing for bone marrow transplantation in humans requires rapid and precise techniques. Currently available alloantisera are not always specific enough to identify single antigenic specificities and are difficult to obtain from multiparous women or from volunteers subjected to planned immunization. Also mouse monoclonal antibodies (mAb) do not always distinguish between HLA class I or class II alloantigens. Conversely, human mAb produced by human B cells from immunized volunteers, after EBV transformation and subculturing, are more powerful than mouse mAb to identify HLA alloantigens and will be investigated. Another approach, which will be pursued, consists of the amplification of small amounts of DNA by polymerase chain reaction and subsequent hybridization by allele or sequence specific oligonucleotide probes. This research on cell typing will be carried out in cooperation with Prof. G.B. Ferrara from the Cancer Institute in Genoa.

Head of Project 1: Prof. Doria

II Objectives for the reporting period

We have investigated whether murine recombinant IL-3 injected in mice exposed to sublethal irradiation (100-200 cGy) can accelerate the recovery of thymocytes and peripheral T and B lymphocytes.

III Objectives for next period

1. Determination of the optimal dose of murine recombinant IL-3 to accelerate complete recovery of T and B lymphocytes in mice exposed to 300 and 400 cGy.
2. Effect of murine recombinant IL-2, IL-4, IL-5, IL-6, IL-7 on the recovery of T and B lymphocytes in sublethally irradiated mice.
3. Effect of murine recombinant IL-3 on the survival of lethally irradiated mice injected with suboptimal doses of bone marrow cells.

IV Progress achieved including publications

Our program is part of the European Network of Experimental and Clinical Research and is focussed on Radiation damage and recovery of the immune system, with the main object of designing appropriate strategies for medical intervention in radiation accidents and radiotherapy.

Studies on mice. We have previously reported that the synthetic nonapeptide VQGEESNDK, position 163-171 of human IL-1 beta, when injected in mice immunodepressed by sublethal irradiation induces restoration of T helper cell activity and IL-2 production. The immunorestorative activity of the nonapeptide is similar to that of the human recombinant (hu r) IL-1 beta, but with no IL-1-like inflammatory effects which hamper the use of the whole protein as immunomodulator. Based on these findings we compared the protective and restorative activities of the 163-171 nonapeptide and hu r IL-1 beta on the 30 day-survival of lethally irradiated mice. When mice were given a single injection of different doses of the nonapeptide or hu r IL-1 beta 20 hrs before total-body irradiation, both molecules were found able to increase the percent survival of mice exposed to 750 or 850, but not to 950 cGy. The nonapeptide, however, was less effective than hu r IL-1 beta and displayed a different dose-response relationship, suggesting that the two molecules act through different radioprotective pathways. When mice were injected with the nonapeptide or hu r IL-1 beta immediately after exposure to 850 cGy, the percent survival was also increased but restoration was lower than protection in both cases. The nonapeptide was less effective than hu r IL-1 beta also in restoration but the two molecules displayed a comparable dose-response relationship as if they

shared similar mechanisms. These findings altogether indicate that the 163-171 nonapeptide is able to protect from lethal radiation injury and to restore viability. The nonapeptide is less effective than hu r IL-1 beta but, as it does not exhibit the IL-1-like side effects of the whole molecule, it appears as an interesting compound for medical intervention in radiation accidents and radiotherapy.

IL-3 is a colony-stimulating factor that regulates hemopoiesis. This cytokine, indeed, is involved in the differentiation of pluripotent stem cells to mature cells of several lineages, such as neutrophils, macrophages, erythrocytes, eosinophils, megakaryocytes and mast cells. IL-3 also promotes limited self-renewal of the multipotent stem cells which give rise to splenic colonies. Yet, there is no convincing evidence that IL-3 regulates the generation and growth of mature T and B lymphocytes. We have, therefore, investigated whether murine recombinant (mu r) IL-3 injected into mice exposed to sublethal irradiation can accelerate the recovery of thymocytes and splenic T and B cells. In a first series of experiments, mice were given 300 cGy and daily injections of mu r IL-3 starting immediately after irradiation for 5 consecutive days. Seven days after irradiation, thymuses and spleens were assayed for cellularity, thymocyte mitotic response to ConA, splenocyte mitotic responses to ConA and LPS, splenocyte antibody response to the hapten trinitrophenyl (TNP) and helper activity to the carrier horse red blood cells (HRBC) in cultures with the conjugate TNP-HRBC. Irradiated mice of other groups were similarly treated with mu r IL-3 for 10, 15, or 20 days and sacrificed 14, 21, or 28 days after irradiation, respectively. Under these conditions, IL-3 treatment did not accelerate the recovery from radiation damage in the thymus and spleen. In a second series of experiments, mice were given 100, 200, 300, or 400 cGy and daily injections of mu r IL-3 starting immediately after irradiation for 5 consecutive days. Mice were sacrificed 7 days after irradiation, and thymuses and spleens were assayed as indicated above. Results indicate that daily injections of 5 ug mu r IL-3 induced complete recovery of thymocyte cellularity and mitotic responsiveness to ConA in mice exposed to 200 but not to 300 or 400 cGy. Lower doses than 5 ug were not efficient in mice exposed to 200 cGy, whereas 1 ug was very effective after 100 cGy. Similar results were obtained for splenocyte count and mitotic responses. Fluorimetric analysis performed on thymocytes indicate that 5 ug mu r IL-3 completely reversed to normal values the CD4 and CD8 cell distribution altered by 200 cGy. Also the antibody response and helper activity of spleen cells were depressed by 200 cGy but could be recoverd up to the level of unirradiated controls if mice were treated with 5 ug, but not 0.5 ug, mu r IL-3. In conclusion these findings indicate that injection of mu r IL-3 may induce complete recovery of T and B cellularity, and responsiveness to mitogens and antigens in sublethally irradiated mice provided larger amounts are injected after higher radiation doses. Thus, IL-3 regulates the generation and growth of mature T and B lymphocytes and appears as a powerful molecule that can be successfully used in radiation accidents and radiotherapy.

Studies on humans. Treatment of irradiated persons by bone marrow transplantation may be successful if host and donor are HLA compatible. Cell typing techniques must be rapid and very precise in the fine recognition of antigenic specificities. The reagents now routinely used, such as alloantisera from multiparous women or from volunteers subjected to planned immunization and mouse monoclonal antibodies (mAb) to HLA class I and II molecules, are not easily available and do not always distinguish between alloantigens. Conversely, human mAb produced by human B cells from immunized volunteers, after EBV transformation and repeated subcultures, are much more powerful than mouse mAb in the fine typing of the HLA antigenic repertoire. Also DNA amplification by polymerase chain reaction (PCR) and subsequent hybridization by allele or sequence specific oligonucleotide probes is a valid approach to cell typing. Prof. G.B. Ferrara, Head of the Immunogenetics Laboratory at the Istituto Nazionale per la Ricerca sul Cancro in Genova, is collaborating with our laboratory in the application of human mAb and recombinant DNA techniques to the identification of HLA allelic specificities. Fourteen cell lines (MP1-14) secreting cytotoxic human IgM alloantibodies of restricted HLA specificities have been established. By use of mAb MP8 the HLA-DP polymorphism was analyzed and a supertypic determinant encoded by 7 DPB genes was detected. Furthermore, the HLA-DP typing was also performed using dot-blot analysis with 14 synthetic oligonucleotide probes. Each probe was tested against genomic DNA amplified by PCR using DP beta-specific primers. A total of 45 HLA homozygous B cell lines of known DPw specificities was analyzed and different hybridization patterns were found for each DPw specificity. The oligonucleotide hybridization performed on DPw negative B cell lines exhibited a pattern distinct from those of known DPw specificities, indicating the presence of novel DP allelic sequences. Thus, the use of these technologies has allowed reliable typing of HLA-DP antigens, which may play an important role in allogeneic bone marrow transplantation and in susceptibillity to autoimmune diseases.

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Progress Report

Contract: Bi6-347e Sector: B22

Title: The reduction of the risks of late effects from incorporated radionuclides (NRPB Association)

1	Stradling	NRPB
2	Volf	KfK Karlsruhe
3	Métivier	CEA - FAR
4	Burgada	ADFAC (Univ. Pierre et M Curie)
5	Peetermans	Univ. Ziekenhus Antwerpen

I. Summary of Project and Global Objectives

(i) Aims and objectives of the project

To evaluate the reduction in risk of late effects from incorporated radionuclides by the administration of chelating agents. To provide practical guidance to those responsible for the treatment of accidental exposures.

(ii) Aims and objectives of reporting period

(a) To investigate the efficacy of the siderophore analogues code names DRO-HOPO and DTPA-DX for enhancing the removal of Pu and Am from the body.

(b) To synthesise and test the siderophore analogue 3,4,3 - LIHOPO.

(c) To investigate treatment regimens for the decorporation of transportable forms of thorium and uranium.

(d) To assess the effects of DTPA administration on the induction of bone tumours by 239Pu.

(e) To develop methodologies for investigating the influence of chelation therapy on the reduction of the risk of late effects to bone.

(iii) The results obtained so far with the siderophore analogues support the strategy of examining the use of such compounds for increasing the excretion of Pu and Am from the body and it hoped that further improvements can be made during 1991.

At present no chelates are available which substantially increase the limitation of Th and U from the body and this remains a potentially serious problem in radiological protection. It is hoped that with the expertise available within the participating organisations and the willing cooperation of other bodies, significant progress can be made in this area in future.

If has been shown that an appreciable reduction in bone tumour incidence can be achieved with chelation treatment. Studies with human adult and fetal bone marrow should also provide valuable information on the reduction of the risk of late effects after the administration of chelating agents.

Head of Project 1: Dr Stradling

II. Objectives for the reporting period

To investigate the efficacy of the siderophore analogues code named DFO-HOPO and DTPA for enhancing the elimination of plutonium and americium from the body, to examine the efficacy of DTPA for the decorporation of thorium and Tiron for the decorporation of uranium.

III. Objectives for next period

The siderophore analogue 3, 4, 3-LIHOPO has been synthesised by Dr R Burgada at the Pierre and Marie Curie University, Paris. This compound will be tested for its ability to enhance the elimination of Pu and Am from rats - screening experiments conducted in the USA suggest that this ligand is likely to be superior to DFO-HOPO for Pu.

Diphosphonates are well established complexing agents for uranium in-vitro, but little information is available on their ability to remove the metal from the body. Some of these compounds, provided free of charge by Albright and Wilson plc (UK) and Norwich Eaton Pharmaceuticals Inc (USA) will be examined for this purpose during 1991 but the study is expected to continue into 1992/93.

To date there appear to be no effective chelating agents available for removing inhaled thorium from the body. Professor Sun of the Shanghai Medical Institute has demonstrated that, in animals, substituted phenol aminocarboxylic acid derivatives can substantially increase the excretion of thorium after its intravenous injection. It is hoped that some of these compounds will be tested further during 1991 but the study is expected to continue into 1992/93.

IV. Progress achieved including publications

A hydroxypyridone derivative of desferrioxamine (DFO-HOPO), a dihydroxamic derivative of DTPA (DTPA-DX), and DTPA were tested at dosages of 30 μmol kg^{-1} for their ability to remove ^{238}Pu and ^{241}Am from rats after their intravenous injection as citrate or inhalation as nitrate. The two siderophore analogues were provided free of charge by Professor K Raymond, Dept of Chemistry, Univ. of California, Berkeley.

The most effective treatment regimen for injected Pu was the repeated administration of DFO-HOPO. By 7d the body content was reduced to 8% of that in untreated animals; the value after DTPA treatment was 15%. Repeated dosages of 3 μmol kg^{-1} DFO-HOPO were as effective as those of 30 μmol kg^{-1} DTPA. After inhalation of Pu, repeated treatment with DTPA, DTPA-DX or DFO-HOPO reduced the body contents by 7d to respectively 10, 15 and 31% of controls. After inhalation of Am, DTPA-DX and DTPA were equally effective, the body contents being reduced to 7% of control values with repeated treatment. Injection of DFO-HOPO was ineffective for enhancing the elimination of inhaled or injected Am.

These results confirm the strategy of examining the use of siderophore analogues for the decorporation of Pu and Am. However, at present, DTPA should remain the agent of choice, particularly after inhalation.

The efficacy of Ca DTPA and Zn DTPA have been evaluated for removing thorium from the rat after its deposition as nitrate in the lungs. When the initial mass concentration in the lungs simulated human exposure to four times the ALI for ^{232}Th, the prompt (300 or 1000 μmol kg^{-1} body weight at 30 min) or repeated (30 or 300 μmol kg^{-1} body weight at 30 min, 6 h, 1, 2, 3d) administration of Ca DTPA were at best only moderately successful for enhancing the elimination of Th. By 7d after exposure, the body contents were respectively 74%, 65%, 90% and 74% of those present in untreated animals. When the mass concentration in the lungs simulated acute exposure to 1.7 x 10^{-3} times the ALI for ^{232}Th, the efficacy of treatment was not increased appreciably despite the substantial reduction in mass. After the repeated administration of Ca DTPA at dosages of 30 and 300 μmol kg^{-1} using the protocol above, the body contents of thorium by 7d were respectively 69% and 51% of those in untreated animals. Under comparable conditions, the efficacy of Zn DTPA was less than Ca DTPA.

The results of these experiments suggest that more effective chelating agents are needed for the treatment of workers overexposed to water soluble thorium compounds.

In the past decade, several phenolic compounds have been investigated in animals for their ability to prevent fatal uranium poisoning. The most promising derivative appears to be Tiron (sodium 4, 5-dihydroxybenzene-1, 3-disulphonate). In experiments conducted at NRPB, the compound was administered to rats in dosages of 30, 300 or 1000 μmol kg^{-1} at 20, 60 and 180 min after the intratracheal instillation of uranyl nitrate. The amounts of uranium deposited in the lungs of rats were equivalent to intakes by workers of about 12 times the permitted daily limit of 2.5 mg. The average body contents of uranium 5d after exposure using the treatment regimens above were respectively about 100%, 78% and 65% of those in untreated animals.

These results suggest that the administration of Tiron is of limited practical value for enhancing the elimination of uranium from the bodies of overexposed workers.

Publications covering work of reporting period

(a) Stradling, G N, Gray, S A, Moody, J C, Hodgson, A, Raymond, K N, Durbin, P W, Rodgers, S J, White, D L and Turowski, P N. The comparative efficacy of DFO-HOPO, DTPA-DX and DTPA for enhancing the elimination of plutonium (IV) and americium (III) from the rat. Proc. 3rd Int. Symp. on Chelating Agents, Pilsen, July 10-12, 1990. Pilsen Medical Report (in press).

(b) Stradling, G N, Moody, J C, Gray, S A, Hodgson, A and Ellender, M. The efficacy of DTPA treatment after deposition of thorium nitrate in the rat lung. IBID.

(c) Stradling, G N, Moody, J C, Gray, S A, Hodgson, A and Ellender, M. The efficacy of DTPA treatment after deposition of thorium nitrate in the rat lung. Human Toxicology (in press).

(d) Stradling, G N, Gray, S A, Moody, J C, Hodgson, A, Raymond, K N, Durbin, P W, Rodgers, S J, White, D L and Turowski, P N. The efficacy of DFO-HOPO, DTPA-DX and DTPA for enhancing the excretion of plutonium and americium from the rat. Accepted by International Journal of Radiation Biology.

(e) Stradling, G N, Gray, S A, Moody, J C, and Ellender, M. The efficacy of Tiron for enhancing the excretion of uranium from the rat. Accepted by Human Toxicology.

Head of Project 2: Dr Volf

II. Objectives for the reporting period

To investigate the efficacy of the siderophore analogues code named DFO-HOPO and DTPA-DX for enhancing the removal of plutonium and americium from the body of the rat after intravenous injection of the actinides. To complete studies of the reduction of bone tumour risk in plutonium-239 treated rats through the near lifetime administration of Zn-DTPA in drinking water.

III. Objectives for next period

(a) Further studies of dose and time relationships for DFO-HOPO.

(b) Comparison of chelate effectiveness with Zn, Na, H or Ca salts of DTPA-DX.

(c) Studies of the biliary excretion of Pu-238 and Am-241 following administration of DTPA and DTPA-DX.

(d) Dose and time relationships for injected and oral LI-HOPO.

IV. Progress achieved including publications

The study of the effects of continuous oral Zn-DTPA on bone tumour induction in male Sprague-Dawley rats injected with 37 kBq Pu-239/kg body weight was completed when the last rat died. Preliminary analysis of the results indicates that treatment, with 10E-3 M Zn-DTPA as drinking water, beginning 4 days after Pu-239 injection reduced the bone tumour incidence from 3.75+/-2.0% to 15.0+/-2.5%. When the start of treatment was delayed until 30 days post Pu-239 injection the tumour incidence was reduced only marginally but the survival time to death with a bone tumour was increased by about 17% as compared to untreated rats.

The effects of the two new chelators, DFO-HOPO and DTPA-DX, were compared with that of DTPA in male Sprague-Dawley rats following a single intravenous injection of Pu-238 and Am-241. Following subcutaneous injection of 30 umoles/kg at 1 hour after radionuclide injection, DFO-HOPO reduced plutonium retention in skeleton and liver, at 7 days post radionuclide injection, to 19 and 6%, respectively, of that in untreated animals, similar treatment with Ca-DTPA was less effective reducing the retention skeletal retention to 39% and that in liver to 25% of that found in the untreated controls. DFO-HOPO was without effect on the retention of Am-241. The combination of DFO-HOPO with Ca-DTPA was even more effective than DFO-HOPO alone in reducing retention in skeleton and liver. DFO-HOPO given orally at a dose of 100 umoles/kg 3 minutes after the nuclides was as effective as the injected substance in reducing retention of Pu-238 in the skeleton and liver.

DTPA-DX, following either subcutaneous injection or oral administration, was less effective than Ca-DTPA in removing plutonium from the skeleton and liver or americium from the skeleton. Injected DTPA-DX did reduce the liver retention of americium by a factor of about 3 compared to Ca-DTPA.

The effectiveness of DFO-HOPO was found to decrease exponentially with time after Pu-238 injection, the approximate half-times for the mobilisation of the nuclide from skeleton and liver were 6 and 12 hours, respectively. Within the dose range 0.3 to 10 umoles/kg, a negative linear relationship was observed when the mobilising effect was plotted against the logarithm of the injected dose of chelator.

Continuous chelate infusion, for 14 days after radionuclide injection, was achieved by means of implanted "mini-pumps", in general tissue retention was reduced to a greater extent than by the single injections of the chelators. However, with DFO-HOPO the continuous infusion was no more effective than the single injection for mobilising plutonium from skeleton and liver.

Publications covering work of reporting period

Taylor, D M and Volf, V, Oral treatment of plutonium incorporation, Plzensky Lekarsky Sbornik (Pilsen Medical Reports), in the press.

Head of Project 3: Dr Metivier

II. Objectives for the reporting period

To test the efficacy of the siderophore analog 3, 4, 3-LIHOPO containing four hydroxypyridinone groups in comparison with the diethylene-triaminepentaacetic acid (DTPA) for enhancing the decorporation of ^{238}Pu as tributylphosphate complex (^{238}Pu-TBP) after inhalation in Sprague Dawley rats.

III. Objectives for next period

Treatment assays with LIHOPO will be continued in 1991. Different schedules for the injections of the chelating agent will use to detect the efficacy of the therapy to decorporate the Pu-TBP after inhalation without inducing toxic effect.

IV. Progress achieved including publications

The first purified sample of 3, 4, 3-LIHOPO (50 mg) has been available at the end of November and it has been tested by intravenous injections in rats previously exposed to an aerosol of ^{238}Pu-TBP. Three groups of rats were used: group 1, 5 rats exposed to Pu-TBP as untreated control animals, groups 2 and 3 exposed to Pu-TBP and injected respectively by DTPA and 3, 4, 3-LIHOPO. The average initial lung burden was about 3, 7 kBq (5, 7 ng Pu). Unique intravenous injection of the different chelates is performed one hour after the end of inhalation at a concentration of 30 $\mu mole\ kg^{-1}$. The rats have been killed 7 and 14 days after contamination and lungs, livers, kidneys and femurs collected. The different organs are actually counted and results are not yet available.

Head of Project 4: Dr Burgada

II. Objectives for the reporting period

To synthesise and purify the siderophore analogue code named 3, 4, 3-LIHOPO. To provide a sufficient amount of the substance for other laboratories within the CEC to investigate its efficacy for removing plutonium and americium from the body of laboratory animals.

III. Objectives for next period

A further batch of 3, 4, 3-LIHOPO will be provided for NRPB to undertake experiments on the efficacy of the substances after the inhalation of Pu and Am in amounts which correspond to human intakes of up to about 20 times the ALI. The treatment regimens will involve acute and repeated treatment at different dosages. Diphosphonates with a known high affinity for uranium will be synthesised for the CEA Pierrelatte to investigate their ability for removing the metal from experimental animals.

IV. Progress achieved including publications

The synthesise of 3, 4, 3-LIHOPO is time consuming and the yield is low, about 5%. To data sufficient material has been provided for experiments to be conducted at KfK Karlsruhe on the efficacy of intravenous and oral administration for removing plutonium and americium from the body after their intravenous injection, and for an experiment at CEA Bruyeres-le-Chatel to examine its efficacy for these actinides after the inhalation of amounts which correspond to human intakes considerably in excess of the ALI.

Publications covering work of reporting period

None, but staff involved with the synthesis will be co-authors of publications resulting from the studies with 3, 4, 3-LIHOPO.

Head of Project 5: Dr Peetermans

II. Objectives for the reporting period

Aims and objectives for Reporting Period 1990 has been a preparation phase of the study. This is due to specialized technical needs of the project. We also could only start the work late because of the lack of supplementary funds to appoint a scientific collaborator. Nevertheless good work has been performed in the double field of Flow Cytometry and Bone Marrow Cultures as a basis for the study using ionizing radiation.

III. Objectives for next period

External irradiation of Long Term Human Bone Marrow Cultures and its subpopulations with collaboration of the department of radiotherapy (Prof Scalliet, University of Antwerp) Effect of Growth factors on recuperation of bone marrow cultures after irradiation.

IV. Progress achieved including publications

Report on the activities of the "Flow Cytometry and Sorting" Unit:

This unit has mainly been involved in the study of the effect of haematopoietic growth factors on highly progenitor enriched bone marrow (BM) cell suspensions.

Enrichment is achieved by means of immunofluorescent labelling of the BM Cells for CD34 antigen and subsequent cell sorting. Purities of > 95% are routinely obtained.

These progenitor enriched suspensions are followed in semi-solid, liquid and semi-solid + liquid cultures upon addition of one or more growth factors (IL3, GM-CSF, G-CSF, M-CSF, IL4, IL2). In this manner stimulatory, inhibitory and synergistic action of growth factors can be followed without the interference of accessory cells.

First results point to an inhibitory action of IL-4 on IL-3 and GM-CSF induced proliferation and to a stimulatory action of IL-4 on G-CSG induced proliferation. A first article on this topic is envisaged to be prepared in the beginning of 1991.

Using the BromodeoxyUridine (BrdUrd) - Hoechst quenching technique the unit also tries to unravel the initial kinetic response of CD34+ progenitors upon growth factor addition. Preliminary experiments to evaluate the toxicity of BrdUrd are carried out at the moment. For these experiments it is also necessary to optimalise serum deprived culture conditions: this work is now in progress.

Bone Marrow Cultures: Effect of Growth Factors

Study in semi-solid cultures. Single and combined action of IL-3, GM-CSF and M-CSF on mononuclear cells of human bone marrow. Combined action of erythropoietin and IL-3 has also been performed.

We found a synergistic effect between IL-3 and GM-CSF, IL-3 and G-CSG, GM-CSF and G-CSF, IL-3 and GM-CSF and G-CSF, IL-3 and M-CSF, GM-CSF and M-CSF.

IL-3 is more effective than 5637-CM in supporting erythroid colony growth.

Publications covering work of reporting period

None.

Contract: Bi6-063 **Sector: B23**
Title: Early and late effects of radiation on skin

1 Hopewell Univ. Oxford

I. Summary of Project and Global Objectives

The studies carried out by the Research Group by the Research Institute (University of Oxford) from part of a broad programme of work to evaluate 'Radiation Effects on the Skin and Subcutaneous Tissues' with respect to radiological protection cirteria and the treatment of localised accidental over-exposure of the skin. Work is carried out in collaboration with the Laboratoire de Radiobiologie Applique, CEA/IPSN/DPS/SPE, Gif sur Yvette, France and the Department of Radiobiology, Medical College of St. Bartholomew's Hospital, London, United Kingdom.

The global objective of the Oxford Laboratory can be described under two broad headings:-

a) research related towards the provision of data required for the further improvement of radiological protection cirteria for the skin and

b) a better understanding of the pathophysiological mechanisms responsible for the development of both early and late radiation responses of the skin as a guide to improvements in the treatment of such lesions.

Head of Project 1: Dr. Hopewell

II. Objectives for the reporting period

In studies required for the improvement of radiological protection criteria work has been carried out on the effects of low dose-rate irradiation from $^{90}Sr^{90}Y$ on the acute radiation response of the skin and to compare these findings with previous studies with high dose-rate irradiation.

Studies as to the possible depth of target cell populations responsible for late radiation damage to the skin have been continued with an evaluation of the time-course of the development of dermal atrophy after ^{170}Tm irradiation.

III Objectives for next period

Studies will be continued into the effects of dose-rate on the radiation response of the skin, with more emphasis being placed on the evaluation of late changes. Work will also be initiated in relation to the treatment of both early and late radiation responses after accidental over-exposure.

IV Progress achieved including publications

Studies have continued into the effects of variations in dose-rate, from sources of $^{90}Sr/^{90}Y$, on the acute radiation response of the skin of the pig. Irradiation was with a standard 22.5mm diameter plaque and the dose-rates that have been used to date have ranges from 2.2cGy/min to 10.7cGy/min. The results from these exposures have been compared with those produced by a high dose-rate source of 300cGy/min. For the low dose-rate studies, $^{90}Sr/^{90}Y$ plaques were mounted in nylon holders which could be sutured to the skin for the period of irradiation, which ranged from a few hours to ~3.5 days. Pigs were only anaesthetised for the times of application and removal of the plaques.

After irradiation the sites of irradiation were assessed at weekly intervals for 9 weeks, by at least three independent observers, for to the presence or absence of moist desquamation and the severity of erythema. The results for the dose-related incidence of moist desquamation are presented in this report (Figure 1). For each of the low dose-rates investigated there was a clearly defined dose-effect relationship, however, the dose effect curves were progressively shifted to the right as the dose-rate was reduced. At the dose levels associated with the 50% incidence of moist desqumation (ED_{50}) the dose modification factors, when compared with the high dose-rate 300cGy/min source, were 1.5, 1.7 and 2.4 for sources with dose-rates of 10.7, 5.2 and 2.2cGy/min, respectively. At the ED_{10} and estimated threshold doses for moist desquamation, the dose-rate effect was, on the basis of the experimental data, less clearly defined. However, the fitted dose-effect curves still suggested modification factors of a similar order of magnitude. Information on the late tissue responses are required before the impact on these results for radiological protection can be fully evaluated.

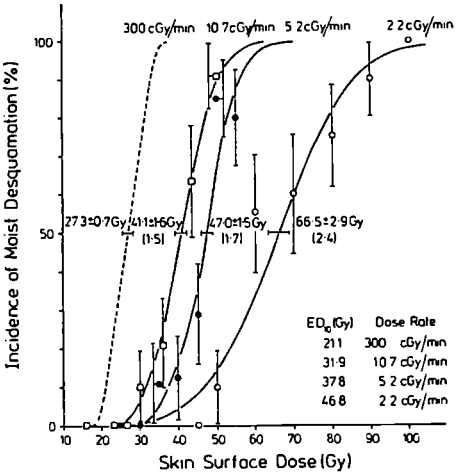

Figure 1. Dose-related changes in the percentage incidence of skin sites developing moist desquamation after irradiation with 22.5mm diameter $^{90}Sr/^{90}Y$ plaques of dose rate 300cGy/min (-----); 10.7cGy/min (□ - □); 5.2cGy/min (● - ●) and 2.2cGy/min (○ - ○). Error bars indicate ±SE. ED_{50} and ED_{10} values with associated dose modification factors are indicated.

Late changes in the skin of pigs, assessed by a measurement of the relative changes in dermal thickness, have been evaluated after irradiation from a 20 x 40mm ^{170}Tm plaque. Exposure was to single doses of 30, 40, 80 and 120Gy at the skin surface at a dose-rate of 1Gy/min. Doses of 30, 40 and 80Gy have previously been shown to be associated with a 15%, 25% and 35% reduction in relative dermal thickness, respectively, based on measurements of dermal thickness in histological sections 104 weeks after irradiation from a 19mm diameter source. No previous studies had been carried out as to the likely severity of damage after a dose of 120Gy.

Serial assessments as the severity of any late dermal changes were obtained at intervals of 12, 14, 16, 18, 20, 22, 26, 44 and 52 weeks after irradiation, using a non-invasive 'A' scan ultrasound technique. These time intervals were selected as previous studies after $^{90}Sr/^{90}Y$ irradiation had indicated two distinct phases of reduction in relative dermal thickness; the initial phase between weeks 12 and 22 and a later phase at approximately 52 weeks.

Observations carried out for up to 52 weeks after ^{170}Tm irradiation have shown a pattern of changes qualitatively similar to those seen after $^{90}Sr/^{90}Y$ exposure (Figure 2). There was an initial reduction in relative dermal thickness between 12 and 22 weeks after irradiation the severity of which was dose dependent, the reduction ranging from between 10% and 15%. There was then evidence for a second phase of reduction in relative dermal thickness at approximately 52 weeks after irradiation but additional measurements, at later times after irradiation, will be required before the magnitude of the effect can be fully evaluated. It is proposed to continue to assess changes for periods up to 104 weeks after irradiation.

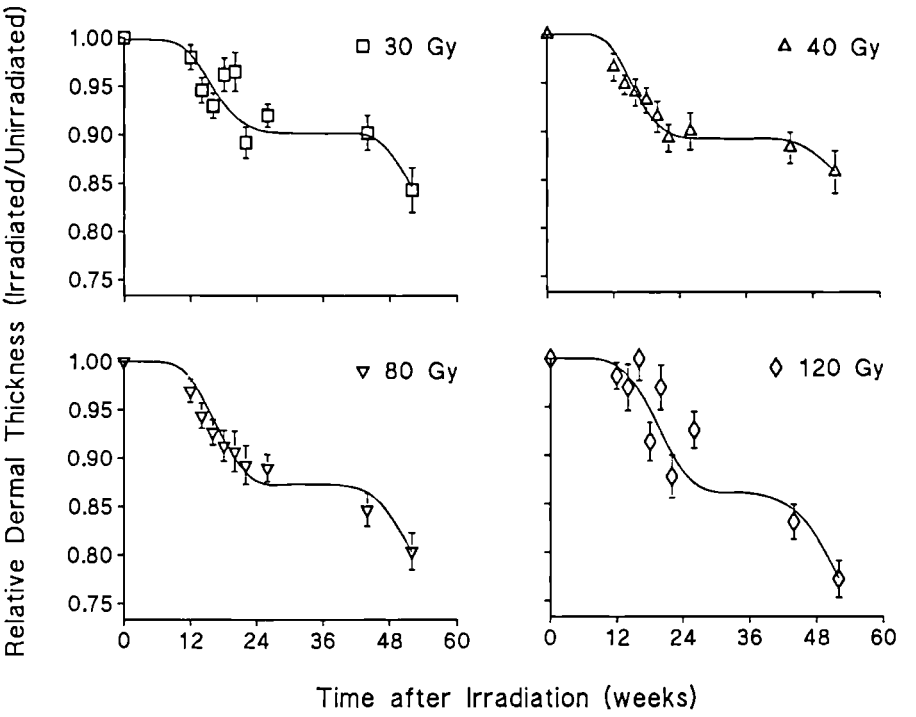

Figure 2. Time-related changes in the relative thickness of the dermis of pigs (ratio of the thickness of the dermis in irradiated skin to that of adjacent areas of unirradiated skin) after irradiation with single doses from ^{170}Tm of 30Gy (□); 40Gy (△); 80Gy (▽) and 120Gy (◇). Error bars indicate ±SE.

Full publications:-

Hopewell, J.W. (1990) The skin: its structure and response to ionizing radiation. Int. J. Radiat. Biol. 57, 751-773.

Sieber, V.K. and Hopewell, J.W. (1990) Radiation-induced temporary partial epilation in the pig: a biological indicator of radiation dose and dose distribution in the skin. Radiat. Protect. Dosim. 30, 117-120.

Morris, G.M. and Hopewell, J.W. (1990) Epidermal cell kinetics of the pig: a review. Cell Tiss. Kinet. 23, 271-282.

van den Aardweg, G.J.M.J., Arnold, M. and Hopewell, J.W. (1990). A comparison of the radiation response of the epidermis in two strains of pig. Radiat. Res. 12, 283-287.

Mortimer, P.S., Simmonds, R.H., Rezvani, M., Robbins, M.E.C., Hopewell, J.W. and Ryan T.J. (1990) The measurement of skin lymph flow by isotope clearance: reliability, reporducibility, injection dynamics and the effects of massage. J. Invest. Derm. 95, 677-689.

Progress Report

Contract: B16-C-058-F Sector:
Title:

PROBLEMS RELATED TO SKIN AND UNDERLYING TISSUES AFTER ACCIDENTS INVOLVING LOCAL IRRADIATION. EXPERIMENTAL STUDY IN THE PIG.

I. Summary of Project and Global Objectives

Acute localized irradiation accidents have been rather scarce during the past decades, but early diagnosis and treatment remain difficult problems for physicians up to now. Research and development of an experimental animal model are important for studying the pathogeny of such injuries and for testing new diagnosis methods with experimental and well codified protocols. The pig, used for a long time in radiobiological research, was choosed for diagnosis and surgical studies; the rabbit, for economical reasons, was choosed for pharmacological studies, when a large number of animals had to be used.

Diagnosis and prognosis cannot be separated in the first approach of the management of the patients. As far as non invasive methods are a necessity, the biophysical methods considered had to make obvious and better, to quantify, the clinical and pathological reactions of the irradiated tissues. Thermography was proposed for the characterization of the early inflammatory reaction, X-ray CT and NMRI for making obvious the early oedema and gammascintigraphy for delimitating the necrotized and, later on, the fibrous tissues when a healing process is possible.

Treatment attempts involved either surgical or pharmacological approaches. With regards to surgery the major problem is the early delimitation of tissues which are going to be necrotic and which have to be shed: some of the diagnosis methods above-mentioned may be useful for this objective (NMRI, gamma scintigraphy). The most adapted time and extent of tissue removal can be determined accurately on the animal model.

Post-irradiation fibrosis is a severe complication of acute localized irradiation, because of the its disabling characteristic and its tendency to spread out in surrounding tissues, weakly or not irradiated. Studies on the cultural characters of the fibroblasts isolated from fibrotic tissues, their cytogenetical anomalies, their synthesis changes and their particular responses to growth factor are means to try to understand and control this pathological process.

Head of Project : F. DABURON

II Objectives for the reporting period

- Relations between fibrosis extension in the irradiated muscle and the dose according to the depth.
- Assessment of the functional ability of the skeletal muscle after irradiation (gamma scintigraphy, electromyography).
- Possible role of TGFb as activation factor of the development of post irradiation fibrosis.

III Objectives for next period

- Improvement of NMRI of the early and late irradiation injuries in the rabbit (using contrast media) .
- Functional exploration of the inflammatory reaction after irradiation in pigs using a 99m Tc labeled lipoglycopeptide, with and without anti-inflammatory treatments (systemic injections or lymphographies).
- Electromyography: exploration of the muscular blood flow with electrical stimulation in pigs; studies of changes in muscular fatigability after irradiation.
- Expression of TGFb and TNFa by irradiated fibroblasts, either in cellular culture or in the tissues.

IV Progress achieved including publications

IV-1 Pathophysiology of fibrosis extension.

- Experimental models for localized irradiation studies (all doses are given at 2cm depth i.e. 4 times higher at the skin basal layer level):

Large White pigs, 40-60 kg, were irradiated on the external side of the right thigh (m. biceps femoralis) with a collimated source of 192 Ir at 30, 40, 64 Gy (n=8 for each dose), and on the back (m. iliospinalis) with six irradiation areas per animal: 4, 8, 12, 16, 20 and 24 Gy (n=8).

The animals were sacrificed 30 weeks after irradiation and the depth of the muscular fibrosis measured.

- Results:

It must be pointed out that the irradiated protocol, delivered very closely to the skin surface (17mm) involved radiolesions more similar to those observed after exposition to "hot particles" than those classicaly observed after overexposure to 60 Co gamma rays or X-rays with a SSD of 50-80cm.

No microscopic modification of the muscular structures was observed after 4, 8 and 12 Gy; between 16 and 64 Gy fibrosis thickness, assessed 30 weeks after irradiation, increased with the dose (Table 1). In pigs locally irradiated either on the thigh or on the back, fibrosis developed in irradiated muscle only when moist desquamation was observed on the skin lesion: the animal surface dose was comprised in these cases between 48 and 64 Gy (12-16 Gy at 2cm depth); fibrosis spread in tissues irradiated at lower doses when applied doses were higher (Table 1). In fact, the importance of the early inflammatory processes after irradiation would control fibrosis extension in depth, .

although the early skin symptomatology gave no information on the pathology at depth and on the importance of late effects.

Applied Dose Gy (2 cm depth)	Fibrosis Limit mm (mean ± sd)	Irradiation Dose (at fib.limit) Gy (mean ± sd)
64 (n=8)	69 ± 4	10 ± 2
40 (n=8)	53 ± 6	10 ± 3
30 (n=8)	41 ± 5	12 ± 3
24 (n=8)	33 ± 5	13 ± 3
20 (n=8)	25 ± 4	15 ± 3
16 (n=8)	18 ± 3	19 ± 3

Table 1 : Fibrosis depth limit and corresponding dose according to the single dose applied.

Conclusion:

The limit of the deep extension of the fibrosis in the skeletal tissue correspond to a deep dose of 14 Gy (sd=4) whatever was the applied dose if higher than 12 Gy at 2cm depth.

IV-2 Assessment of muscle functionnal ability

a) Scintigraphy

The characterization of tissues involved in necrotic and fibrotic process was performed with 201 Tl scintigraphy (see 1989 final report). The influence of blood flow changes during these explorations was shown off using 133 Xe scintigraphy injected intra-arterially. The accumulation phases of 201 Tl are, to a large extent, independent from blood flow changes.

Specific anti-inflammatory drugs were used concomitantly with a 99m Tc labeled lipoglycopeptide to make obvious its property of revealing activated macrophages.

Conclusion:

We can dispose now of two markers very useful in the follow up of the different phases of the evolution of acute lesions (inflammation, necrosis and fibrosis).

b) Modelization of muscular fatigue

Most of the biophysical methods used up to now for the dosimetry of acute localized irradiation had a high threshold in the dose-effect relationship: thermography, RMN imaging, scintigraphy. We tried to develop a non invasive method for assessing lower doses.

Fatigue was induced by electro-stimulation of the nervous branch of m. flexor carpi radialis which was rather easy to study individually in pigs.

Performance change were estimated with indwelling electrodes and strain gauge set on a restraint device.

Conclusion:

Using periodic sequences working at one fixed frequency we are able to characterize a decrease of the excitibility due to fatigue induced by high frequency and a change in excitation-contraction coupling induced by low frequency. Works are in progress to assess the effect of irradiation between 5 and 20 Gy on the characteristic of that fatigue.

IV-3 Fibrosis studies

Objectives

Post-irradiation fibrosis exhibits, particularly in non spontaneously healing lesions, a tendency to spread out in surrounding tissues, weakly or not irradiated; fibroblasts isolated from radiation-induced fibrotic tissues exhibits in culture an abnormal and activated phenotype. Previous cytogenetic studies exhibited that the "pre-transformation" of fibroblasts extracted from post-radiation fibrosis would be induced by irradiation.

On the other hand post-irradiation fibrosis spreads in a modified extracellular matrix and an inflammatory tissue, and keeps a proliferative capacity which remained high even after 2 years. Then what are the factors involved? We studied first TGFb (Transforming Growth Factor b) which controls the extracellular matrix accumulation.

Methodology

Fibrotic tissues were removed from the normal dermis and the thigh lesion at 1, 4, 6 and 16 months after irradiation; RNA were hybridized with a molecular probe coding for 1 exon of TGFb.

Results

An intense hybridization could be seen in all fibrosis, much higher than in normal dermis. When RNA was isolated from confluent primary cultures the expression was weak and variable in dermis and high and stable in fibrosis; in the later no diminution of the TGFb expression could be observed up to 2 years later.

TGFb stimulated collagen synthesis either in fibrosis or in normal dermis, modified culture aspect (hills and valleys) in monolayer fibrosis cultures.

At low dose TGFb stimulated the synthesis of all proteins, especially the non-collagenic ones; at high dose collagen was still synthetized when non collagen proteins were inhibited.

Conclusion:

It did exist an autocrin stimulating process in post-radiation fibrosis fibroblasts which could be a mecanism of cellular activity persistence in chronical fibrosis. On the other hand further mediators, especially the extracellular matrix and its degradation fragments, might interfere.

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Progress Report

Contract: Bi7-049 **Sector:** B23

Title: European clinical research on practical protocols for the diagnostics and treatment of localized overexposure

1	Gongora	Institut Curie
2	Strambi	ENEA
3	Herranz-Crespo	Hospital General Marañón

I. Summary of Project and Global Objectives

Les brûlures radiologiques représentent la pathologie la plus fréquente engendrée par les accidents radiologiques. Elles sont différentes des brûlures thermiques et chimiques ; elles posent des problèmes diagnostiques et thérapeutiques spécifiques. Le traitement optimal des brûlures radiologiques implique que la quantité d'énergie délivrée aux différents tissus soit parfaitement connue ; aussi le programme de recherche porte sur la mise au point de protocoles relatifs à la dosimétrie biologique et sur des protocoles d'investigations paracliniques. Ces derniers font appel essentiellement à la termographie I.R. , à la capillaroscopie et aux méthodes nucléaires. Le traitement des brûlures radiologiques est, en fonction de la gravité, soit médical, soit chirurgical. Sur le plan médical les protocoles de recherche concernent les méthodes d'isolement segmentaire ou total, les problèmes de la douleur qui présentent des caractères spécifiques, les traitements par enzymes fondés sue des données physiopathogéniques. Le traitement chirurgical pour être aussi conservateur que possible doit faire appel aux données dosimétrique physiques et paracliniques pour définir les volumes d'excision et choisir judicieusement les méthodes de recouvrement (greffe, lambeau, peau artificielle). Le programme de recherche porte sur ces différents domaines.

Head of Project 1: Prof. Gongora

II Objectives for the reporting period

- Apport de la capillaroscopie au diagnostic, au traitement et à la surveillance à long terme des brûlures radiologiques.
- Apport de l'étude de la vascularisation par scintigraphie au diagnostic et à la surveillance à long terme
- Physiopathologie de la peau irradiée
  - Immunohistochimie
  - Culture d'explants de peau irradiée
- Approche thérapeutique
  - Traitement des fibroses post radiques par la superoxyde dismutase
  - Prévention des fibroses post radiques par la superoxyde dismutase

III Objectives for next period

-Poursuite du travail sur la physiologie de la peau irradiée notamment par hybridation in situ (TGFα, TGFβ, FGF) et Immunohistochimie (récepteurs de PDGF)
- Poursuite du travail sur la prévention et le traitement des fibroses postradiques par la superoxyde dismutase
- Traitement de la douleur

La douleur est une constante dans la symptomatologie des radiolésions ; elle est présente à tous les stades de la maladie ; par son intensité et par sa durée, parfois de plusieurs mois, elle constitue une difficulté thérapeutique majeure; elle ne peut pas être traitée à la demande. Il est donc nécessaire d'analyser l'efficacité des différentes médications cliniques et physiques, d'en préciser les modalités quantitatives et chronologiques d'utilisation et d'association.

- Traitement chirurgical

La conduite du traitement chirurgical des brûlures radiologiques repose sur la connaissance approfondie de l'évolution de ces lésions ; lorsque le chirurgien est sollicité, généralement quand apparaît la nécrose, d'une part, il ignore le volume potentiel de nécrose, d'autre part, il ne connaît pas parfaitement la viabilité réelle des tissus apparemment sains sur lesquels il pourrait déposer greffe ou lambeau. Les méthodes biologiques et physiques d'évaluation de ces données doivent être mises en jeu. Leur efficacité demande encore à être testée. De même, le moment le plus favorable pour intervenir, pressenti par des études expérimentales chez l'animal, demande à être précisé. Enfin, le choix des modalités de recouvrement après excision de tissus nécrosés ou en voie de l'être, greffe ou lambeau doit faire l'objet d'essais cliniques.

Une étude particulière doit porter sur l'utilisation de peaux artificielles.

IV Progress achieved including publications

IV - I Capillaroscopie

L'étude des modifications capillaroscopiques après irradiation aiguë a porté sur 29 observations. Il s'agit d'irradiation des mains accidentelles dans la plupart des cas.

- Les anomalies capillaires son fréquentes.

On observe deux catégories de modifications :

Les unes importantes consistent en raréfaction des anses, zones désertes, hémorragies, ectasies diverses et néogénèse . Ces anomalies sont souvent associées et sont en relation directe ou indirecte avec la zone exposée ce qui confère une certaine spécificité et permet le diagnostic différentiel avec des maladies systémiques.

Les autres sont plus subtiles : capillaires sinueux ou dilatés

Il est important de prendre en considération le moment où l'examen est pratiqué par rapport au moment de l'exposition. En effet, comme pour d'autres moyens d'investigations (thermographie et scintigraphie vasculaire), les résultats diffèrent en fonction du délai qui sépare l'examen du moment de l'exposition. Mais alors que thermographie et scintigraphie montrent des modifications qui s'estompent par la suite, à l'inverse, la capillaroscopie, le plus souvent ne montre pas de modifications significatives immédiatement ; les anomalies n'apparaissent que plus tardivement. (Dans certains cas, cependant, une modification capillaroscopique est le seul témoin de l'exposition). Aussi, la capillaroscopie présente davantage un intérêt pronostique que diagnostique. Elle est toutefois utile au diagnostic topographique.

En outre, les modifications capillaroscopiques morphologiques sont très longues à restaurer et peuvent constituer tardivement le seul témoin d'une exposition.

Bien que les modifications capillaroscopiques ne soient pas très précoces, il est très important de pratiquer un examen précoce de référence notamment dans le cas où il y a une pathologie associée.

Les expositions des mains engendrent une symptomatologie capillaroscopique qui prend tout son intérêt dans le contexte clinique et dans le contexte paraclinique (thermographie, scintigraphie vasculaire.)

IV - II Examens radioisotopiques

Ils portent essentiellement sur l'étude des modifications vasculaires et l'étude des réactions osseuses.

Les méthodes d'investigations de la vascularisation utilisant des traceurs radioactifs ont essentiellement deux objectifs : étude des modifications des débits vasculaires, étude des modifications de la perméabilité vasculaire après irradiation. Largement étudiées sur le plan expérimental chez l'animal, leur appréciation en clinique est relativement restreinte.

Comme pour la thermographie, les modifications de la microcirculation et de la circulation en général sont dépendantes de multiples facteurs affectant les territoires de référence et peuvent contribuer à accroître artificiellement les différences de débit entre tissus irradiés et tissus sains.

Ces méthodes présentent un intérêt majeur pour le diagnostic précoce, pour le bilan préchirurgical, pour le suivi des séquelles et des complications.

Lors d'une irradiation accidentelle, c'est généralement un ensemble de formations tissulaires qui est affecté et non pas exclusivement le revêtement cutané. L'atteinte osseuse est fréquente ; elle dépend bien entendu de l'intensité et de l'énergie du rayonnement ; or, dans les irradiations accidentelles, les énergies en cause sont généralement relativement élevées ; c'est le cas pour la gammagraphie industrielle.

Des modifications précoces peuvent être enregistrées par scintigraphie à l'aide de molécules ostéotropes. Il s'agit généralement d'hyperfixation osseuse des territoires irradiés. Exceptionnellement, on peut observer à l'inverse pour des doses très élevées une "sidération" des fonctions métaboliques et l'absence de fixation physiologique.

IV - III Physiopathologie de la peau irradiée

L'étude a porté sur les modifications immunohistochimiques et physiopathologiques de la peau mammaire humaine irradiée.

1 - Immunohistochimie de la peau irradiée

L'objectif de l'étude immunohistochimiques est de définir, en fonction du délai après l'irradiation,

l'évolution du phénotype des fibroblastes activés et les modifications biologiques en rapport avec le développement de la fibrose pathologique.
Des prélèvements cutanés mammaires ont été collectés chez 30 femmes opérées à des délais variables (3 mois à 71 mois) après irradiation thérapeutique de la glande mammaire par 60Co. (dose peau 25 à 40 Gy en 5 à 6 semaines) soit dans le champ d'irradiation (peau irradiée) soit en dehors du champ (peau non irradiée).

1.1 - Protéines du cytosquelette
- La vimentine
Tous les fibroblastes dermiques examinés expriment la vimentine, que les peaux aient ou non été irradiées. Cependant, au niveau du derme normal, les fibroblastes visualisés par l'anticorps anti-vimentine sont en nombre modéré et répartis à travers le derme superficiel et profond. Dans le derme irradié, une accumulation de cellules vimentine-positives est observée au niveau de la zone superficielle, immédiatement adjacente à la jonction dermo-épidermique, le long de la membrane basale.
- L'alpha-actine de muscle lisse
Au niveau des peaux non irradiées, l'anticorps anti-alpha actine marque essentiellement les médias musculaires des vaisseaux ainsi que les cellules myoépithéliales des glandes sudoripares. De surcroît, un certain nombre de fibroblastes de derme irradié expriment l'alpha actine de muscle lisse ; par ailleurs, le marquage de la média et de l'adventice fibreuse des vaisseaux télangiectasiques est particulièrement intense, sur les coupes de peau irradiée.
Ce phénotype alpha actine - positif est généralement identifié comme caractéristique de fibroblastes "activés", réactionnels ou "myofibroblastes".
- La desmine
L'anticorps anti-desmine n'a visualisé que les formations musculaires périvasculaires ou quelques fibres musculaires éparses dans le derme des peaux irradiées ou non. Il n'a pas été observé de marquage des cellules fibroblastiques du derme.
En conclusion, les fibroblastes de derme irradié présentent donc un phénotype V+A+D- caractéristique des myofibroblastes dans 22/30 cas. Les peaux non irradiés présentaient un faible marquage des fibroblastes dermiques par l'alpha actine de muscle lisse dans 3/9 cas (desmoplasie?).

1.2 - La fibronectine
- Dans les peaux non irradiées
L'anticorps antifibronectine marque faiblement les espaces intercellulaires au niveau de la partie profonde de la couche basale, en regard de la membrane basale. La fibronectine est également visualisée autour des parois vasculaires et le long de membranes cytoplasmiques d'un petit nombre de cellules fibroblastiques du derme superficiel. L'intensité de marquage est globalement faible.
- Dans les peaux irradiées
A niveau de l'épiderme, le marquage, d'intensité plus forte, s'étend aux couches basale, prabasale et parfois intermédiaire. La fibronectine, essentiellement intercellulaire et quelquefois membranaire, est également visualisée dans le cytoplasme sous-membranaire des cellules épithéliales des couches parabasale et intermédiaire, dans 6/30 cas.
Au niveau du derme, la fibronectine apparaît essentiellement inter- et péricellulaire, marquant la quasi-totalité des fibroblastes observés. Un marquage des parois vasculaires est également observé.

1.3 - Le TGF Beta
- Dans les peaux non irradiées
Au niveau de l'épiderme, le TGF Beta est visualisé sur les membranes cellulaires cytoplasmiques, parfois nucléaires, des cellules de la couche basale de l'épiderme ; exceptionnellement, l'anticorps anti-TGF Beta marque faiblement les membranes cytoplasmiques de rares fibroblastes dermiques.
Un marquage vasculaire, d'intensité variable, est également observé à la limite entre l'intima et la media des vaisseaux intradermiques, évoquant ainsi une localisation du TGF Beta sur la membrane basale sous-endothéliale.
- Dans les peaux irradiées
Les cellules de l'épiderme présentent un marquage nucléaire prononcé par l'anticorps anti-TGF

Beta, précisément localisé dans la couche intermédiaire, lorsque l'épiderme est de faible épaisseur et le derme épais et apapillaire, étendu aux couches parabasale et superficielle lorsque l'épiderme est plus épais et le derme papillaire. Une large majorité des fibroblastes du derme montre un marquage intranucléaire par l'anticorps anti-TGF Beta.
Le marquage vasculaire reproduit celui observé dans le derme des peaux non irradiées.

1.4 - Le Récepteur de l'EGF
- Dans les peaux non irradiées
L'épiderme est faiblement marqué au niveau des couches basale et plus rarement parabasale profonde ; ce marquage est uniquement membranaire.
Dans le derme, seules sont quelquefois marquées les membranes cytoplasmiques des cellules glandulaires des glandes sudoripares.
Les cellules endothéliales vasculaires sont négatives.
- Dans les peaux irradiées
Le Récepteur d'EGF est également membranaire, mais visualisé au niveau de la totalité des couches cellulaires de l'épiderme jusqu'aux couches superficielles. Le marquage est beaucoup plus intense généralement que dans les épidermes de peaux non irradiées, en particulier lorsque le prélèvement est pratiqué entre 3 et 6 mois après la fin de l'irradiation.
La topologie et l'intensité de marquage apparaissent caractéristiques d'une irradiation antérieure, même ancienne de la peau. Dans le derme, il n'a pas été observé de différence entre le derme irradié et non irradié.
Ainsi l'analyse immunohistochimiques de la peau irradiée montre un phénotype activé des fibroblastes, surtout dans la zone adjacente à la jonction dermo-épidermique. Les cellules épidermiques montrent une surexpression de récepteurs de facteurs de croissance et de facteur fibrosant (TGF Beta). L'ensemble des résultats suggère une interaction dermoépidermique de type paracrine.

2 - Physiopathologie du fibroblaste irradié
La mise en culture d'explants des peaux irradiées ou non permet d'observer :
- l'extension retardée des fibroblastes de peau irradiée
- la migration et la colonisation à distance des fibroblastes de peau irradiée,
contrairement à la prolifération contiguë des fibroblastes de peau non irradiée
- la formation de structures nodulaires après confluence dans les cultures de peau irradiée, l'absence de ces formations pour les peaux non irradiées (inhibition de contact)
- la capacité des seuls fibroblastes irradiés de croître sans sérum.
Ces résultats suggèrent l'acquisition d'un caractère "transformé" des fibroblastes irradiés.
Ceci a conduit à l'étude de la surexpression de certains facteurs potentiellement liés à la prolifération autocrine. L'analyse par Northern blot de mRNA extrait des cultures d'explants et de prélèvements de derme irradié ou non a été entreprise. Les sondes utilisées sont celles du TGFß, TGFα, PDGF. Cette étude n'est pas terminée.

IV - IV Approche thérapeutique des lésions radiques localisées

1 - Traitement de fibroses constituées
47 malades ayant développé une fibrose post radique après traitement radiothérapique pour cancer du sein ont été traités par superoxyde dismutase. La dose tumeur était supérieure à 50 Gy. Ces malades présentaient une fibrose mammaire ou axillaire étendue douloureuse s'accompagnant de gêne fonctionnelle. Le traitement a comporté l'application locale biquotidienne d'une préparation liposomale de superoxyde dismutase érythrocytaire bovine à 4000 unités par mg. La durée du traitement était de trois mois et la quantité totale de superoxyde dismutase administrée par malade était de 100mg. L'efficacité du traitement a été évaluée par rapport à la situation initiale par l'aspect clinique (score de Laugier modifié), par téléthermographie infrarouge et par pHmétrie cutané. Les contrôles ont été effectués avant traitement, à 6 semaines, 3 mois, 6 mois et un an de la fin du traitement. On observe une amélioration objective caractérisée par une réduction du score qui en un an passe de 69,7±29,2 à 37,9±17,45. Les réponses portent dans l'ordre chronologique sur la douleur (à partir de la 2ème semaine), sur l'assouplissement de la fibrose (à partir de la

$4^{ème}$ ou $5^{ème}$ semaine), sur la diminution de la pigmentation et des télangiectasies (à partir de 6 mois) et sur l'amélioration fonctionnelle (entre 9 et 12 mois). Cette amélioration paraît liée à la quantité totale de superoxyde dismutase avec un seuil de réponse à 30 mg, une réponse optimale pour 90 à 100 mg.

2 - Traitement préventif des radiolésions par la superoxyde dismutase
30 malades soumises à une curiethérapie intrautérine ont reçu un traitement préventif des réactions et des complications rectales.
Le traitement a comporté l'application locale de superoxyde dismutase ; la surveillance clinique a porté sur la douleur, la diarrhée, les hémorragies ; l'évaluation paraclinique sur les données de la fibroscopie et de l'histologie sur biopsie. Le recul est de un an.

3 - Application de l'EGF au traitement des radiodermites
Récemment, a débuté une étude de l'utilisation de l'EGF (Epidermal Growth Factor) en application locale pour le traitement des radiodermites. 5 patients, présentant des séquelles récentes d'une irradiation aiguë localisée (thérapeutique) sous forme de radiodermite ulcérée évolutive ont été traitées par application locale biquotidienne (4 semaines) de pommade contenant 10 microgrammes/g d'EGF recombinant humain.
Une amélioration clinique a été constatée dans les 5 cas traités. L'amélioration n'étant définie actuellement que par comparaison avec l'évolution spontanée habituelle de ces lésions, il convient maintenant de rendre plus objective l'évaluation de l'efficacité thérapeutique et de préciser les indications du traitement. Aucun effet de l'EGF recombinant n'a été observé sur la fibrose sous jacente. Ce résultat est en accord avec la localisation immunohistochimiques intra-épidermique du récepteur de l'EGF.

Head of Project 2: Dr. Strambi

II Objectives for the reporting period

II.1 Capillary microscopy.

The research has been aimed to study and standardize reliable techniques for monitoring radiation effects in cutaneous and in mucous tissues, as well in acute as chronical exposure.

II.2 Thermography.

Cutaneous blood flow alterations, particularly in chronically radiation exposed skin, can also be studied by means of telethermography associated with a standardized thermal stimulation technique. This method give us Thermal Recovery Times (TRT) which are more meaningful than the traditional baseline thermal gradients.

III Objectives for next period

III.1 Capillary microscopy.

Utilization of a computer system to process the images of microvessels directly from the microscope lens through a telecamera CCD B/N Sony, which is connected to an appropriate image analyzer for transformation from analogic to digital form. These images will be quantified according to size, number per field, morphometry, relative relationship. etc. and stored such that, over time, a profile of the structure under study can be defined.

III.2 Thermography.

Analysis of TRT mean values in normal subjects as well as in radiation workers, particularly those employed in medical radiology. This analysis is aimed to assess a possible relationship between skin radiation damage and TRT variations, also when clinical evidence is still lacking. That circumstance will be of value in the early diagnosis, as well for acute

(accidental) as for chronic exposure, and it is particularly important when dosimetric evaluations are difficult or lacking.

IV Progress achieved including publications

IV.1 Capillary microscopy.

We used binocular stereomicroscope Nikon SMZ-X with optic fibre illuminator and a microcamera. This apparatus permits a tree-dimensional visualization of the microvessels. The chosen sites were the nail fold and the bulbar conjunctiva. The examination of conjunctiva is of a particular interest because it allows observation of the complete terminal microvessel network: capillaries, venule, arteriole, and arterio-venous anastomosis. The clinical investigation, still in course, is carried out in cooperation with the Occupational Medicine Institute of the University of Naples. It concerns a group of some 350 radiation workers, chronically exposed since many years, with scarce or lacking dosimetric data.

The collected biomicroscopic results are scored in four groups as follows: 0 = normal; 1 = slight alterations; 2 = middle alterations of capillary loops; with reference to morphology, number, calibre and distribution of the microvessels; 3 = major alterations. In about 6% of the examined workers we found alterations of group 2 and in about 5% of group 3.

IV.2 Thermography.

In traditional thermography, the various energetic quanta are visualized in thermal points, with a minimal difference of only 0.1 °C.

To improve the sensitivity of the thermographic method we utilize a thermal stimulus applied on the skin and then we record the subsequent Thermal Recovery Time (TRT).

For the present study the following equipment is employed: a computerized AGEMA 870 Thermograph and a Surgicon Thermostimulator. This later is a latex balloon (thermal probe) connected by a rubber pipe to a composite refrigerating-heating system containing distilled water in its reservoir. The

reservoir has a 5 liter capacity while the balloons can be o various sizes (from 100 to 500 ml). Once the liquid has reached the selected temperature in the reservoir (range 5 °to 50 °C, with a 0.1 °C accuracy) it is sent to the balloon by means of a pump; then it fills the balloon and continuously recirculates at high speeds from the reservoir to the balloon and viceversa. The system permits maintaining the temperature of the liquid in the balloon at the same level as the reservoir, with a difference of no more than 0.1 °C. The device is also equipped with a temperature control and a timer to determine both the time of the probe application and the TRT. This later is monitored on the thermograph in real time.

The temperature selected for this study was 5 °C for an application time of 20 seconds. This technique does not request the climatisation of the ambient and of the patient.

The clinical investigation, still in course, is carried out in cooperation with the Hospital S. Gallicano in Rome; it concerns a group of radiation workers chronically exposed since many years, with or without clinical signs of skin (aspecific) damage. In these workers physical dosimetry was scarce or not always available.

At this moment 45 radiation workers have been examined.

In 19 subjects with a slight onicopathy we observed a complete hypothermia immediately after the thermostimulation and TRT of 15 min ± 2min.

In 12 subjects with cutaneous atrophy and onicodistrophy we had a complete hypothermia after the thermal stimulation and a prolonged thermal recovery time (8 min ± 1min).

In 4 subjects an incomplete hypothermia after thermostimulation was observed, followed by a very precocious thermal recovery (30 sec +- 5 s).

Thus the degree of the microvessels damage can be distinguished in severe (no response to thermal stimulation, moderate (with protracted hypothermia after thermostimulation), and mild (with very protracted hypothermia).

Head of Project 3: Dr. R. Herranz

I Dosimetrie Biologique

On a étudié un total de 31 personnes au Centre de Radiopathologie et Radioprotection, de l'Hôpital General Gregorio Marañón.

La répartition selon l'origine et la profession, est la suivante:

- 16 (52%) : public en général
- 9 (29%) : Radiologie Industrielle
- 5 (16%) : Institutions Sanitaires
- 1 (3%) : Institutions de recherche.

Les résultats globaux sont les suivants:

- Dans 23 cas (74%) : dose 0. Sous les limites de détection de la méthode.
- Dans 7 cas (23%) : le nombre d'aberrations chromosomiques indiquent des doses en-dessous de 0.15 Gy.
- Dans 1 cas (3%) la dose estimée par Dosimétrie Biologique est 1.3 Gy.

Parmi les 8 cas d'irradiation, 5 (62,5%), procèdent de la Radiologie Industrielle, et 3 (37,5%), d'Institutions Sanitaires.

La dose moyenne estimée à l'aide de la Dosimétrie Biologique par des méthodes Cytogénétiques est 89 mSv (entre 0 et 160 mSv) dans 4 cas; 141 mSv (entre 45 et 238 msv5) dans 2 cas; et 1260 mSv (entre 1170 et 1350 mSv5) dans 1 cas.

Dans le cas restant 1. l'estimation de dose n'a pas été possible car il s'agissait d'un médecin professionnellement exposé à l'action des Radiations Ionisantes au cours de 28 ans de son activité professionnelle qui était traité pour un carcinome épidermoide du second doigt de la main gauche avec chirurgie et chimiothérapie.

Parmi les 7 cas quantifiés, il existait une concordance entre l'estimation de dose par des méthodes physiques et biologiques en 4 cas (57%), il n'en existait pas dans 2 cas (29%). Dans 1 cas (14%) il n'existait pas de données physiques.

Dans le cas où la dose estimée est de 1260 mSv, on continue le suivi de l'évolution.

Actuellement le laboratoire de Dosimétrie Biologique développe de nouvelles techniques appliquées à d'autres tissus, principalement la peau et les annexes cutanées.

II Aspects Cliniques

On a développé un protocole pour la prévention et traitement des lésions dermiques chez les malades soumis à des traitements radiothérapiques qui a pour but de diminuer, retarder et traiter la radiodermite.

Actuellement on travaille avec une double étude en utilisant d'une part de l'extrait d'Aloa Vera sous forme de Gel, d'autre part un composé de Vit. E associé à un agent oxydant qui inhibe la formation de radicaux libres dans la zone irradiée, après le moment de l'irradiation.

L' évaluation de ces données sera effectuée selon des critères statistiques significatifs.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-C-060-UK

Medical Research Council
20 Park Crescent
GB-London W1N 4AL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. S.B. Field
MRC Cyclotron Unit
Hammersmith Hospital
Ducane Road
GB-London W12 OHS

Telephone number: 01-743 2030 (Ext. 3720)

Title of the research contract:

RBE for normal tissues at low doses and low doses fraction in normal and potentially sensitive populations, with emphasis cn parenchymal and vascular damage in late and chronic radiation damage.

List of projects:

1. RBE studies at low doses/fraction on the CNS and the development of vascular related damage.

Title of the project no.:

1

RBE studies at low doses per fraction on the CNS and the development of vascular related damage.

Head(s) of project:

Shirley Hornsey D.Sc, FIBiol, CBiol

Scientific staff:

R. Myers PhD; M.A.Rogers MIBiol; G.Tozer PhD.

I. Objectives of the project:

To establish the RBE at low doses per fraction for the CNS. To establish the relationship between parenchymal and vascular damage in the development of late and chronic damage to the CNS following irradiation with X-rays or neutrons. The effect of adjuvant chemicals which may affect the parenchymal or vascular damage selectively or differentially will be used to investigate the pathogenic process and to elucidate factors which may enhance late or chronic radiation damage.

II. Objectives for the reporting period:

1. RBE measurements at low doses/fraction in the cervical cord with X-rays and neutrons of high and intermediate energy.

2 Measurements of vascular permeability as a marker of injury to the blood brain barrier.

3. Attempts to reduce radiation injury to the CNS by the use of vasoactive drugs given post irradiation.

III. Progress achieved:

Irradiation of the spinal cord or brain may lead to white matter necrosis, a very serious complication, which can result even in death. It has been suggested that the injury follows damage to oligodendrocytes leading to demyelination, however the evidence is weak and the pathway through which demyelination would by itself lead to widespread necrosis is not clear. The purpose of the present study is to examine the possibility that radiation induced white matter necrosis follows oedema resulting from disruption of the blood brain barrier, the consequential reduction in blood flow causing infarction. This is studied by measuring changes in permeability of blood brain barrier in presymptematic rats following irradiation of the cervical cord.

A radiation induced increase in vascular permeability raises the possibility of ameliorating the damage by the use of drugs which limit such leakage or may reduce its deleterious effects. In the present study a number of possible compounds have been tested for this purpose.

In addition the RBE of high energy neutrons in use for radiotherapy has been measured at low doses per fraction using the "topping up" method.

METHODOLOGY

The cervical cords of 3-4 month old CFHB male rats were irradiated with 250 kVp X-rays or neutrons of energies d(16)-Be or P(62)-Be. Animals were lightly anaesthetized with sodium pentobarbitone at 45 mg per kg body weight. The radiation beams were restricted to 2.5cm of cord length.

For histological studies rats (only those not showing clinical signs) were sacrificed between 17 and 21 weeks post irradiation by deep anaesthesia followed by perfusion with 4% formaldehyde and 1% glutaraldehyde. Following dissection cords were embedded in JB4 plastic medium, 2um sections cut and appropriately stained.

Capillary permeability was measured by the leakage of

^{14}C-labelled-α -amino-isobutyric acid (AIB) from the blood into the tissue of the spinal cord. Following inoculation of 20 uCi ^{14}C-AIB into the femoral vein, rats were sacrificed by perfusion 30 minutes later. Cords were immediately dissected out and partitioned into regions approximating to C1-C2, C3-C4, C5-C6, T2-T3, T5-T6, and T7-T8. The trigeminal ganglia were also removed. Three animals were used at each time point. In some cases autoradiographs were also produced.

Drug treatments were all started at 17 weeks post irradiation and given by inoculation intramuscularly or subcutaneously into the hind limbs or flanks 3 times per week. All animals were observed carefully for the onset of ataxia in the fore limbs and if noted were immediately sacrificed.

The drugs used included :

1. Dipyridamole - 1.5mg in 0.3mg carrier per inoculation
2. Verapamyl hydrochloride - 0.75mg in 0.3 mg carrier per inoculation
3. Desferrioxamine mesylate - 30 mg in 0.3 mg carrier per inoculation together with a low iron diet of less than 10mg/kg, starting at 12 weeks. All other necessary nutrients were supplied.

The packed cell volume and haemoglobin of animals on the low iron diet and treated with desferal and from age related control animals were measured.

RESULTS

The incidence of ataxia as a function of time after irradiation and dose is shown in figure 1. With larger doses the onset is earlier and the maximum incidence is also reached earlier. Animals which had not become ataxic by 220 days were deemed eligible to be used for vascular leakage studies.

RBE

The time course of development of ataxia was similar after X-rays or fast neutrons. Six doses in 25 days of 0.6

Gy per fraction 250 kVp X-rays or 1.5, 1.0, or 0.6 Gy per fraction 16d-Be neutrons or 1.37 Gy per fraction 62p-Be neutrons were given and "topped up" with a range of doses of X-rays. The resulting dose response curves were compared and gave RBE values at the equivalent level of injury to 2 Gy X-rays of 4.7 for 16d-Be neutrons and 4.0 for 62p-Be neutrons respectively, i.e. a ratio of effectiveness between the two neutron beams of 1.17. The data also gave limiting values of RBE at vanishingly small doses of approximately 9 and 8 respectively.

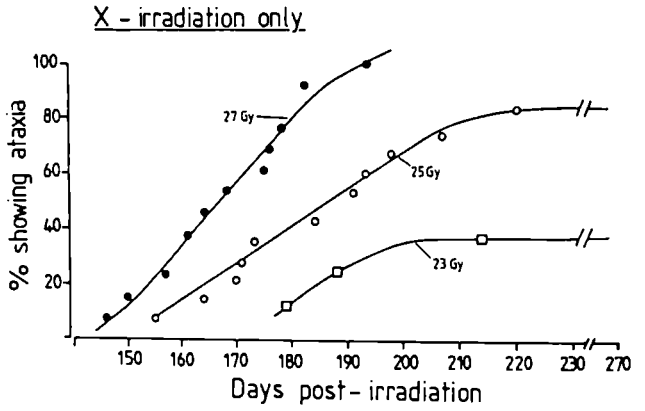

Fig 1. Development of ataxia in the forelimbs of rats following X-irradiation of the cervical spine.

Histology

Following 30 Gy no pathological changes to the cervical cord were observed until 18 weeks when extravasation of red blood cells and some oedema were seen in the dorsal horns of the grey matter. Vascular disturbances became evident by 19 weeks in the dorsal columns of the white matter and dorsal horns of the grey matter. Venous thrombi were observed also in the dorsal pial vessels. Some focal necrosis became evident in the white matter. By 20 weeks these changes had progressed despite no signs of clinical changes. Regions of necrosis were always noted in the dorsal half of the cross section of the cervical cord and usually confined to the gracile and

cuneate tracts of the dorsal white matter columns and the outer laminae of the dorsal horns. The corticospinal tract of the dorsal column was often spared.

Vascular Permeability

AIB is a marker for alterations in the blood brain barrier although quantitative information is unreliable when the tissue is undergoing pathological changes. In this case scintillation counting is used instead. Figure 2 illustrates the relative concentrations of AIB in irradiated and unirradiated cord. Changes became apparent from 17 weeks.

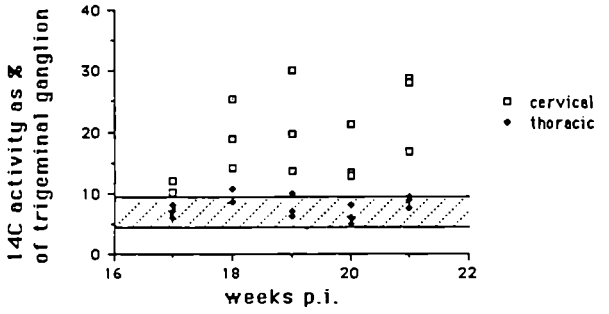

Fig 2. ^{14}C-AIB activity in irradiated cervical and unirradiated thoracic rat spinal cord as a percentage of the activity of the trigeminal ganglia of the same animal. Each point is a mean of 3 animals. Standard deviations were typically less than 10% of the mean for cervical cords and 2% for thoracic cords. The hatched band shows the 95% confidence limits of control values.

For comparison, activity in some ataxic rats was measured and was shown to increase substantially in the cervical region (by up to 100%) and was also increased in the unirradiated thoracic cord indicating a "tracking" of damage away from the site of injury.

Drug Treatments

The effects of desferrioximine combined with a low iron diet

are shown in figure 3. Clearly there is a reduction in effectiveness of the irradiation by subsequent application of this regime. The same is true for dipyridamole as illustrated in figure 4. However treatment with verapamyl had little effect on the outcome of the development of ataxia. Observations were carried out for 8 months post treatment.

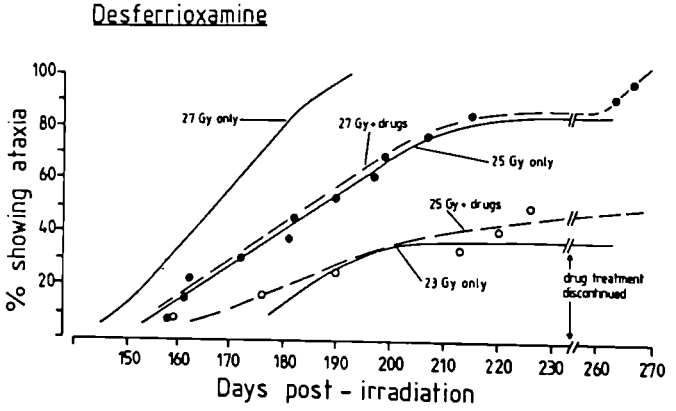

Fig 3 Development of ataxia in the forelimbs of rats following X-irradiation of the cervical cord. The full lines are for X-rays only taken from figure 1. The dotted lines are for animals treated with a low iron diet plus desferrioxamine.

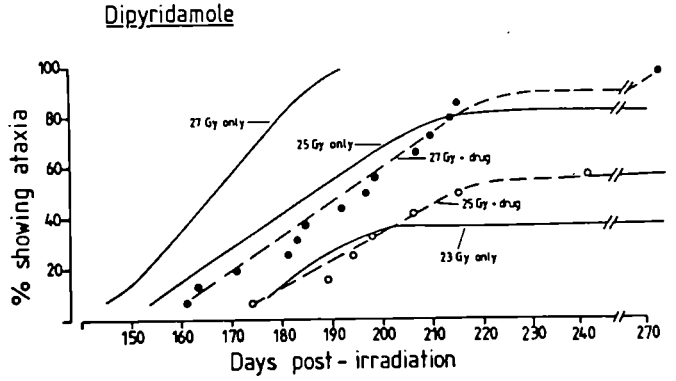

Fig 4 Development of ataxia in the forelimbs of rats following X-irradiation of the cervical cord. The full lines are for X-rays only taken from figure 1. The dotted lines are for animals treated with dipyridamole.

The results for all 3 drug combinations are combined in figure 5 for comparison from which it is clear that dipyridamole and the combination of a low iron diet together with desferrioxamine are effective in reducing the effects of irradiation whereas verapamyl had no significant effect.

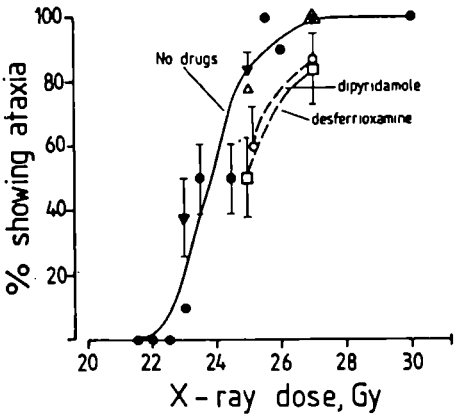

Fig 5. The percentage of rats developing ataxia by 265 days after irradiation. ▼ and ● refer to X-rays only; △ X-rays plus verapamyl; ◐ X-rays plus dipyridamole; □ X-rays plus a low iron diet and desferrioxamine.

DISCUSSION

It is clear from the fractionation studies that the RBE for damage to the CNS at low doses per fraction is higher than for most other tissues. It was observed that the very high energy beam was only slightly less effective than at more moderate energies. These results emphasize the need to take great care when using fast neutrons to treat regions including the CNS.

In the histological study the lack of ability to follow an individual animal through the development of the radiation syndrome means that it is not possible to identify with certainty the precise cause of the increase in vascular permeability. It is known that ischaemia resulting from

vascular occlusion can lead to disruption of the blood brain barrier. In addition, oedema resulting from disruption of the blood brain barrier, can cause ischaemia. Thus the observed changes in vascular permeability are probably part of a cascade of ischaemic related events caused by pathology in a critical arterial supply leading to white matter necrosis following irradiation.

If an increase in vascular permeability resulting from or leading to ischaemia is a critical phase in the development of radiation induced white matter necrosis the possibility exists of ameliorating such damage by drugs which either directly limit the leakage or limit their effects.

Verapamyl, a calcium channel blocker, was used in an attempt to reduce permeability and hence oedema. Clearly it was not effective, possibly because it is the sodium channels which are opened by radiation injury and the damage is therefore unaffected by calcium channel blockers.

Dipyridamole, a muscle relaxant, was used to increase blood flow and thus reduce vessel blockage and transient ischaemia. It also reduces the thrombotic activity of the blood. As seen in figures 4 and 5 this drug was effective in reducing the level of radiation damage.

Reperfusion injury is damage resulting from the production of superoxide radicals when previously ischemic tissue is exposed to oxygen. Iron is a catalist to the reaction. Desferrioxamine, a chelating agent, was given in combination with a low iron diet in order to decrease reperfusion injury. The low iron diet was necessary to reduce the replacement of free iron in tissue which was removed by the desferrioxamine. As seen in figures 3 and 5 this drug combination was also effective. The result is also important in that it provides evidence that reperfusion injury may play a role in the development of radiation damage.

These studies demonstrate that it is possible to interfere with and ameliorate radiation damage by appropriate treatments post irradiation. The consequences for both radiation therapy and radiation protection could be considerable.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Professor L.W. Duchan and Dr S. Love
Department of Neuropathology
Institute of Neurology and The National Hospital
Queen Square
London WC1N 3BG

V. Publications:

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Progress Report

Contract: Bi7-005 **Sector:** B24
Title: Irradiation and thyroid disease.

1	Dumont	Univ. Libre de Bruxelles (ULB)
2	Malone	St James Hospital
3	Smyth	Univ. College of Dublin, Belfield

I. Summary of Project and Global Objectives

It is well known that because of its avidity for iodine the thyroid is open to significant radiation exposure in the event of nuclear accidents as 131-I and nuclides of iodine are relatively abundant fission products. Further it is well known that the thyroid has a significant incidence of cancer, benign disease, and hypothyroidism in consequence of radiation. As well as exposure after nuclear incidents the thyroid is also at risk during many diagnostic and therapeutical medical procedures, and from accidents and occupational exposure among laboratory and medical workers. The level of incidence of thyroid disease per unit of radiation dose is unusually high, and although most of the disease is not fatal it can lead to significant loss in quality of life, worry and anxiety among those effected.

As the thyroid cannot distinguish between radioactive and stable iodine one of the major factors influencing uptake of radioiodines is the dietary content of stable iodine. Another factor influencing risk from radioiodines is the mass of the thyroid gland exposed to irradiation.

The project presents 3 main objectives :

1) to establish baseline data on iodine dietary supply, and radioiodine uptake in Europe in order to allow evaluation of the consequences of radioiodine contamination. In particular detailed baseline data on dietary iodine intake and thyroid volume will be established in Ireland to determine the parameters involved.

2) To determine the appropriateness of absorbed dose as an index for use in cancer risk estimation in mass. To evaluate fetal thyroid dose after radioiodine ingestion.

3) To develop new models for the study of radiobiology and radiation carcinogenesis of the thyroid:

a) immortal differentiated human thyroid cell lines
b) transgenic mice with thyroid targeted oncogenes.

Head of Project 1: Prof. Dumont

II Objectives for the reporting period

1) Feasibility of a model of differentiated human thyroid cell line for radiobiological studies.
2) Development of a model of transgenic mice for specific gene expression in the thyroid.
3) Further development of our knowledge of the control of proliferation in human thyroid cells (effects of growth factors).
4) Collection of data on thyroid radioiodine uptake in European regions.

III Objectives for next period

1) Development of a model of differentiated human thyroid cell line for radiobiological studies. Definition of the steps of in vitro immortalization.
2) Development of a model of transgenic mice for autonomous functioning thyroid nodules and for dedifferentiated thyroid tumors.
3) Further development of our knowledge of the control of proliferation in human thyroid cells (role of TSH and cyclic AMP).

IV Progress achieved including publications

1) Development of a model of human thyroid cell line.
The study of the radiobiology of the human thyroid has had until now to rely on unsatisfactory models

a) animal thyroids (mostly rats and mice) in vivo, providing reliable but relatively imprecise data on cells of other species; even in such studies measurements of risks for low doses are scarce.
b) human thyroids studied in vivo : epidemiological data are available for moderate doses but are almost impossible to obtain for low doses because of the huge numbers of patients required.
c) human and animal cells primary cultures which because of their short lifespan and low plating activity can only give inaccurate and even doubtful data.
d) cell lines derived from animal tissues (eg FRTL5) which provide accurate and reliable data, but on a material that may have little relevance for human cells in vivo.

The need for differentiated cell lines has therefore been recognized as a major aim of radiobiological research at the EEC, Dublin ""Cell Transformation and Radiation induced Cancer" meeting. Some attempts have already been made to create such a line either using thyroid cancer cells from surgical samples or using normal cells transfected with oncogenes. However the cell lines obtained are completely dedifferentiated.

Relatively well differentiated human keratinocyte cell lines have been obtained using the immortalizing genes of human papilloma viruses. We have therefore studied the feasibility of applying this methodology to human thyroid cells.

A subgenomic fragment of the tumorigenic HPV-16 has been cloned in the pML2 vector (derived from pBR322). The DNA fragment containing the full sequences of the immortalizing genes E_6-E_7 is under the control of the powerful LTR promoter of Mo-Mu LV. Several protocols have been tried. The final protocol is described hereafter. Human thyroid cells transfected by the lipofection method have been cultured for one week, trypsinized, plated again and treated with a general cyclic AMP enhancer (forskolin) for 3 weeks, then passaged again for another 2 weeks. While many cells then degenerate and die, some foci of cells with epitheloid morphology appear. These cells have been passaged 19 times with alternative treatment with forskolin and TSH for 11 months after the transfection. These cells multiply with a doubling time of 60-70hrs in medium with 0.2 % serum. They exhibit a typical epitheloid morphology. They secrete thyroglobulin and this secretion is stimulated by a factor of 3 by thyrotropin (from 5 to 15 ng of TG/pg DNA/48 hours). (The results are similar to those obtained in primary cultures of human thyroid cells). Their cyclic AMP levels are enhanced by a factor of 3 in response to TSH 500 µU/ml (from 0.4 to 1.3 pMole/ug DNA).
New preparations are now developed.

2) Development of a model of transgenic mice for thyroid tumorigenesis.

The thyroglobulin promoter has been placed upstream of chloramphenicol acetyl transferase cDNA. When injected into mouse oocytes within a pBR322 vector, this promoter has been shown to be able to target the expression of the gene specifically in the thyroid of injected mice and of their offspring. Depression of the thyroid by triiodothyronine treatment decreases while stimulation by antithyroid drugs increases the expression of the gene. The same type of construction has been used to target SV40 Large T (LT) immortalizing gene. In the transgenic mice and their progeny LT expression in the thyroid was confirmed by Northern blotting and immunohistochemistry. Moreover these mice developed progressive goiters immediately after birth. The growth of the goiter with its clinical consequences (difficulty to breathe) was restrained by T3 administration, which also delayed the death of the animals. However, tumors progress resumed after a few weeks showing that they become independent of TSH. The follicular tumors concentrated radioiodide but the oxidation of this iodide was greatly decreased. They involved the whole thyroid. Autopsies of demonstrated metastases. Thus these animals represent faithful experimental models of the human thyroid tumors progression. These results must now be extended to a larger series of animals.

3) Control of thyroid cell proliferation.

The study of the control of thyroid cell proliferation and differentiation has been pursued at the cell level by in situ hybridization on dog cells. The stimulatory effects of TSH, cyclic AMP enhancers, epidermal growth factor, and phorbol esters on proliferation were confirmed. On the other hand, TSH and cyclic AMP enhancers induced both proliferation and differentiation (as evaluated by in situ hybridization of thyroglobulin mRNA) in the same cells at the same time. This shows conclusively that in these specialized differentiated epithelial cells cyclic AMP, contrary to a popular concept, activates the two supposedly opposite processes.
In human thyroid cells in culture, it was shown that TGF β inhibits proliferation but also differentiation expression. Thus TGF β could in the human thyroid be the negative factor controlling growth.

4) Epidemiology of iodidine deficiency in the Community.

Data have been obtained on the normal radioiodine uptake and urinary iodide excretion in various European centers. These data, to be published in the J. of Endocrinological Investigation, demonstrate a wide range of dietary iodine supply in Europe with large areas of wild to severe iodine deficiency. The resulting thyroid radioiodide uptakes vary by a factor of 3. As effects of radioiodine contamination are directly proportional to the relative thyroid uptake it can be shown that general iodine prophylaxis in Europe would, besides its clinical beneficial effects, prevent more than 2/3 of the eventual population radiation burden from nuclear accidents, or otherwise.

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TATON, M., ROGER, P.P., LAMY, F., VAN SANDE, J., DUMONT, J.E.
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In: Cell Transformation and Radiation-Induced Cancer (eds Chadwick, K.H., Seymour, C., Barnhart, B.) pp 117-126, 1989.

MALONE, J., UNGER, J., DELANGE, F., LAGASSE, R., DUMONT, J.E.
Thyroid consequences of Chernobyl in the countries of the European Community.
Journal of Endocrinological Investigation, (accepted).

Head of Project 2: Dr. Malone

II Objectives for the reporting period

1. To ensure optimum use of resources in respect of determining the transformation rate in the thyroid.

2. To develop a formal approach to the question of the appropriateness of absorbed dose as an index for risk in the thyroid.

3. To resolve some residual questions in the issue of fetal thyroid dose arising from maternal ingestion of 131-I.

4. To continue the established collaborations in respect of dosimetry parameters and iodine kinetics.

III Objectives for next period

1. To continue the work in respect of the appropriateness of absorbed dose with particular reference to determination of number of cells/g of thyroid throughout Europe using approaches based on (a) tissue digestion; (b) modelling; and (c) literature/existing laboratory data.

2. To continue developing a formal analytical statement of the above problem. This development will bear in mind the fact that the problem is probably not confined to the thyroid and will seek to introduce the approach to breast tissue.

3. To reach a conclusion of the present phase of the study of Fetal Thyroid Dose.

4. To re-establish cell culture work in respect of issues that are complimentary to and/or supportive of the major in vivo study in this area to be undertaken by the NCI in the USA.

5. To continue the established collaborations and apply the results acquired viz a viz dosimetry and iodine kinetics.

IV Progress achieved including publications

1. Biological Effectiveness of 131-I :

Early in 1990 a small group was convened by Dr. Bruce Wacholz (Head,Radiation Effects Division, National Cancer Institute, Washington). The group consisted of US experts in macro/micro dosimetry of incorporated radionuclides, and experts in the Radiobiology/Epidemiology of 131-I, 125-I and related phenomena. In addition Prof. G. Walinder and Prof. J. Malone were invited. A core group was charged with preparing recommendations on investigations , with the objective of resolving the question of biological effectiveness of 131-I Irradiation for Carcinogenesis. The investigations were planned to be performed in animals, and possibly supplemented in cell cultures. These recommendations will be supplemented by further epidemiological input.

Should the investigations which are planned take place, there will be a major programme in the US concentrating on the problem of the biological effectiveness of 131-I irradiation in carcinogenesis. This is a part of the programme in our current contract as originally envisaged. The scale of the US investigation will, if undertaken, be very large. Hence it is best that our work play a complimentary and/or supportive role to it. Because of this we have deferred work in this area pending clarification of the scope and direction of the NCI initiative.

2. Fetal Thyroid Dose:

The estimate of fetal brain dose from maternally ingested 131-I in the fetal thyroid has been extended from 8 - 16 weeks to the entire gestational period. The sources of anatomical and biokinetic data and the method of estimating the dose are the same as those reported previously (Gilligan and Malone 1991). On the basis of these anatomical sources we have inferred that at no stage in gestation does the range of 131-I electrons exceed the minimum brain thyroid distance. The data for fetal anatomical distances was modelled by linear regression. The data for absorbed fractions have been obtained by modelling the data of Berger(1968) using the "pcnonlin package".

The average brain dose estimate is entirely due to gamma emissions from the fetal thyroid and ranges from 0 - 60 µGy/MBq maternally ingested 131-I (figure 1). It is comparable to the permanently present dose from maternal organs. The dose peaks in the weeks 18-24. A maximum local dose has also been calculated using modelled minimum thyroid brain distances and the biokinetic data for each stage of gestation. The dose is shown in figure 2 and varies from 0 - 800 µGy/ MBq maternally ingested 131-I. This dose is intended to represent the upper limit of the dose to any part of the brain during the entire gestational period.

The method of calculation used in this estimation will have associated errors . It is unlikely that these errors would be greater than an order of magnitude which are comparable to those associated with other dose estimates. Extrapolation of the these or any fetal dosimetry estimates should be done with care as they are based on data drawn from a small number of fetuses. The estimates reported here should not be treated as absolute but as an approximate reflection of the dose in the fetal brain.

Figure 1. Average absorbed dose in brain as a function of maternally ingested 131-I using modelled anatomical parameters. (Legends refer to sources of biokinetic data).

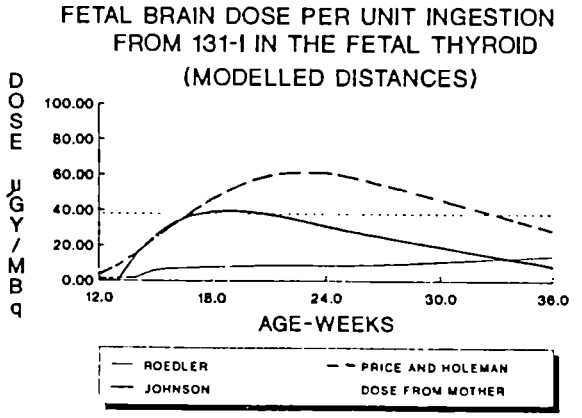
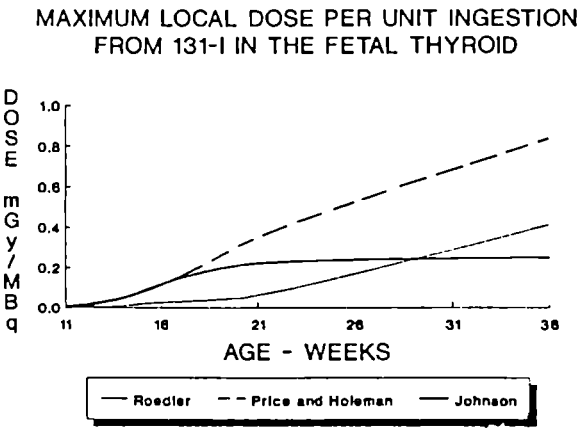


Figure 2. Maximum local brain dose from the fetal thyroid as a function of maternally ingested 131-I.(Legends refer to sources of biokinetic data).



3. Appropriateness of Absorbed Dose as an Index for Use in Risk Estimation:

An examination of the appropriateness of thyroid absorbed dose as a concept to use with risk estimates has been undertaken. In broadest terms the limitations of the use of organ absorbed dose were found to be:

1) Inappropriate biokinetic and anatomical parameters
2) Break down of conventional dose estimation at a cellular level and for small organs.
3) Exclusion of detriment to other organs from radiation emanating from that organ which recieves highest dose.
4) Incompatibility with a truly stochastic model of radiocarcinogenesis.

The first three problems are dealt with in detail by many authors and have been summarised by this group in Malone and Gilligan (1990). In addition some of the parameters required in these areas are the subject of a collabarative investigation with Dr. Smith. The fourth has also been examined in this reporting period. The incompatibility with a stochastic model of radiocarcinogenesis arises from the exclusion of number of cells exposed as a parameter contributing to risk in conventional risk estimation. To enable the inclusion of number of cells exposed it is suggested that this has to be related to a more accessible parameter such as mass (Gilligan and Malone 1991). This problem was addressed in this reporting period and includes mass determinations by ultrasound in the associated project.

Methods of measuring cells/g were examined. The following conclusions were reached (a) section studies could provide

accurate information on absolute figures of number of cells per gram of tissue (b) cell yield from digestion was a relative reflection of cell number per gram (c) modelling using spherical follicles was found to give a poor reflection of morphologic composition of the glands.

Data on number of cells in a gram of human thyroid tissue is not widely available. However a figure of 3×10^8 for epethilial cells/g was calculated from section data by Coclet et al. (1989) for Belgian euthyroid human tissue. This is similar to data for other species. Examination of the literature found that animal studies suggest that upon TSH stimulation the number of cells/g remains approximately constant but that it may rise on involution. Cell yield from digestion experiments trebled for colloid goitre tissue also suggesting a different number of cells/g for certain thyroid tissue types.

References:

Coclet J., et al., 1989, Cell population kinetics in dog and human adult thyroid, Clin. Endocrin.,31.

Presentations/Publications:

Gilligan P., Malone J.F.,1991,Estimate of 131-I fetal brain dose from fetal thyroid in gestational weeks 8-16, Poster presented at HPA annual meeting 1990 ,Clin. Phys. and Physiol. Meas.,1991 (Abstract: In Press).

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Malone J.F., Gilligan P.,1990, Problems in Dosimetry including consideration of special groups: in utero, the neonate, children and adults. In: Iodine Prophylaxis after Nuclear Accidents, edited by E. Rubery and E. Smales, (London : Pergamon Press),pp. 65-79.

Gilligan P., Malone J.F.,1991, The appropriateness of absorbed dose as a concept to use with risk estimates, Proceedings of the 23rd meeting of the ESRB ,London: Taylor and Francis ,(In Press).

Gilligan P. , Malone J.F.,1991, Absorbed dose: is it an appropriate index to use in thyroid risk estimation ?, Paper presented at 1990 Annual Scientific Meeting of the Association of Physical Scientists in Medicine, Galway, Ireland, Journal of Biomedical Sciences, (Abstract: In Press).

Gilligan P., Malone J.F.,1989, Stochastic risk estimation for 131-I in the thyroid, Paper presented at 1989 Annual Scientific Meeting of the Association of Physical Scientists in Medicine, Kilkenny, Ireland,

Malone J.F., Gilligan P. ,1988, Thyroid irradiation following the Chernobyl accident in CEC countries: an assessment of the projected fatal and non-fatal harm, In: Frontiers in Radiation Biology, edited by E. Riklis, Weinheim: V.C.H., pp. 575-588.

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Malone J.F., Unger J., Delange F., Lagasse R.,Dumont J.E., 1991, Thyroid consequences of Chernobyl in the countries of the European community, J. Endocrinol. Invest. (In Press).

Malone J.F.,1990,The r.b.e. of 131-I in the thyroid: new observations in cell culture and a hierarchial model of the response of the gland, Invited paper at NCI radiation effects branch workshop, Radiation dosimetry of the thyroid- 131-I and external X-rays,Washington, March 1990.

Head of Project 3: Dr. Smyth

Project IE3 has as its overall objective the establishment of baseline data on dietary iodine intake and thyroid volume in study populations.

II Objectives for the reporting period

1. To study urinary iodine excretion, used as an index of dietary iodine intake, in populations residing on either side of the Irish Sea.
2. To study seasonal variations in urinary iodine excretion and to relate these to dietary milk iodine content.
3. Using ultrasound scanning to study the thyroid volume and the frequency of thyroid nodules in the study populations.
4. To report on the kinetics of administered iodine and to make recommendations on iodine dosage and the frequency of administration required to maintain optimal levels.

III Objectives for next period

Although diminished in scale, the major targets outlined in the technical annex of the original proposal have been retained.

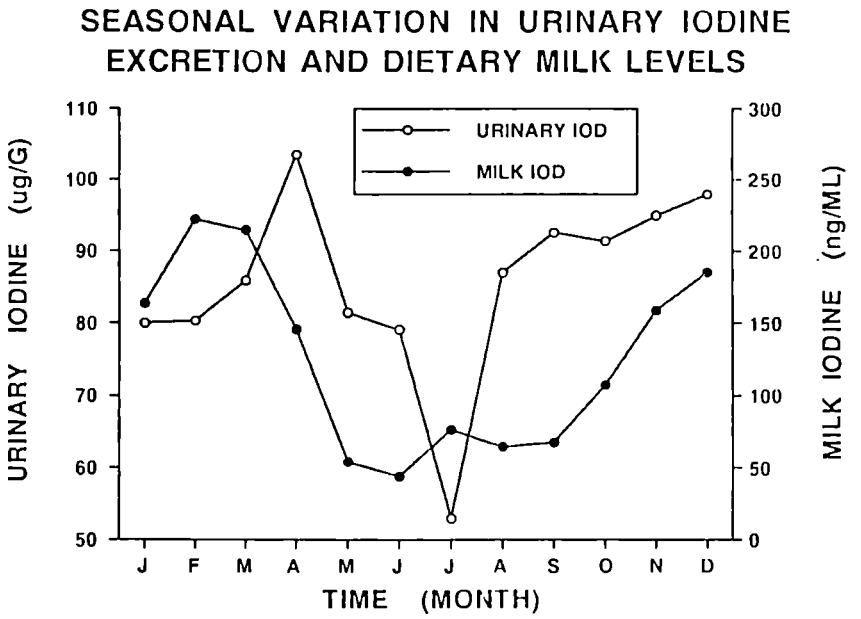
1. To continue the study of urinary iodine excretion with particular emphasis on: a) Different geographical regions in Ireland and Wales. b) Studies on urinary iodine excretion in "at risk" groups, i.e. Mothers during pregnancy and postpartum, neonates and school children.
2. To study normal variations in iodine excretion in regions other than Dublin.
3. To expand sonographic thyroid volume measurements to include the three trimesters of pregnancy, the postpartum period, neonates and school children.
4. Preliminary studies on iodine kinetics have yielded most interesting and usefu information. To reach fruition it will be necessary for this work to expand beyond the scale and duration of the present project.

IV Progress achieved including publications

1. Urinary iodine excretion was measured in random urine samples obtained from subjects residing in the Dublin area. Although this group cannot be taken as being absolutely representative of the total Dublin population, it is large enough and drawn from sufficiently wide sources to provide a reasonable indication of the iodine status of that population. The mean urinary iodine excretion of 106±73 ug/G creatinine (Median 83), while excluding urinary iodine dificiency in the study population, obscured the fact that 18.6% had values suggestive of iodine deficiency (<50 Ug) and 40.8% <70 ug). A disturbing finding for which no immediate explanation was forthcoming, was that mean I/C values calculated for various centers showed wide variations (72-204 ug/G). It was however noted that urine samples had been obtained at different times of the year.

2. As milk has been reported to form an important source of dietary iodine intake in Northern European countries, it was decided to look for possible seasonal variations in dietary milk iodine content in Ireland. We had previously shown that the iodine content of milk sampled at 31 farms in different parts of Ireland was 61± 59 ug/Kg in Summer and 101±65ug/G in Winter (P<0.001).

In view of the differences in milk iodine values recorded between Summer and Winter it was decided to conduct sequential studies on the iodine content of bulk liquid milk provided for human consumption in the Dublin area over a one year period. Monthly mean values were calculated for individual milk samples. It can be seen that the results confirm our earlier report from separate farms. As shown in the accompanying Figure, the iodine content of milk was at its highest during the winter months (February-April) when cattle were housed and receiving food supplements. Iodine values declined during the summer months when the cattle were once again put out to pasture.



In order to determine if this variation in milk iodine content was reflected in urinary iodine exretion, random urine samples were obtained from 370 patients who were attending the same outpatients clinic over a one year period. Monthly mean values were calculated. The Figure shows that variations in urinary iodine excretion paralleled those observed in milk iodine content being highest in winter showing a maximum of 222 ug/Kg in February and lowest in Summer (44 Ug/Kg in July).

Preliminary studies on urinary iodine excretion in pregnant mothers attending the National Maternity Hospital in Dublin show an increase in excretion over the 3 trimesters of pregnancy. As this was a cross-sectional study and therefore subject to increased error, a prospective study following the same patients through their pregnancies is now underway.

3. As no data exists on thyroid volume in the Irish population a study using an ultrasound scanner fitted with a 7.5 m/hz linear transducer was undertaken. The operator (PS) received training in thyroid sonography at the Medizinisine Universitat Zu Lubeck in Germany. A total of 311 adult on-hospitalized volunteers was studied consisting of 158 females and 153 males. The volume of each thyroid level was calculated using the formula (Width x Depth x Length x II/6) (Brunn et al. 1981)[1] and upper limits for normal thyroid volume suggested by Gutekunst et al.(1988)[2] (18 mls for females, 25 mls for males) were used. The mean thyroid volume for 158 females was 12.2± 7.7 Mls and for 153 males 15.6 ± 5.0 mls. These values were significantly different but the difference could be accounted for on the basis of greater male body weight. The % of enlarged thyroid glands in females was 7.6% and in males 2.6%. However thyroid enlargement was encountered more frequently in older subjects.

4. In order to establish optimum conditions for iodine prophylaxis measured doses of iodine containing preparations to healthy volunteers Preliminary findings on iodine kinetics were obtained using sequential urine and serum iodine measurements. It is hoped that this data will provide an improved scientific basis for the dosage and frequency of administration required to maintain optimal levels of circulating iodine.

1.Brunn J, Block V, Ruf G, Boss I, Kunze WP & Scriba PC. Volumetrie der Schilddrusen-lappen mittels Real-time Sonographie. Dtsch Med Wschr. (1981) 106: 1338-1340.

2.Gutekunst R, Becker W,Hehrmann R, Olbricht Th. and Pfannensteil P. Ultraschalldiagnostik der Schilddruse. Dtsch Med Wschr. (1988) 113: 1109-1112.

Communications: Dr. P. Smyth

1. "Factors Determining the Iodine Status of a Population", 9th Joint Meeting of British Endocrine Societies, Glasgow, Scotland Mar, 1990.

2. "Normal Thyroid Volume in Females". 9th Joint Meeting of British Endocrine Societies, Glasgow, Scotland Mar, 1990.

3. Iodine status of a population without Endemic Goitre, International Symposium "Iodine and the Thyroid" Athens, September 1990.

4. Iodine Excretion and Thyroid Volume in Pregnancy. International Symposium "Iodine and the Thyroid" Athens, September 1990.

5. Merck Symposium, "The Thyroid and Pregnancy" Brussels February 1991. (With Dept. Obstetrics and Gynaecology)

PUBLICATIONS

Hetherton AM, McKenna TJ, Counihan TB, Poole DBR, O'Donovan DK, O'Higgins NJ and Smyth PPA, Seasonal Variations in Iodine Intake in Ireland. Irish J Med Sci (1990) 139:127

Moran T. and Smyth PPA. In vitro TSH modulation of thyroid growth responsiveness. Irish J Med Sci (1990) 159:124

Smyth PPA, Hetheron AM, Ryan R, O'Herlihy C. Alterations in Iodine Status and Thyroid Volume during Pregnancy. "Merck Symposium Series (in press) 1991

Contract: Bi7-003 **Sector: B31**
Title: Effects of radiation on the development of the central nervous system

1	Reyners	CEN - SCK
2	Ferrer	Hospital Principes de España
3	Coffigny	CEA - Bruyères-le-Châtel

I. Summary of Project and Global Objectives

The evaluation of the deleterious effects of an accidental exposure to a low dose of ionizing radiations during the fetal development of the central nervous system still represents a much debated issue : even the large consensus over the high radiosensitivity of the developing brain has been recently questioned by Mole (1990) who claimed that the damage could only be the consequence of abscopic effects and particularly of an oxygen deprivation due to impaired erythropoiesis. On the other hand, field studies using the revised DS86 dosimetry have estimated that an exposure to a dose as low as 1 cGy could have induced severe mental retardation in a number of Hiroshima and Nagasaki survivors (Otake et al., 1987).

One of the aims of the present joint project is to cast more lights on the question of low dose exposures by means of a multidisciplinary experimental approach involving in vitro neuroreceptor studies (France), immunocytochemistry (Spain) and automatic image analysis (Belgium). In contrast with the assessment of epidemiological data by Otake et al., animal experiments not only allow a more accurate dosimetry but also a precise knowledge of the fetal age at the time of irradiation.

In addition, and possibly of more practical relevance, a large part of the project now focuses on the evaluation of the risks of low dose rate exposures protracted over a number of days. Very few data are available on this sensitive topic which still today is currently dealt with (see the 1991 UNSCEAR draft-report on irradiation of the developing human brain) by referring to the available information on accidental exposures to a variety of environmental toxic agents (mercury, triméthyltin or even alcohol)! Our data unexpectedly reveal that protracted exposure to gamma rays during selected periods of the pregnancy can produce brain alterations after dose levels (as low as 20 cGy) previously thought to be damaging only under acute exposures. In the future, these studies should be extended to prolonged neutron exposures.

Head of Project : Dr.Reyners

II Objectives for the reporting period

1) Evaluation of the acute effects of an exposure to 600 KeV neutrons on day 15 post-conception (PC) in the Wistar rats :
a) measuring the brain atrophy (brain weight, BrW).
b) measuring the involution of the white matter (by means of an assessment of the volume of the cingulum, CiVol).
2) Evaluation of the protracted effects of a low dose rate of gamma exposure during whole or part of the pregnancy :
a) after : 17, 37 or 89 cGy from day 0 to 20 PC.
b) 7, 11, 17, 27, 34 or 81 cGy from day 14 to 20 PC.
c) 16, 34 or 58 cGy from day 12 to 16 PC.

III Objectives for next period

1) Evaluation of the effects of the DOSE RATE after an exposure to 40 cGy of Cs137 gamma rays on day 15 PC (Wistar rat) by measuring : a) brain atrophy ; b) the involution of the cingulum.
2) Comparison of the effects of irradiation between different rat STRAINS : Wistar and Sprague-Dawley.
3) Automatic Image Analysis of the MYELINATION in the cingulum after protracted low dose gamma exposures (from 7 cGy).
4) Automatic quantification of the SYNAPTIC DENSITY at the electron microscope level after neutron exposure on day 15 PC.
5) PUBLICATIONS.

IV Progress achieved including publications

1) Acute effects of an exposure to 600 KeV neutrons on day 15 post-conception.

Brain weights of prenatally irradiated rats decrease linearly according to dose in 3, 15 and 24 month old rats ; the phenomenons are significant down to the 2.5 cGy dose. However, after transforming these data in the percentage of their respective control means and pooling the results of the 3 age groups, the slight microcephaly (a 2% brain weight loss) found after an exposure to only 1 cGy (50 mSv ; Fig.1) resulted significant : $F_{1,104} = 6.6$; $P < 0.012$.

2) Evaluation of the protracted effects of a gamma exposure (at a low dose rate) during whole or part of the pregnancy.

a) after 17, 37 or 89 cGy from day 0 to 20 PC.
b) 7, 11, 17, 27, 34 or 81 cGy from day 14 to 20 PC.
c) 16, 34 or 58 cGy from day 12 to 16 PC.
N.B. : the "heterogeneity" of the above doses results from the fact that they represent the measured mean doses actually received by the exposed animals.

Fig.2 shows some results from these protracted gamma irradiations ; the effects of an acute X-irradiation are also shown for comparative purposes. In spite of the large amount of superposition between the numerous dose-effect curves reported, some interesting conclusions can be drawn : If, in general, protracted exposures produce less effects than acute ones, it also appears that a careful selection of the period of irradiation during the pregnancy, e.g. by irradiating between day 12 and day 16 PC, produces a brain atrophy at least similar to the one produced by an acute exposure to the same dose given in 20 seconds during day 15 PC!

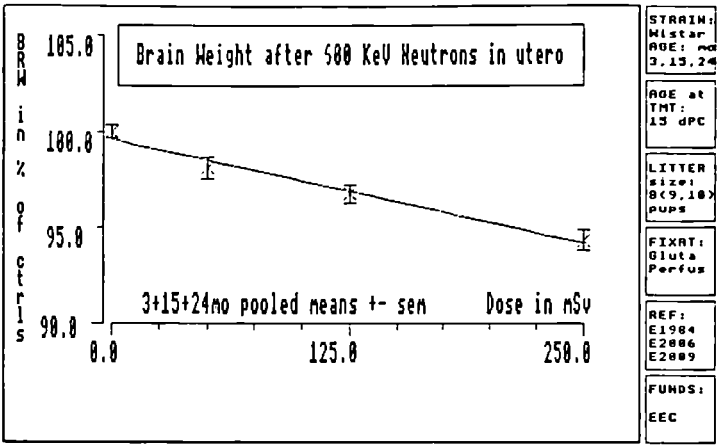


Fig:1 Ref: Hubert REYNERS et al. DTPL.MTHPOOLPC2 CEN-SCK, Mol(B)

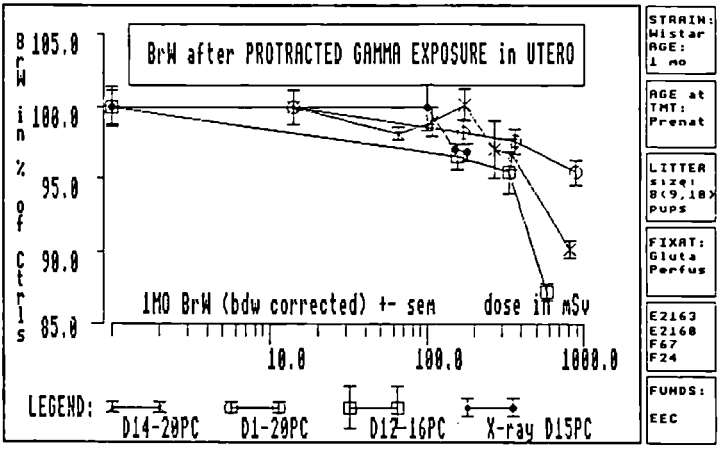


Fig:2 Ref: Hubert REYNERS et al. DTPL.GASv9091oo CEN-SCK, Mol(B)

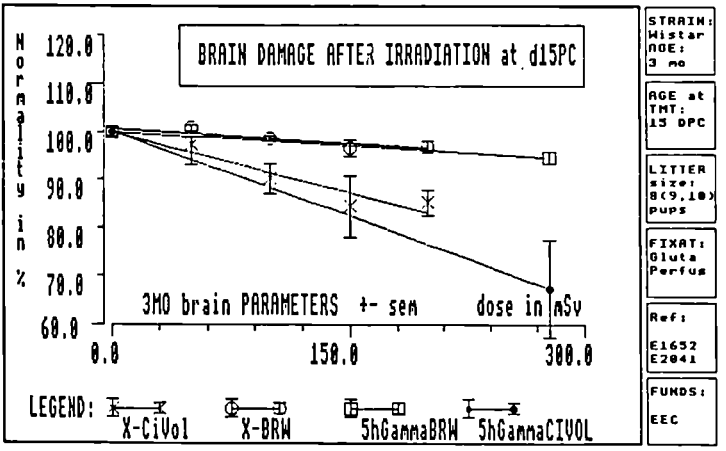


Fig:3 Ref: Hubert REYNERS et al. X03MCIBRW CEN-SCK, Mol(B) May1991

Brain atrophy may not always represent the best available estimator of the effects of a prenatal irradiation of the brain (Fig.3). The decrease of the volume of the cingulum (CiVol ; a purely white matter area located above the corpus callosum) in relation to the dose can be much more important than the loss in BrW. Here also, an exposure of a few seconds to X-rays does not cause more effect than a 5h gamma irradiation. The reasons for the very high radiosensitivity of the cingulum are unclear. Many hypotheses can be formulated ; a particularly attractive one arises from a recent observation by I. Ferrer, the Spanish cocontractant to this project, who found a reduction of a neuronal population (calbindin positive) selectively occurring in the cingular cortex, an area of the cerebral cortex located just above the cingulum.

3) Recent publications over these topics and closely related fields :

Ultrastructural and kinetic changes in glial cells after irradiation. H. Reyners in : "Frontiers in Radiation Biology", E. Riklis editor. VCH Weinheim, p.347-353 (1990).

Bimodal effects of X-rays on adult microglia. E. Gianfelici de Reyners and H. Reyners. XXIII Congress of the European Society of Radiobiology. Book of Abstracts, Dublin (1990).

Effects of in utero gamma ray irradiation on the development of the rat brain : low versus high dose rates. H. Reyners, E. Gianfelici de Reyners, L. Regniers and J.-R. Maisin. XXIII Congress of the European Society of Radiobiology. Book of Abstracts, Dublin (1990).

An ultrastructural analysis of the development of the late effects of X-irradiation in a brain area at major risk : the Fimbria hippocampi. H. Reyners, E. Gianfelici de Reyners, T. Yeung and J.W. Hopewell. XXIII Congress of the European Society of Radiobiology. Book of Abstracts, Dublin (1990).

Bimodal response of microglia after X-irradiation of the adult rat brain. E. Gianfelici de Reyners and H. Reyners. Belg. J. Zool. 120. S1, p.33 (1990).

see also in : I. Ferrer's references.

Head of Project 2: Dr. Ferrer

II Objectives for the reporting period

Study of naturally occurring cell death in the cerebral cortex during postnatal development in prenatally-irradiated animals. Development of dendritic spines on cortical pyramidal cells after prenatal X-ray exposure.
Development of local-circuit neurons (interneurons) in the cerebral cortex of prenatally-irradiated animals.

III Objectives for next period

Development and fate of interneurons in the cerebral cortex of rats subjected to X-irradiation during the fetal period.
Study of radial glial cells and migration patterns of cortical neuroblasts after X-ray exposure during development.

IV Progress achieved including publications

Study of naturally occurring cell death during postnatal development in the cerebral cortex of the normal rat and in micrencephalic rats induced by prenatal X-irradiation. Development of dendritic spines on cortical pyramidal cells in rats after X-ray exposure during the fetal period.
Technical approach to the immunocytochemical identification of local-circuit neurons in the cerebral cortex using antibodies to parvalbumin, calbindin, GABA and different neuropeptides. Preliminary studies in rats irradiated with 50 and 100 cGy at embryonic ages 15, 17, 19.

Publications :

1. - Naturally occurring cell death in the subicular complex and hippocampus in the rat during development. I. Ferrer, T. Serrano, E. Soriano. Neuroscience Research 8 (1990) 60-66.
2. - Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. I. Ferrer, E. Bernet, T. Del Rio, M.E. Soriano, M. Fonseca. Neuroscience 39 (1990) 451-458.
3. - Development of dendritic spines in the cerebral cortex of the micrencephalic rat following prenatal X-irradiation. I. Ferrer, E. Soriano, E. Marti, E. Digon, H. Reyners, E. Gianfelici de Reyners. Neuroscience Letters (in press).
4. - Naturally occurring, postnatal cell death in the cerebral cortex of the micrencephalic rat induced by prenatal X-irradiation. I. Ferrer, E. Soriano, E. Marti, E. Laforet, H. Reyners, E. Gianfelici de Reyners. Submitted.

Head of project 3: Dr Coffigny

II Objectives for the reporting period

In the first part of the contract, an *in vitro* model was developed to study the radiosensitivity of the mesencephalic and striatal rat cells freshly isolated. This radiosensitivity was assessed using three parameters:
- nerve cells lethality
- neurites growth
- dopamine(DA) and gamma amino butyric acid(GABA) uptake in 3- day-old cell culture irradiated on day zero.

Preliminary results on the identification of neurons and glial cells by immunohistochemistry of their particular cytoskeleton proteins have been obtained.

III Objectives for next period

The reported work will continue by measuring the lethality and neurite growth on identified DA and GABA neurons using immunohistochemical methods. In parallel with the *in vitro* irradiation, an *in vivo* exposure of 0.75 Gy will be carried on 14- day- old fetuses prior to the isolation and culture of the nerve cells during three days. This comparison will give informations on the influence of the cell environment at the time of exposure.
In order to study possible late repairs of the irradiation effects, the three parameters measured in 3- day-old culture will be studied in 7- day-old culture.

IV Progress achieved including publications

In embryos, the DA neurons of the mesencephalum innervate the striatum structure. In adult animals, more complex relationships are present between these parts of the brain.

Methods

To culture nerve cells with success, they must be taken up just at the end of the mitotic period but before the differentiation period. Fortunately, in rat fetuses, this transition between the two stages for the mesencephalum and the striatum occured at the same time, on day 14 of gestation. Nerve cells from both structures were mechanically isolated . The neurotransmitters uptake assay needed up to 2 10^5 cells / cm^2 but the lethality and neurite growth measurements needed only 4 10^4 cells / cm^2.
The cells, freshly isolated, were gamma irradiated with 0.25, 0.50, 0.75, 1.5 and 3 Gy and cultured 3 days in serum free medium before being analysed.
The lethality was assessed by counting of the trypan blue negative living cells . The longest neurites were measured on micrographs. These two parameters were studied only on striatal cells. The ^3H DA and ^{14}C GABA uptakes were determined. All results were expressed as a percentage of control values.

Results

The relative number of living cells decreased significantly after 0.50 Gy or higher doses (figure 1). The longest neurite was reduced with as little as 0.25 Gy

(figure 2). The DA uptake by mesencephalic cells and the GABA uptake in cells of both structures were decreased after 0.25 Gy or more (figure 3). The effect was dose dependent.

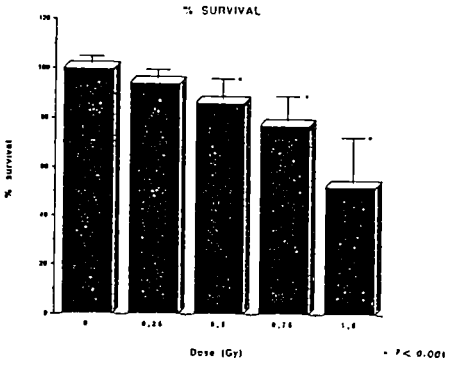


Figure 1.Percentage of the striatal cells survival as a function of exposure dose

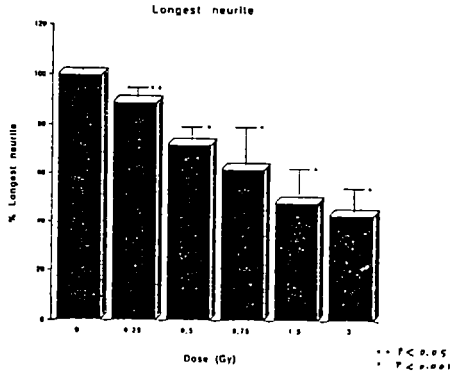


Figure2. Percentage of the longest neurite of striatal cells as a function of exposure dose

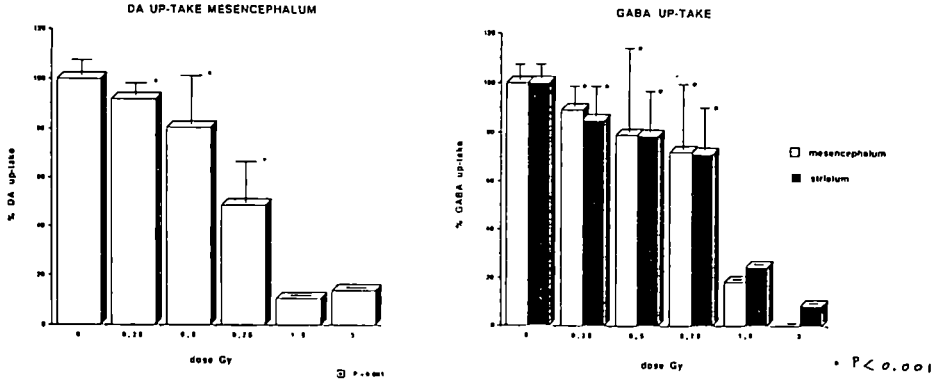


Figure 3. Percentage of DA uptake in mesencephalic cells and GABA uptake in mesencephalic and striatal cells as a function of exposure dose

Discussion-Conclusion

The exposure dose of 0.25 Gy with gamma rays seems to be near the threshold value for a deleterious effect on nerve cells.The neurotransmitters uptake and neurite growth seem to be parameters more sensitive than the cell lethality.

Publications

Coffigny H.,Beauvallet M. and Court L. -Irradiation effects on mesencephalic and striatal nerve cells in culture. 23rd Annual Meeting of the European Society for Radiation Biology. Dublin,Ireland, september 24-26, 1990.

Court L.,Coffigny H.,Fatome M.,Gueneau G.,Laget P.,Menetrier F. and Vernois Y. - Effets des rayonnements ionisants sur le système nerveux central en voie de développement (aspect électrophysiologique) et comparaison avec d'autres facteurs tératogènes. In "effets tératogènes des rayonnements ionisants" EDF-Comité de radioprotection, 1990, 6, 17-28.

Coffigny H. -Irradiation effects on neurite growth and transmitter content in cultured rat brain neurons. EULEP Newsletter, 1991,61, 9-10.

Progress Report

Contract: Bi7-001 Sector: B32

Title: Dysfunction and neoplasis of haemopoietic and osteogenic tissue following external irradiation or bone-seeking radionuclide contamination in utero or during neonatal development

1. Humphreys	MRC Radiobiological Unit
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I. Summary of Project and Global Objectives

The relative sensitivities to radiation of tissues in the developing compared with those in the adult animal are of crucial current importance. Recent experiments in mice have demonstrated an apparently greater sensitivity to α-particle irradiation of foetal tissues than of those of adult animals from measurements of haemopoiesis made in neonate and juvenile animals by *in vivo* and *in vitro* techniques.

The aims of the contract therefore are to determine:

1. The most radiosensitive period during pre- and post-natal development for both the response of stromal and haemopoietic marrow cells and for the dysfunction or induction of neoplastic change in bone and bone marrow following contamination with α-particle emitting radionuclides or comparable doses of external low LET irradiation.

2. The functional quality of mature cells generated from haemopoietic tissue damaged by such radiations.

3. The identity and location of the sensitive cell populations.

4. The role of haemopoietic growth factors in regulation and recovery of irradiation damaged tissue.

5. The features of the stromal populations identified by cellular and molecular techniques.

Head of Project 1: Dr. Humphreys

II Objective for the reporting period

To begin a long-term experiment in CBA/H mice demonstrating the late effects of ^{239}Pu administered *in utero* by introducing into the experiment approximately 1000 male offspring from CBA/H females given 16, 32 or 64 Bq g^{-1} ^{239}Pu on either day 4 or day 13 of gestation.

III Objectives for the next period

To continue the observation of the mice and to begin assessing the effects of the *in utero* contamination.

On the basis of the results of parallel studies on the distribution and effects of ^{228}Th administered paratibially to pregnant female and adult male mice respectively, to consider the injection of ^{228}Th paratibially into pregnant mice to simulate a continuous exposure of the foetuses to ^{224}Ra.

IV Progress achieved including publications

Materials and methods

^{239}Pu was obtained from Amersham International as a solution of the element (99% ^{239}Pu, 1% ^{240}Pu - other isotopes not detected) in 3MHNO_3. This material was standardized against a ^{239}Pu standard (also obtained from Amersham International) by counting in a Beckman Model LS5000CE liquid scintillation counter.

Solutions of ^{239}Pu in 1% trisodium citrate solution (adjusted to pH 5.5 by the addition of 3MHNO_3) were prepared, containing concentrations of ^{239}Pu of 1600, 3200 and 6400 Bq g^{-1} solution enabling each mouse to be injected with one-hundredth of its body mass of injection solution to achieve the required activities of 16, 32 or 64 Bq g^{-1} body mass respectively.

Female CBA/H mice were mated with male CBA/H mice and on day 4 or day 13 of gestation (conception was considered to take place on day zero) injected intravenously via lateral tail vein with the appropriate injection solution.

The offspring were sexed as soon as possible after birth and only the males entered into the experiment. These were weaned after 21 days, housed four to a box, fed and watered *ad libitum* and subsequently examined at frequent intervals by a standard procedure (Humphreys *et al.* 1989).

Results

Table 1 shows the current status of the experiment. To date only 5 animals are dead out of 953 introduced; no significant pathology has been seen.

Table 1
In utero injection of ^{239}Pu
Status May 1991

^{239}Pu (Bq g^{-1})	16	32	32	64	64
Days gestation	13	13	4	13	4
No. of offspring entered	195	158	200	200	200
No. of offspring dead	3	0	0	1	1
Mouse-days exposure	54792	21607	56799	41145	76367

Publication

Humphreys, E.R., Major, I.R., Stones, V.A. (1989). Myeloid leukaemia/osteosarcoma ratio in CBA/H mice given ^{224}Ra - interim results. In: "Risks from Radium and Thorotrast" (edited by D.M. Taylor, C.W. Mays, G.B. Gerber, and R.G. Thomas). British Institute of Radiology Report 21, Butterworths, Sevenoaks, Kent, England; Stoneham, Mass. USA, pp.36-39.

Head of Project 2: Dr.Vandenheuvel

II Objectives for the reporting period

In order to identify a radiosensitive stage in development, Balb/c mice are radiocontaminated with 241-americium at different developmental ages and in different ways. The effect of this internal irradiation on haemopoietic and stromal cell populations in the bone marrow is studied.

Long-term cultures (LTC) allow the *in vitro* study of radiation-induced defects to haemopoietic precursor cells and to their micro-environment. Cellular and extracellular matrix components in these cultures are analyzed to find changes related to persistent haemopoietic dysfunction after *in utero* contamination with ^{241}Am. The haemopoietic capacity of the stromal layer is evaluated by reseeding the stroma with haemopoietic pluripotent stem cells.

III Objectives for next period

1. A new experiment has already been started in which male mice are contaminated with different activities of ^{241}Am to define a dose-effect relationship in the offspring.

2. We will search for the radiosensitive component in the LTC. Initially we will focus on growth factor production.

3. Cell populations of haemopoietic organs will be separated by flow cytometry to search for the cell fraction which contains the stromal precursors (in collaboration with J. Visser, Rijswijk). Purified cell populations will be characterised.

4. Completion of the study of the survival and pathology of Balb/c mice to investigate the possibility of enhanced tumour rate after *in utero* contamination with ^{241}Am.

IV Progress achieved including publications

Methods

1. Balb/c mice were radiocontaminated with 241-americium. Different ways of contamination were used to irradiate specific stages which are important in the development of haemopoietic organs, including:
- single i.v. injection of pregnant mice on 14th day of gestation (14 kBq/mouse); offspring were fostered by contaminated mothers during the first 3 weeks after birth;
- continuous infusion using osmotic pumps between 7th and 14th day of gestation (11.15 kBq/mouse); offspring transferred to fostermother;
- continuous infusion using osmotic pumps between 14th and 19th day of gestation (7.5 kBq/mouse); offspring transferred to fostermother;
- contamination of neonates via lactation during 3 weeks after birth (fostermother received 14 kBq/mouse; i.v. injection);
- single i.p. injection of male mice (6.2 kBq/mouse) 32 days before mating with female mice.

13, 24 and 32 weeks after birth, haemopoietic and stromal stem cell studies were performed on the offspring or neonates;

- the number of CFU-s, CFU-GM and CFU-f were evaluated using short-term clonal assays;
- qualitative evaluation of haemopoietic function using long-term bone marrow cultures (LTC) which is a culture system to maintain haemopoiesis *in vitro*.

13 and 24 weeks after contamination; analysis of CFU-GM output in adherent and non-adherent phase using 24-multiwell dishes.

32 weeks after contamination, analysis of CFU-GM output in non-adherent phase using culture flasks.

95 weeks post-contamination stem cell studies were performed following 241-americium *in utero* (injection at the 14th day of gestation) and via lactation.

2. In order to assess the functional capacity of stromal cells to maintain haemopoiesis *in vitro* after radiocontamination, we will recharge stromal layers of LTC from control and radiocontaminated mice with haemopoietic pluripotent stem cells (HPSC) (Rh-123 dull cells, Facs sorted by Jan Visser, TNO, Rijswijk). The CFU-GM output is a measure of the haemopoietic activity (Vandenheuvel 1990, Vandenheuvel *et al.* 1991a).

Initially, we tested if HPSC (from Balb/c mice of TNO, Rijswijk) could proliferate and differentiate on our stromal layers (from Balb/c mice, SCK, Belgium). 4 weeks after initiation of LTC, stromal layers were X-irradiated (10 Gy) to destroy endogenous haemopoiesis and were reseeded with unpurified haemopoietic cells or HPSC.

3. The stromal layer in LTC from control and 241-Am contaminated mice was characterized. The presence of different cell types (macrophages, alkaline phosphatase and acid phosphatase positive cells) and extracellular matrix components (sulphated- and non-sulphated glycosaminoglycans) was studied using histochemical staining techniques. The latter components are believed to have a function in the regulation of haemopoiesis (Vandenheuvel *et al.* 1991b).

Results and Discussion

1A. Cellularity and number of haemopoietic and stromal stem cells

Neither the bone marrow cellularity nor the number of pluripotent haemopoietic stem cells were changed in the offspring after the different ways of *in utero* contamination.

The amount of progenitor cells for granulocytes and macrophages (CFU-GM) tended to decrease (equal to control values or significantly lower [t-test, $P \leq 0.05$]), while in contrast the number of stromal stem cells had the tendency to increase (equal or higher than control values).

1B. Long-term bone marrow cultures

13 weeks post partum no differences were seen in the haemopoietic activity of LTC, which reflect the functional capacity of stromal cells to maintain haemopoiesis *in vitro* from either control mice or contaminated mice.

24 weeks post partum the haemopoietic activity of LTC was significantly diminished; the CFU-GM output in cultures from contaminated mice (regardless of the way of 241-Am contamination) was lower compared with the CFU-GM yield in cultures derived from control mice.

32 weeks postnatally, the CFU-GM yield in LTC derived from offspring contaminated *in utero* using osmotic pumps either between the 7th and 14th day of gestation or between the 14th and 19th day of gestation was significantly lower than the CFU-GM content in LTC from control mice. CFU-GM output in LTC from the other groups was similar to the CFU-GM output in LTC from control mice.

The associated absorbed α-irradiation dose to the femur was approximated, resulting from previous retention studies in fostered and non-fostered offspring of mice contaminated at the 14th day of gestation with 14 kBq of 241-americium (Schoeters *et al.* 1990).

The cumulative dose to the femur in offspring reared by their own contaminated mother reached 1.7 cGy at 13 weeks postcontamination, 2.6 cGy 24 weeks after contamination and 3.3 cGy at 32 weeks postcontamination.

Mice fostered during the lactation period by a contaminated mother accumulated respectively 0.9 cGy after 13 weeks and 1.5 cGy after 24 weeks due to 241-Am contamination of the milk. In the male mice contaminated 32 days before conception, the cumulative dose to the testis amounted to 2.1 cGy.

1C. Effect at long-term

At 95 weeks after contamination *in utero* on the 14th day of gestation, LTC derived from 241-Am contaminated mice showed a decreased CFU-GM output compared with cultures from control offspring (two-way ANOVA, $P \leq 0.01$). Nevertheless, no significant differences ($P \leq 0.01$) were seen between the control and radiocontaminated mice, in bone marrow cellularity and stem cell concentrations (CFU-s, CFU-GM, CFU-f). The associated cumulative dose to the femur at that time is 8.5 cGy.

2. Preliminary results indicate that HPSC can be maintained on a stromal layer.

3. Phenotypic characterization of the confluent adherent stromal layer yielded no obvious differences in cell types and extracellular matrix components between stromal layers derived from control animals and ^{241}Am contaminated mice. Therefore we will apply other techniques (i.e. factor dependent cell lines, *in situ* hybridization) to study growth factor and extracellular matrix production.

Conclusions

We have seen quantitative and qualitative effects on the bone marrow cells after contamination of mice at different developmental stages. To define the most radiosensitive stage:

1. Information on the transplacental transfer, on the distribution of ^{241}Am in the offspring and on the associated α-dose after different ways of ^{241}Am contamination is required.

2. We shall analyse the nature of the damage in the LTC after different contamination procedures. Which cell population (the haemopoietic or stromal cells) is damaged? Is there

a change in the production of e.a. growth factors and extracellular matrix components or in the presence of cell adhesion molecules?

Working with purified haemopoietic populations and separated stromal populations will be of great benefit.

Publications

Van Den Heuvel, R.L. (1990). Bone marrow of Balb/c mice radio-contaminated with 241-Am *in utero* shows a deficient *in vitro* haemopoiesis. International Journal of Radiation Biology, 57, 103-115.

Van Den Heuvel, R., Schoeters, G., Leppens, H., Vanderborght, O. (1991a). Stromal cells in long-term cultures of liver, spleen and bone marrow at different developmental ages have different capacities to maintain GM-CFC proliferation. Experimental Haematology, 19, 115-121.

Van Den Heuvel, R., Mathieu, E., Schoeters, G., Leppens, H., Vanderborght, O. (1991b). Characterisation of stromal cells derived from murine (fetal and postnatal) haemopoietic liver and bone marrow: comparison between stromal stem cell assay (CFU-f) and long-term cultures. International Journal of Developmental Biology, 35, 33-41.

Schoeters, G., Van Den Heuvel, R., Leppens, H., Vander Plaetse, F., Vanderborght, O.(1990). Distribution of 241-Am in offspring from Balb/c mice injected with 241-Am at 14 days of gestation: relation to calcium and iron metabolism and comparison with distribution of 241-Am after injection of adults. International Journal of Radiation Biology, 58, 371-382.

Van Den Heuvel, R.L., UIA University of Antwerp, Belgium, 22/6/1990. Ph.D. thesis: Study of murine stroma in fetal and postnatal haemopoietic organs and after radiocontamination with 241-americium *in utero* and as adults.

Head of Project 3: Dr. Lord

II Objectives for the reporting period

To investigate the transplacental uptake of maternal ^{239}Pu contamination and its effects on the development and maintenance of haemopoietic tissue.

To compare repopulation of haemopoietic and stromal precursor cell populations by 6 months after γ-irradiation at age 1 week, 4 weeks or 11 weeks.

III Objectives for next period

To extend the long-term observations on haemopoiesis in the offspring of ^{239}Pu contaminated mice. To consider comparative uptakes and effects of neonatal contamination. To start up comparable radium contamination from single injections of ^{224}Ra and from paratibial injections of ^{228}Th. To assess residual injury in terms of deficiencies in megakaryocyte precursor cells and in marrow repopulating ability.

IV Progress achieved including publications

Approximately 1% of ^{239}Pu used to contaminate pregnant mice at mid-term gestation appears in the newborn offspring - most of this is in the foetal liver. When contamination occurs early in gestation, uptake in the new born offspring is ten fold lower. Effects on haemopoiesis in the long-term however, are comparable. Reduced numbers of stem cells are required to maintain a higher level of proliferation in order to maintain cell output. The mechanisms in the two situations are different: the one is determined primarily by effects on the haemopoietic stem cells; the other by effects primarily on the stromal microenvironment.

In control animals, the number of marrow cells per femur increased from about 10^7 at 4 weeks of age to 1.8×10^7 at 37 weeks. The corresponding increases for day 8 CFU-S were from 3000 to 4000, for day 12 CFU-S 3000 to 5000, and for iv-CFC 30,000 to 55,000. In contrast, CFU-F numbers declined from around 750 at 4 weeks of age to 400 at 15 weeks, and then increased to 650 at 37 weeks. There are greater increases in these parameters with time for younger mice compared with adults. If growth is reduced by irradiation, this indicates the greater potential for greater residual injury in the younger animals.

Regarding CFU-S response and recovery after external irradiation: (a) there was no significant dose-rate effect between 6 and 60 cGy/min for acute survival, (b) acute survival levels were consistently higher for day 12 than for day 8 CFU-S, but the difference disappeared by 6 months post irradiation recovery, (c) long-term recovery at 6 months was equally good in mice of all ages, except perhaps after the highest dose (4.5 Gy) used in the 1 week old mice. Regarding CFU-S per colony (a) the recovery levels were in general lower in 1 week old mice than in the other ages, (b) the effects of radiation on this endpoint were greater than for CFU-S numbers, in mice aged 1 week or 10-12 weeks, but not 4-5 weeks, (c) there was a tendency for better recovery after lower doses delivered at the lower dose-rate (6 cGy/min) in mice aged 1 week or 4-5 weeks, but this was not seen in adults. Surprisingly, recovery in adults was poorer after higher doses at the lower (compared to the higher) dose-rate.

Concerning recovery of i.v.-CFC there was: (a) no significant dose-rate effect, (b) better recovery than CFU-S in 4 week old mice but with similar levels in 1 week old mice or in adults.

Regarding CFU-F (a) an acute dose-rate effect was observed for 4-5 week old mice but the reverse was found for adult mice, (b) recovery was in general poorer in 1 week old mice than in the other ages, (c) a marked dose rate effect was observed for the levels of recovery in 4 week old mice. This was not so marked for adult mice, and there was a tendency towards the reverse effect in 1 week old mice.

Publications

Baird, M.C., Hendry, J.H., Dexter, T.M., Testa, N.G. (1991). The radiosensitivity of populations of murine haemopoietic colony-forming cells which respond to combinations of growth factors. Experimental Haematology (in press).

Baird, M.C., Hendry, J.H., Testa, N.G. (1990). Radiosensitivity increases with differentiation status of murine haemopoietic progenitor cells selected using enriched marrow subpopulations and recombinant growth factors. Radiation Research, 123, 292-298.

Bierkens, J.G., Hendry, J.H., Testa, N.G. (1991). Recovery of the proliferative and functional integrity of mouse bone marrow in long-term cultures established after whole-body irradiation at different doses and dose-rates. Experimental Haematology (in press).

Hendry, J.H., Roberts, S.A. (1990). Analysis of dose-incidence relationships for marrow failure in different species, in terms of radiosensitivity of tissue-rescuing units. Radiation Research, 122, 155-160.

Lord, B.I., Molineux, G., Humphreys, E.R., Stones, V.A. (1991). Long-term effects of plutonium-239 and radium-224 on the distribution and performance of pluripotent haemopoietic progenitor cells and their regulatory microenvironment. International Journal of Radiation Biology, 59, 211-227.

Mason, T.M., Humphreys, E.R., Lord, B.I. (1991). Alpha-particle irradiation of haemopoietic tissue in pre- and post-natal mice. 1: Distribution of plutonium-239 after mid-term contamination. International Journal of Radiation Biology, 59, 467-478.

Qi, D.Y., Hendry, J.H. Testa, N.G. (1991). Interactions in recovery and in residual injury from sequential treatments of mouse haemopoietic and stromal marrow cell populations, using X-rays, cyclophosphamide and busulphan. Radiotherapy and Oncology, 20, 46-52.

Head of Project 4: Dr. J.W.M. Visser

II Objectives for the reporting period

The collaboration with the SCK/CEN in Mol in order to analyse and purify stem cells from mouse bone marrow and from fetal liver with respect to their long-term culture capability on irradiated stromal layers is the main goal of the project in the first terms. In addition, the project aims at the purification of the stem cells of the stromal layers by applying a similar strategy which has been successful for the isolation of the pluripotent haematopoietic stem cell. Possible experiments to study the interaction between purified stem cells and stromal cells at the molecular level are to be discussed and planned in the first part of the project.

III Objectives for next period

The development of a protocol to isolate the stem cells of the stromal layers that support haemopoietic growth, will be continued in close collaboration between the research teams at the SCK/CEN in Mol, and the Radiobiological Institute-TNO in Rijswijk. Procedures employing antibodies and negative selection with immunomagnetic beads in combination with culture have to result in a detailed phenotyping of the stromal stem cells. A combination of immunomagnetic bead and fluorescence-activated cell sorting techniques will be developed to prepare stromal layers of a well-defined composition. These will be employed for detailed studies on the regulation of haemopoiesis by the interaction between stem cells and stromal elements.

IV Progress achieved including publications

In a first series of experiments it was established that pluripotent haemopoietic stem cells could be highly purified from adult mouse bone marrow at the TNO institute in Rijswijk, and that these cells could be transported to Mol to be cultured there at the SCK/CEN laboratory on a variety of stromal layers to produce haemopoietic progenitor cells. In a preliminary series of experiments the enrichment for the cell producing CFU-GM on stroma was found to be a factor of 1.8. Further experiments are necessary to improve the isolation procedure applying recently published new data (Jones *et al.*, 1990, Nature 347, 188) in our combination of sorting techniques, and to compare different sorted cell fractions and control groups on the stromal layers.

The first experiments to isolate stromal progenitor cells in Rijswijk were not successful in that the sorted cells did not give rise to stromal layers after transport to Mol. Unfractionated cells sent from Rijswijk to Mol gave normal stromal layers. Attempts to obtain stromal layers from sorted mouse cells failed, whereas in Rijswijk good layers could be obtained from monkey bone marrow using fluorescence-activated cell sorting of antibody-labelled cells. Not only did mouse cells that were passed through the fluorescence-activated cell sorter, fail to produce a stromal layer, but also those cells that were separated using immunomagnetic bead procedures. The bead selection procedure is now being modified in Mol to prevent transportation problems. As soon as the reason for this unexpected result has become clear, further attempts will be undertaken to isolate the murine stromal stem cells by fluorescence-activated cell sorting.

Publications

Bayer, J.A., Bauman, J.G.J. (1990). Flow cytometric detection of β-globin mRNA in murine haemopoietic tissues using fluorescent *in situ* hybridization. Cytometry, 11, 132-143.

Van Dekken, H., Arkestein, G.J.A., Visser, J.W.M., Bauman, J.G.J. (1990). Flow cytometric quantification of human chromosome specific repetitive DNA sequences by single and bicolour fluorescent *in situ* hybridization to lymphocyte interphase nuclei. Cytometry, 11, 153-164.

Visser J.W.M., De Vries, P. (1990). Identification and purification of murine haematopoietic stem cells by flow cytometry. In: Methods in Cell Biology, Vol. 33 Flow Cytometry (Darzynkiewicz, Z. and Crissman, H., eds.) Academic Press, Inc., San Diego, pp. 451-468.

Visser, J.W.M., De Vries, P. Hogeweg-Platenburg, M.G.C., Bayer, J., Schoeters, G, Van Den Heuvel, R., Mulder, A.H. (1991). Culture of haemopoietic stem cells purified from murine bone marrow, Seminars in Haematology, 28: 117-125.

Visser, J.W.M., Hogeweg-Platenburg, M.G.C., De Vries, P., Bayer, J., Ploemacher, R.E. (1990). Culture of purified pluripotent haemopoietic stem cells. In: The Biology of Haematopoiesis (Dainiak, N., Cronkite, E.P., McCaffrey, R., Shadduck, R.K., eds.), Wiley-Liss, Inc., New York, pp. 1-8.

Visser, J.W.M., Van Bekkum, D.W. (1990). Purification of pluripotent haemopoietic stem cells: past and present. Experimental Haematology, 18, 248-256.

Head of Project 5: Dr. Tejero

II Objectives for the reporting period

The objective for the reporting period was to determine whether residual damage is manifest by functional disorder in mature granulocytes (phagocytosis dysfunction) after 5 Gy whole body X-irradiation. The study is focused on O_2^- anion production in granulocytes obtained from peripheral blood and long-term bone marrow culture (LTBMC).

III Objectives for next period

The objective for next period is to extend our investigation with respect to O_2^- anion production from different doses, (single and repeated), types of radiation (X-rays, γ-rays) and age in mice (neonate and adult). The effect of age will also be studied in foetal and neonatal mice contaminated with Pu-239. The study will be carried out on granulocytes obtained from peripheral blood and LTBMC from B6D2F1 and C57Bl x Balb/c F1 mice over long periods after irradiation or contamination.

IV Progress achieved including publications

Our investigation has been carried out during a one year observation period following 5 Gy total body irradiation (X-rays, 300 kV, 1.21 Gy/min). All experiments have been performed on individual animals and in parallel with age-matched controls (C57Bl x Balb/c females).

Methodology

The study was carried out on granulocytes obtained either from peripheral blood or from LTBMC. Granulocytes from peripheral blood were used in preference to those from a casein-stimulated peritoneal exudate to avoid the problem of superoxide generation associated with the latter method. Our results are the first reported for granulocytes from peripheral blood in mice. Isolated granulocytes were obtained by sedimentation of erythrocytes with 1.5% T-500 Dextran and centrifugation on Ficoll-Paque 1600 x g, 20 min, 20°C. The contaminated erythrocytes were removed by hypotonic lysis (25 sec).

Long-term bone marrow cultures were established using one femur and one tibia in 10 ml of Fischer's medium supplemented with 20% horse serum (pretested batch) and 10^{-5}M hydrocortisone sodium hemissuccinate. Cultures were fed weekly and the recovered cells (90-95%, mature granulocytes) subsequently assayed for superoxide anion production.

GM-CFC were assayed in semi-solid agar. For the assay, 10^5 bone marrow cells were plated. Supernatants of LTBMC, free of cells, at 10, 20 and 40% of WEHI-3B conditioned medium at 10% were used as the source of colony-stimulating-activity.

Table 1. Superoxide anion production in granulocytes from peripheral blood at 6 and 12 months postirradiation

		Time post-irradiation (months)	
		6	12
nmol O_2^-/min/10^6 cells	C	0.72 ± 0.04	1.10 ± 0.04
		n = 3	n = 6
		p < 0.05	p < 0.05
	I	1.36 ± 0.02	2.30 ± 0.40
		n = 3	n = 7

C: control; I: irradiated. Values expressed x ± SEM. n = number of experiments; each experiment corresponds to individual animals; p = significance of difference from control using student's t-test

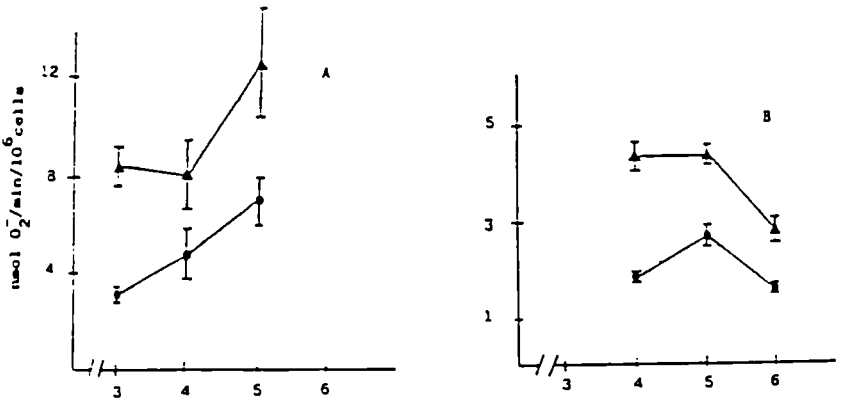

Fig. 1. Superoxide anion production in granulocytes from LTBMC obtained from irradiated (▲) and age-matched control mice (■). The experiments have been performed at 6 (A) and 12 (B) months postirradiation and at different weeks of culture. The values expressed X ± SEM. Net 3-11 experiments for each point.

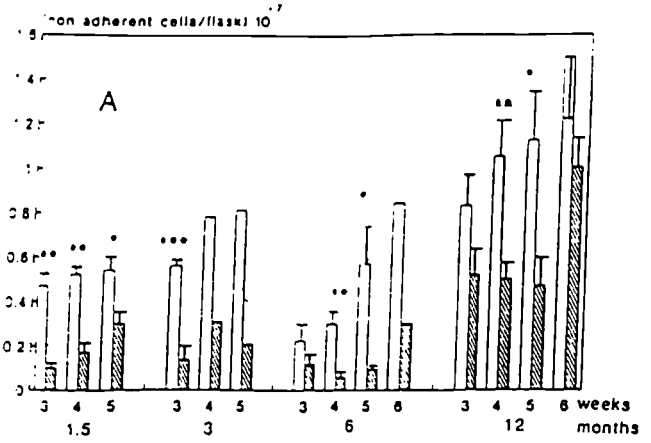

Fig. 2. Cell production of LTBMC from irradiated (■) and age-matched control animals (□). The measurements have been performed at different months postirradiation and weeks of culture. The values are expressed as x ± SEM. Net 3-15 experiments for each point. Insert in the figure statistical significance of difference from control using Student's t-test. *p<0.05; **p<0.01; ***p<0.001.

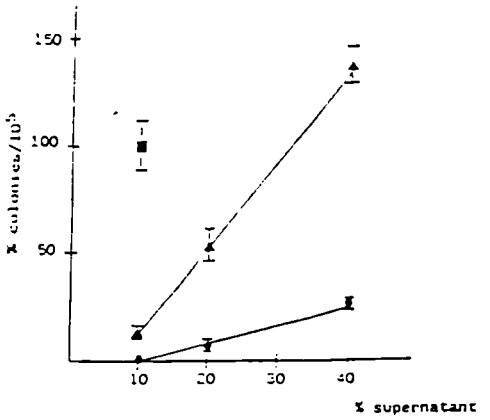

Fig. 3. Stimulation of GM-CFC progenitors by 4 week-old LTBMC supernatants established 12 months postirradiation. Irradiated (▲), age-matched control (●) and WEHI-3B conditioned medium (■). Values are x ± SEM. Similar patterns have been obtained at different weeks of cultures and time postirradiation.

Superoxide anion production was determined by the continuous spectrophotometric measurement of the superoxide dismutase-inhibitable reduction of ferricytochrome c at 550 nm. Cells were stimulated with 1 mg PMA and change in absorbance at 550 nm was continuously recorded at 37°C.

Results and Discussion

The long-term effects of radiation are the same for granulocytes obtained from peripheral blood or LTBMC; an enhancement of superoxide anion production in cells from irradiated animals. The difference is statistically significant at both 6 and 12 months postirradiation (Table 1, Fig. 1). The production of granulocytes in LTBMC is shown in Fig. 2. The number of non-adherent cells in the supernatant was always less in LTBMC from treated animals caused probably in these mice because the adherent layer never became properly established over the period studied.

Fig. 3 shows that the cell-free supernatant obtained from LTBMC prepared from irradiated animals was able to stimulate GM-CFC, showing that some CSF is produced in these cultures which is not detectable in cultures of normal marrow cells

Conclusion

In summary, our data suggest that total body irradiation (5 Gy) of mice produce a compensatory mechanism probably in stromal cells, so that they release excess CSF. This factor could prime granulocytes in such a way that their capacity to produce superoxide anion is increased.

Scientific staff: S. Gaitán, E. Cuenllas, J.M.J. Herranz, P. Sancho, S Escribano.

Publications

Cuenllas, E., Gaitán, S., Escribano, S., Bueren, J.A., Tejero, C. (1990). Long-term haemopoietic injury in mice granulocytes after 5 Gy irradiation. Cell Biology International Reports, 14, Suppl. p. 314.

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Grande, T., Tejero, C., Bueren, J.A. (1990). Long-term haemopoietic damage in adult and newborn irradiated mice. 23rd Annual Meeting of the European Society for Radiation Biology. September. Book of Abstracts, Dublin (Ireland).

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Head of Project 6: Dr. Bueren

II Objectives for the reporting period

To compare the long-term haemopoietic damage induced by a single acute dose of 7 Gy X-rays in adult and suckling mice.

To achieve an efficient haemopoietic stem cell infection with retroviruses that allow the characterization of haemopoietic stem cell clones.

III Objectives for next period

To characterize the contributions of stromal and haemopoietic injury in adult and suckling mice.

To analyse haemopoiesis of mice following external irradiation of embryonic stages of development.

To analyse the clonal expression of genetically marked haemopoietic stem cells following *in vivo* transplantation in mice or *in vitro* in Long Term Bone Marrow Cultures (LTBMC).

To determine the influence of the irradiation on the expression of haemopoietic stem cell clones.

IV Progress achieved including publications

1. Long-term haemopoietic damage in adult and suckling irradiated mice

Methodology

Twelve week and eight day old F1(C57B1 x Balb/c) mice were irradiated with single acute doses of 7 Gy X-rays (300 kV, 1.12 Gy/min). Groups of three mice were taken at intervals and the numbers of white cells in blood and CFU-S, CFU-GM, BFU-E and CFU-F in bone marrow and spleen compared with similar numbers in age-matched controls; the extent of stromal damage was inferred from the numbers of CFU-F cultured *in vitro*.

Results

Adult mice

The long-term study of haemopoiesis in these mice showed that fewer than 10% of the animals died of irradiation syndrome; those which recovered showed essentially normal leukocyte numbers and haematocrit values in the first year post-irradiation. However, a significant decrease in numbers of leukocytes was observed at later times. In the bone marrow, however, all the haemopoietic progenitors tested were significantly reduced to about 10-40% of age-matched control values with no signs of recovery up to the end of the first year post-irradiation. No evidence was found of a compensatory enhancement of haemopoiesis in the spleen where there was also a 10-40% depletion of haemopoietic progenitors compared with age-matched controls.

Suckling mice

Survival in this group of mice was comparable with that seen in adults. Measurements of peripheral blood cell numbers were normal at all times studied, but, in contrast to the results obtained in adults, a slow but consistent recovery of femoral haemopoietic progenitors was seen throughout the first year post-irradiation. With the exception of CFU-GM, which reached 70% control, all haempoietic progenitors reached normal values in this period. In the spleen there was significant haemopoietic injury (about 50% control) although less than that seen in adults.

An overexpression of CFU-F numbers was seen at later times in adult but not in the 8 day old mice.

A study is now in progress of the self-renewal capacity of CFU-S and of stromal function in irradiated adult and 8-day old mice to investigate the mechanisms which allow the long-term haemopoietic recovery in suckling mice. Irradiation at earlier stages will also give information on the effects of age on haemopoietic sensitivity.

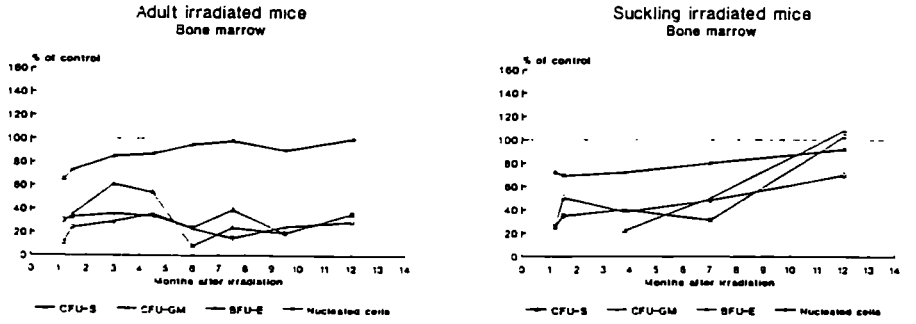

Figure 1. Long-term haemopoietic damage in the femoral bone marrow of 12 week-old and 8 day-old mice, irradiated with 7 Gy of X rays.

2. Transfer of genetic markers in haemopoietic stem cells

Methodology

Retroviruses

The pXT1 retrovirus containing the neomycin phosphotransferase gene (neo^r) under the control of the Long Terminal Repeat of a Moloney Leukaemia derived retrovirus was used.

Development of cell clones producing high titers of defective retroviruses

Defective ecotropic retroviruses were obtained from selected clones of packaging ψ_2 cells transfected with retroviral DNA. ψ_2-pXT1 clones resistant to the neomycin analogue G-418 were grown and retrovirus supernatants titrated by slot-blot and by determining the number of NIH-3T3 cells resistant to G-418. In some experiments, retroviruses were produced by coculture of amphotropic PA-317 cells with the selected ψ_2-pXT1 clones.

Transfer of genetic markers in bone marrow cells

Retroviral producer cells were cocultured for 15 to 48 h with bone marrow cells obtained from 5-FU treated animals in the presence of WEHI-3 conditioned medium, with or without IL-6. In some experiments a 48 h-preincubation of the bone marrow with such factors was carried out.

Determination of the insertion efficiency of the neo^r gene in the haemopoietic stem cells

Southern blots and PCR analyses of DNA extracted from CFU-S colonies and/or haemopoietic organs were performed with probes and oligonucleotides specific for the neo^r gene.

Results

Selection of ψ_2-pXT1 clones producing high titers of defective retroviruses

Fifty clones of the ψ_2 cells transfected with the pXT1 were titrated by a slot-blot assay. The eight best clones were tested by the 3T3 infection assay. Clones producing 2-5.10^5 infective particles per ml were selected for further experiments.

Efficiency of genetic transfer to CFU-S 12d

As the CFU-S_{12d} is one of the most primitive haemopoietic precursors for which a clonal assay is available, the efficiency of retroviral infection of this cell was selected as first indication of genetic transfer in haemopoietic stem cells. Fifteen, 24 and 48 h-cocultures of bone marrow cells with the selected ψ_2-pXT1 clone in the presence of 10% of WEHI-3 conditioned medium, only infected 5-30% of the CFU-S. The inclusion of a 48 h selection step in G418 following the coculture, killed about 80% of the CFU-S population, although increased the efficiency of infection of survival CFU-S to close to 100%.

Experiments in which amphotropic PA 317 cells were cocultured with ψ_2-pXT1 cells increased significantly the virus titer up to ten times. Therefore, this procedure of coculture was carried out to determine whether the CFU-S population could be efficiently infected without a further in vitro selection in G418. The influence that the preincubation of the bone marrow cells in WEHI-3 CM (with or without IL-6) had on the efficiency of infection of these cells has also been examined in these experiments. Table I summarizes the results obtained.

Retrovirus producer cell line	Selection in G-418	Virus titer	Pre-incubation	Factors	% of splenic colonies with genetic mark
ψ_2	-	5.10^5	-	IL-3	5-30 (*,**)
ψ_2	+	5.10^5	-	IL-3	90-100 (*)
ψ_2 + PA317	-	10^6	-	IL-3	80-90 (**)
ψ_2 + PA317	-	5.10^6	+	IL-3	80-90 (**)
ψ_2 + PA317	-	5.10^6	+	IL-3 + IL-6	80-90 (**)

Table I: Analysis of the efficiency of genetic transfer into primitive haemopoietic precursors (CFU-S 12d, * - analysed by Southern blot; ** - analysed by PCR).

From these experiments it was concluded that the infection of this multipotent haemopoietic precursor has been efficiently achieved. The aggressive selection step in the neomycin analogue (between 70% to 90% of CFU-S were killed in this treatment), although efficient to increase the percentage of genetically marked CFU-S, can be omitted when retroviral titers are increased. Preliminary PCR experiments of bone marrow obtained from recipients reconstituted with infected cells, showed a high expression of marked stem cells eight weeks after transplantation, when preincubation was carried out in IL-3 plus IL-6.

At present, insertional analyses by Southern blotting of blood and lymphohaemopoietic organs from recipients transplanted with genetically marked bone marrow are in progress. The long-term reconstitution of these organs by the marked cells will be subsequently used to investigate failures in the differentiation, self-renewal and longevity of the most primitive totipotent haemopoietic stem cells, as a consequence of the irradiation.

Publications

Bernad, A., González, J., Almendral, J.M., Bueren, J.A. (1991). Comparative analysis of parameters influencing the efficiency of retroviral infection of CFU-S 12d. 2nd International Symposium on Molecular Biology of Haematopoiesis, Innsbruck.

Cuenllas, S., Gaitán, E., Bueren, J.A., Tejero, C. Mechanisms towards compensation of long-term haempoietic injury in mice after 5 Gy total body irradiation: *in vivo* and *in vitro* enhancement of superoxide anion production by granulocytes. Experimental Haematology (submitted).

Cuenllas, E., Gaitán, S., Escribano, S., Bueren, J.A., Tejero, C. (1991). Long-term haemopoietic study in mice after 5 Gy irradiation: erythrocyte functionality. 20th Annual Meeting of the International Society for Experimental Haematology, Parma.

Grande, T., González, J., Tejero, C., Maganto, G., Bueren, J.C. (1990). Production of humoral factors that stimulate spleen colony-forming units in mice irradiated with moderate doses of X-rays. Radiation Research, 122, 53-57.

Grande, T., Tejero, C. Bueren, J.A. (1990). Long-term haemopoietic damage in adult and newborn irradiated mice. 23rd Annual Meeting of the European Society for Radiation Biology, Dublin.

Grande, T., Tejero, C., Bueren, J.A. (1991). CFU-S injury and stromal damage long-term after irradiation of adult and newborn mice. 9th International Congress of Radiation Research, Toronto.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: B16-C-310-F

C.E.A.
I.P.S.N. *
Département de Protection Sanitaire
B.P. nº 6
F- 92260 Fontenay-aux roses

Head(s) of research team(s) [name(s) and address(es)]:

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F-91680 Bruyères-le Châtel

Telephone number: (1) 69 26 56 01

Title of the research contract:

Foetal dosimetry: measurement of the effects induced after *in utero* chronic irradiation as a function of dose-rate and gestation age.

List of projects:

1. Foetal dosimetry: measurement of the effects induced after *in utero* chronic irradiation as a function of dose-rate and gestation age.

2. Effects of radiation on the development of the central nervous system.

* This contract initialy signed by Dr H. Métivier (I.P.S.N.) is now conducted by Dr H. Coffigny (DSV).

Title of the project no.: B16-C-310-F

Foetal dosimetry: measurement of the effects induced after *in utero* chronic irradiation as a function of dose-rate and gestation age.

Head(s) of project:
Dr. H. Coffigny
DSV / DPTE / STCE / LRT BP 12 91680 Bruyères le châtel, France.

Scientific staff:
H. Coffigny, P. Fritsch and M. Beauvallet

I. Objectives of the project:

The aim of this project is to estimate irradiation doses delivered to the embryo or the fœtus after internal contamination by radionuclides. For that purpose, we determined a dose-effect relationship between the irradiation doses delivered by protracted external irradiation during the whole intra-uterine life or part of it, and the biological effects measured on different radiosensitive target organs. Thus, irradiation doses delivered after internal contamination could be estimated from the measurement of the induced biological effects.

II. Objectives for the reporting period:

The effects of protracted irradiation during the whole gestation or part of it were studied on brain.The early effects (mortality, body and brain weights) were determined at the end of the gestation period. The late effects (body and brain weight, histology) were studied in 3 month old animals. We plan to establish dose- effect relationships with the different target organs and to observe a specific dose-rate effect.

III. Progress achieved:

Methodology

Rats (Sprague-Dawley) and mice (CBA) were irradiated with a ^{60}Co source (> 23 hours per day) during the whole intra-uterine life and two groups of mice were irradiated during the last 2/3 or 1/3 of gestation. Dose-rates were 0.03, 0.1, 0.25 and 0.375 Gy/day i.e. cumulative doses of 0.6, 2, 5 and 7.5 Gy respectively in rat. In mice, the dose-rates were 0.2, 0.4 and 0.6 Gy /day and cumulative doses were 3.6, 7.2 and 10.8 Gy during the whole intra-uterine life; 2.4, 4.8 and 7.2 Gy during the last 2/3 of gestation; 1.2, 2.4 and 3.6 Gy during the last 1/3 of gestation.

Four groups of 15 day old rat fœtuses received 1 Gy with dose-rates of 0.86, 4.75, 23.9 and 239 Gy/day.

For each point in all experiments, animals came from 4 litters or more.

At the end of gestation, one part of the pregnant rats or mice were killed by overdose of anesthetic. The mortality of embryos and fœtuses was scored with a distinction between pre-implantation death (difference between the number of corpus luteum and the number of implantation points) and post-implantation death (difference between the number of living fœtuses and the number of implantation points).

On day 21 of gestation for the rats or on day 19 of gestation for the mice, fœtus and placenta were weighed and the whole head was fixed in 10% of Becker fixative after incision of the skull for brain histology.

For the late effects study, the other part of pregnant animals were allowed to go to parturition. Post-natal deaths were scored. In 3 month old males and females, the body , the cerebrum (forebrain + midbrain) and hindbrain were weighed and the cerebrum was fixed for the histological study. The tissue sections were coloured with hematoxylin and eosin or with toluidine blue.

Results

2-1 Early effects of protracted irradiation

2-1-1 Pre- and post-natal death

Rat

Prenatal mortality, mainly post-implantation, increased significantly with the at the dose-rates of 0.25 and 0.375 Gy/day (table 1)

dose-rates (Gy/day)	0	0.03	0.1	0.25	0.375
mortality (%)	10.7	16.0	13.5	19.6	22.3

An important neonatal death occured only with 0.375 Gy/day.

Mouse

The mortality of control mice was very important in this strain. The prenatal death was increased only with protracted irradiation during the whole intra-

uterine life and was the consequence of post-implantation death (table 2).

Dose-rates (Gy/day)	Protracted irradiation period	Mortality (%)
0		40.5
0.2	1/3	44.7
	2/3	20.8
	3/3	73.1
0.4	1/3	31.8
	2/3	36.1
	3/3	64.7
0.6	1/3	50.9
	2/3	44.0
	3/3	100.0

All pups irradiated with 0.4 Gy/day during the whole intra-uterine life and with 0.6 Gy/day during the last 2/3 of gestation died.

2-1-2 Fœtal and placenta weights

Rat

The body (figure1) and placenta weights decreased with increasing dose-rates or cumulative doses of irradiation.

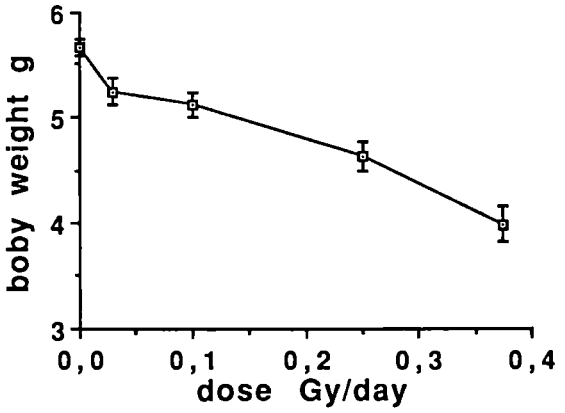


Figure 1- Fœtal body weight

Mouse

For each dose-rate, the body weight decreased with increasing doses received during the whole, last 2/3 or 1/3 of gestation (table 3).

Dose-rates (Gy/day)	Protracted irradiation period	Body weight (mg) ±SE
0		889.1 ± 17.6
0.2	1/3	847.1 ± 16.5
	2/3	736.3 ± 30.6
	3/3	667.4 ± 22.9
0.4	1/3	751.3 ± 19.4
	2/3	672.2 ± 17.5
	3/3	580.0 ± 41.9
0.6	1/3	759.6 ± 22.0
	2/3	505.7 ± 16.8

The placenta weights decreased as cumulative doses increased except for 0.2 Gy/day where no modification was observed.

2-1-3 Fœtal brain weight

Rat

The absolute brain weight decreased with increasing dose-rate or cumulative exposure doses (figure 2). The relative brain weights expressed in mg/100 g of body weight were not different from the control ones except for a decrease with 0.25 Gy/day.

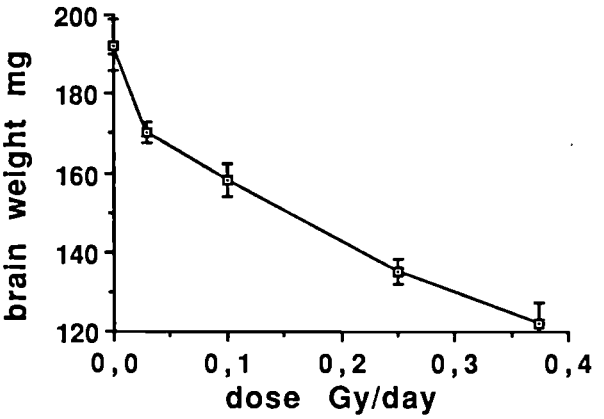


Figure 2- Rat fœtal brain weight

Mouse

The brain weight followed the same pattern of reduction as in rat.

2-2 Late effects of protracted irradiation

2-2-1 Body weight

Rat and mouse

The irradiated female and male body weights were not significantly different from the control ones.

2-2-2 Brain weight

Rat

Female and male cerebrum weights decreased with increasing dose and dose-rate from 0.03 Gy/day (figure 3). No significant change was observed with the hindbrain in the same conditions.

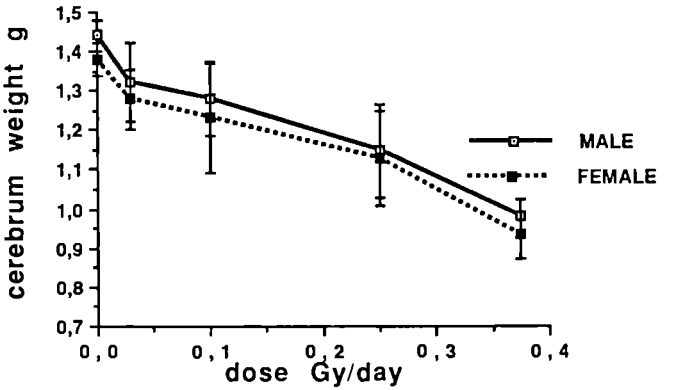


Figure 3- adult rat female and male cerebrum weight

Mouse

The brain weights of females and males were considered together. Ttey decreased with increasing dose-rates or doses received during the last 1/3 of gestation. The effects were similar if irradiation was carried out during the whole or last 2/3 of gestation with 0.2 Gy/day or the last 2/3 with 0.4 Gy/day (figure 4).

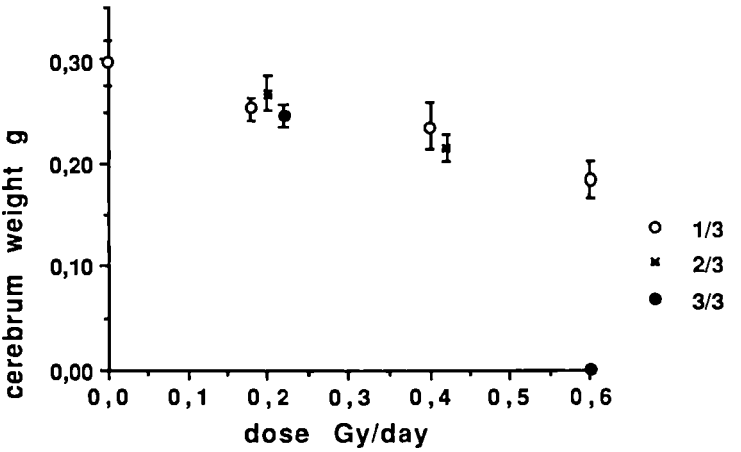


Figure 4- Adult mice cerebrum weight

2-2-3 Brain histology

No gross malformation was observed in rat and mouse brain.

2-3 Late effects of 1 Gy on day 15 of gestation in rat

2-3-1 Body weight

The female and male body weight were not very different from the control ones.

2-3-2 Brain weight

The female and male cerebrum weights were considered together; 1 Gy of exposure on day 15 of gestation induced an atrophy of the cerebral hemispheres. The cerebrum weights decreased more and more as dose-rates increased (table 4).The hindbrain weights were not modified.

Dose -rate (Gy/day) (%)	brain weight (g) ± confident limit (p>95%)	decrease (%)
0	1.339 ± 0.041	
0.06	1.090 ± 0.033	18.6
0.33	1.084 ± 0.040	19.0
1.66	0.958 ± 0.066	28.5
16.66	0.903 ± 0.034	32.6

2-3-3 Brain histology

No gross malformation was noted in rat irradiated with 0.86 and 4.75 Gy/day. *Corpus callosum* agenesis and ectopic gray matter were observed in 50 % of rats irradiated with 1 Gy at 23.9 Gy/day and in 100% of rats with 239Gy. In most animals, perturbations of the aligment of *hippocampus* pyramidal cells in

the region underlying the ectopic gray matter were observed. The cortex thickness did not seem to be modified.

Discussion

Fœtal body weight is a good parameter to estimate the total exposure dose received during the whole intra-uterine life but this is no moreverified in adult animals after growth compensation. Conversely, brain weight is a good parameter for the exposure estimation at the end of gestation but it is more accurate in the adult. Nevertheless, this parameter is working only during the last 1/3 of gestation. In the range of dose-rates studied, no specific dose-rate effect is observed on body and brain weights. Brain histology does not allow more accuracy on a possible dose-rate effect in the limit of animal survival.

In the other part of this work, with a sublethal 1 Gy acute exposure on day 15 of gestation, a dose-rate effect on brain occurs between 4.75 and 23.9 Gy/day. Brain histology is not more sensitive than brain weight to determine the lower limit of the dose-rate effect.

Conclusion

Fœtal body weights (early effect) and adult brain weights (late effect) are good parameters to estimate the dose of exposure received during intra-uterine life.

No dose-rate effect compatible with animals survival occurs with protracted irradiation during gestation.

One Gy acuteexposure in the middle of the brain organogenesis period shows a specific dose-rate effect in adults between 4.75 and 23.9 Gy/day on brain weight and histology.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

J.D. Harrison NRPB UK
D.L. Henshaw Bristol University UK

V. Publications:

Coffigny H., Fritsch P., Vernois Y., Beauvallet M., Court L., Métivier H. and Masse R.- Influence of dose-rate on the biological effects of protracted gamma irradiation during the whole gestation of rats. EULEP Newsletter, 1988, 50, 28-29.

Fritsch P., Coffigny H.,Vernois Y., Beauvallet M., Court L., Métivier H. and Masse R.- Early and late effects of protracted gamma irradiation during intra-uterine life. EULEP Newsletter, 1989, 52 26-27.

Coffigny H., Fritsch P., Beauvallet M.,Court L., Métivier H. and Masse R.- Dose-rate effect of gamma irradiation during the intra-uterine life on brain development. 22nd Annual Meeting of the European Society for Radiation Biology, Brussels, Belgium, September 11-16, 1989. Book of abstracts, 1989, 166.

Progress Report

Contract: Bi6-347 - J Sector:

Title: Dosimetry and effects of fetal irradiation from incorporated radionuclides

1	Harrison	NRPB
2	Coffigny	CEA
3	Henshaw	University of Bristol

I. Summary of Project and Global Objectives

An important aspect of the assessment of risks from incorporated radionuclides is the possibility of intakes by pregnant women and in utero exposure of the developing fetus. The overall objective of the project is to provide experimental data for the development of dosimetric models and assessment of risk. Studies will include measurements of ^{210}Po and $^{239/240}$Pu in human fetal tissues and placentae, animal studies of the biokinetics of radionuclide transfer and effects. Animal biokinetic studies will initially concentrate on comparing the uptake and distribution of Po-210, Pu-238 and Am-241 in rats and guinea pigs for different exposure conditions. The data obtained in these studies will be used, together with the human data, to develop dosimetric models. Studies of the effects of in utero irradiation will be carried out using mice, initially comparing external irradiation at different times during gestation to provide information on changes in the radiosensitivity of particular tissues and allow subsequent comparisons with internal irradiation.

A detailed investigation is underway at the University of Bristol on the distribution within the human fetus of natural alpha-radioactivity using autopsy samples obtained from various stages of development from 18 weeks to stillborn. Where possible, the samples comprised placenta, cord, fetal liver, spleen, thymus and spine. Analyses to date, using TASTRAK, show highest levels of alpha-activity, largely attributable to ^{210}Po, in the bone of the spine with values up to 2 Bq kg^{-1} and an average of 0.75 Bq kg^{-1}. Concentrations in soft tissues, including liver, and in the placenta, are about an order of magnitude lower.

About forty samples of fetal tissues and placentae from abortions at 14-19 weeks have been obtained by NRPB. The mothers' ages were from 15 to 28 years. For initial measurements of ^{239}Pu, combined samples of fetal and placental tissues were analysed. Concentrations of about 20 μBq kg^{-1} were obtained by mass spectrometry. Separate analyses of fetal and placental tissues are now in progress. Measurements of ^{210}Po, by radiochemistry, have shown concentrations to be about three orders of magnitude greater than for ^{239}Pu, with about two-thirds of the total activity in

the placenta. The ranges in concentrations measured in the total of 18 cases analysed to date were 4 - 59 mBq kg^{-1} for fetal tissues and 6 - 138 mBq kg^{-1} for placentae.

The transfer of Pu to the developing embryo and fetus is being studied using rats and guinea pigs. Plutonium-238 was administered to the animals at different stages of pregnancy and transfer of the embryo and fetus determined by autoradiography and tissue analysis. Retention in the total fetoplacental unit (FPU) was similar in the two species and increased with advancing gestation. However, distribution of ^{238}Pu within the FPU was different, with the yolk sac concentrating the greatest proportion in the rat while the placenta showed greater retention in the guinea pig. Transfer to the embryo/fetus accounted for a small proportion of total FPU activity, with greatest concentrations in early and late gestation and lowest in mid-gestation. Within the fetus at late stages, ^{238}Pu accumulated primarily in the skeleton, particularly in guinea pigs, with lower concentrations in the liver. Preliminary data on the transfer of ^{210}Po to the fetus in rats and guinea pigs show that, as for Pu, the greatest concentrations were in the yolk sac. Concentrations of ^{210}Po in the fetus were greater than for ^{238}Pu at mid-gestation but lower in late gestation with no specific uptake in liver and bone.

The animal data have been used to estimate doses to the human fetus from maternal intakes. For a chronic maternal intake of ^{239}Pu throughout pregnancy, taking account of both in utero doses and activity retained by the newborn child, the overall life-time dose to haemopoietic tissue of the offspring was estimated to be two orders of magnitude less than the equivalent maternal dose.

Studies at CEA of the effects of external radiation on in utero development are a continuation of work carried out with the support of CEC Contract B16-C-310-F. Rats were irradiated by ^{60}Co (> 23 hours day^{-1}) during the whole of gestation. Dose rates were 0.03, 0.1, 0.25, and 0.37 Gy day^{-1}. In a separate experiment, rate fetuses were given 1 Gy of acute irradiation on day 15 of gestation at dose rates of 0.86, 4.75, 23.9 and 239 Gy day^{-1}. Changes in the brain and gonads were studied on the last day of gestation or at three months of age. The thickness of the brain cortex was not significantly affected by protracted doses up to 0.37 Gy day^{-1}. After acute irradiation, agenesis of the corpus collosum was observed at dose-rates greater than 4.75 Gy day^{-1} and disruption of pyramidal cell alignment in the hippocampus was observed at 23.9 and 239 Gy day^{-1}. The weights of the ovaries and testes were reduced at the lowest protracted doses but all germ cells were killed at 0.25 and 0.37 Gy day^{-1}. For acute irradiation, the lowest dose rate was the most effective in reducing gonadal weight.

Head of Project 1: Dr Harrison

II. Objectives for the reporting period

1. To collect and analyse human fetal tissue samples and placentae.

2. To study the transfer of plutonium and polonium to the developing embryo and fetus of rats and guinea pigs.

III. Objectives for next period

1. Continue with the collection and analyses of human fetal tissues.

2. Extend animal studies of Pu transfer to consider the microdistribution of alpha-activity and dose in the yolk sac and in embryonic tissues during organogenesis; and to determine Pu transfer after administration prior to conception.

3. Continue animal studies of Po transfer and compare the transfer and distribution of the actinides Am and Np with that of Pu.

IV. Progress achieved including publications

About forty samples of fetal tissues and placentae from abortions at 14-19 weeks have been obtained by NRPB. The mothers ages were from 15 to 28 years. For initial measurements of ^{239}Pu, combined samples of fetal and placental tissues were analysed. Concentrations of about 20 $\mu Bq\ kg^{-1}$ were obtained by mass spectrometry. Separate analyses of fetal and placental tissues are now in progress. Measurements of ^{210}Po, by radiochemistry, have shown concentrations to be about three orders of magnitude greater than for ^{239}Pu, with about two-thirds of the total activity in the placenta. The ranges in concentrations measured in the total of 18 cases analysed to date were 4 - 59 mBq kg^{-1} for fetal tissues and 6 - 138 mBq kg^{-1} for placentae.

The transfer of ^{238}Pu from the maternal circulation to the developing embryo and fetus was studied in rats and guinea pigs. For administration at different stages of gestation, measurements were made after three days in rats and seven days in guinea pigs, or at birth. Transfer was greater after administration at later stages of gestation, up to a maximum of about 0.8 - 0.9% of the injected activity per fetoplacental unit (FPU) and about 0.2% per fetus in both species. The yolk sac retained up to about 80% of the total activity in the FPU in rats, compared with about 25% for the guinea-pig; retention in placental trophoblast was greater in the guinea pig. The concentrations of ^{238}Pu in the yolk sac were generally about two to three orders of magnitude greater than fetal concentrations and of the same order as in maternal liver and bone. In both species, concentrations in the embryo and fetus were greatest early in gestation, lowest around mid-organogenesis, and increased in late gestation. The fetus:mother whole-body concentration ratios in late gestation were about 0.1 and 0.05 in rats and guinea-pigs, respectively. For the guinea pig fetus in late gestation and neonates at birth, the liver accounted for about 6 - 9% of retained activity and similar values for femora indicated skeletal retention of about 60 - 90%. For administration at each stage of gestation, and particularly

at early stages, transfer of ^{238}Pu to the fetus continued throughout gestation but concentrations decreased due to fetal growth.

Studies are in progress on the transfer of ^{210}Po to the embryo and fetus of rats and guinea pigs. The results show lower fetal concentrations of Po after administration later in gestation, consistent with placental discrimination against Po transfer. Within the fetus in late gestation Po distribution appeared fairly uniform. As for Pu, concentrations in the yolk sac were considerably greater than fetal concentrations.

Animal data for the transfer of Pu to the fetus have been used to estimate doses to the human fetus from maternal intakes of ^{239}Pu, concentrating on the calculation of doses to haemopoietic tissues. The main approach adopted was to compare concentrations of Pu in fetal tissues with the corresponding maternal liver concentration. For chronic maternal intake of 1 kBq of ^{239}Pu during the year of pregnancy (1 ALI for members of the public), the in utero dose to fetal haemopoietic tissue was calculated to be about 1 μSv compared to a red bone marrow (RBM) dose to the mother of about 20 μSv in the year. The dose of 1 μSv to the fetus took account of the possibility that haemopoietic stem cells migrate from the yolk sac to embryonic liver and finally to bone marrow. On the basis, about 60% of the estimated in utero dose to haemopoietic tissue was delivered to the yolk sac and about 40% to the bone marrow. Estimating the amount of ^{239}Pu in the offspring at birth and calculating the committed dose equivalent to RBM to 70 y of age gave a value of 12 μSv compared with a maternal RBM dose of 1400 μSv.

Publications covering work of reporting period

Morgan, A, Harrison, J D and Stather, J W (1990). Doses to the human fetus from plutonium intakes during pregnancy. Radiol. Prot. Bull. 114, 10-14.

Morgan, A, Haines, J W and Harrison, J D. The incorporation of plutonium by the embryo and fetus of rats and guinea pigs. Int. J. Radiat. Biol. (in press).

Harrison, J D, Morgan, A, Haines, J W and Stather, J W. The fetal uptake of plutonium and polonium in animals and estimates of doses to humans. Proc. MRC/CEIR Forum on Radionuclides in the embryo and fetus, Nov. 1990. To be published in Int. J. Radiat. Biol.

Head of Project 2: Dr Coffigny

II. Objectives for the reporting period

The aim of this project is to estimate irradiation doses delivered to the embryo or the fetus after internal contamination by radionuclides.

To complete a study of the effect of gamma irradiation on rodent brain histology (as proposed in the last progress report for Contract B16 C-310-F); to extend studies to effects on gonadal tissues.

III. Objectives for next period

The effects of protracted neutron irradiation during the whole of gestation will be studied in rats early (at the end of gestation) and late (in three month old animals), measuring the same parameters in the brain and gonads as for gamma irradiation.

IV. Progress achieved including publications

Methodology

Rats were irradiated using ^{60}Co gamma rays (> 23 hours day^{-1}) during the whole of intra-uterine life. Dose-rates were 0.03, 0.1, 0.25 and 0.37 Gy day^{-1}. In a separate experiment, rat fetuses received 1 Gy acute irradiation on day 15 of gestation at dose-rates of 0.86, 4.75, 23.9 and 239 Gy day^{-1}. Changes in the brain and gonads were studied on the last day of gestation or at three months of age.

Results

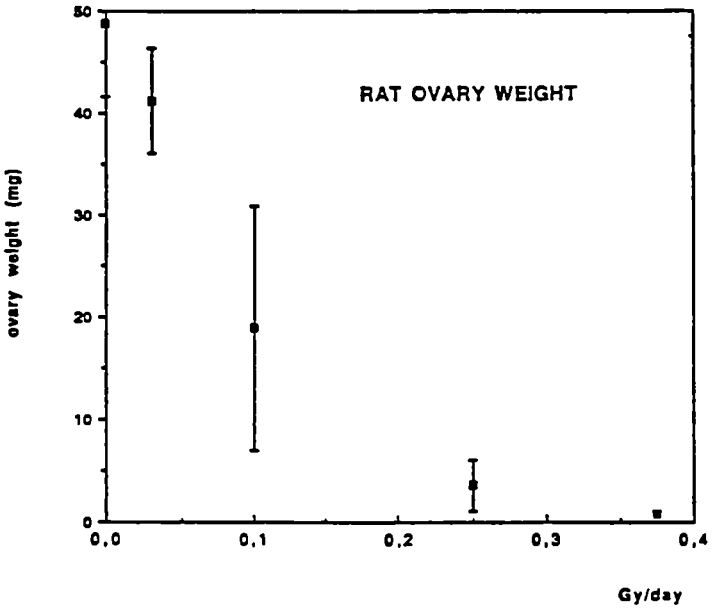
The thickness of the cortex was not significantly affected by protracted irradiation up to 0.37 Gy day^{-1}, a limit for animal survival. But with 1 Gy acute irradiation on day 15 of gestation, compatible with animal survival, higher dose-rates were investigated. Agenesis of the corpus collosum was observed with dose rates greater than 4.75 Gy day^{-1}. With 23.9 Gy day^{-1} and particularly 239 Gy day^{-1}, the hippocampus pyramidal cell alignment was disrupted near to ectopic grey matter.

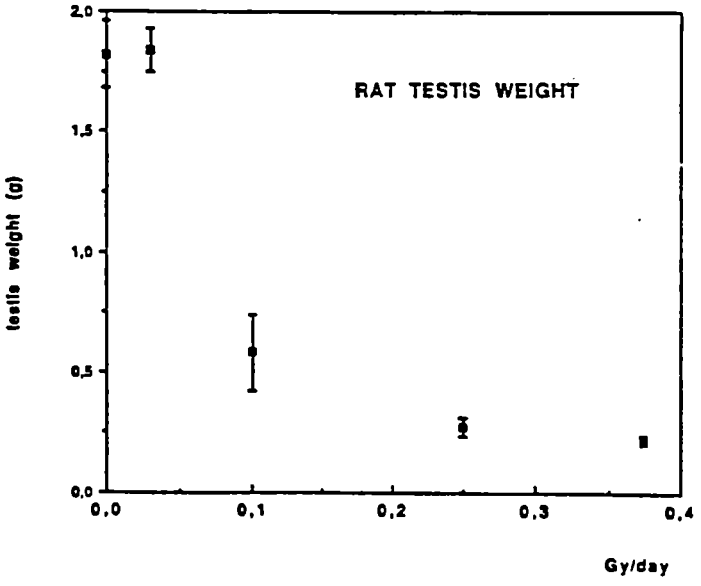
Examination of gonads showed that the weight of the ovaries and testes were decreased after as low a protected dose as 0.03 and 0.1 Gy day^{-1}, respectively, but all germ cells were killed with 0.25 and 0.37 Gy day^{-1}. After acute irradiation of 1 Gy on day 15 of gestation, a specific dose-rate effect on the testes was evident with only 0.87 Gy day^{-1}. With higher dose-rates, the weight of the testes and their histology were normal. A dose-rate of 0.87 Gy day^{-1} was also most effective in reducing the weight of the ovaries.

Discussion

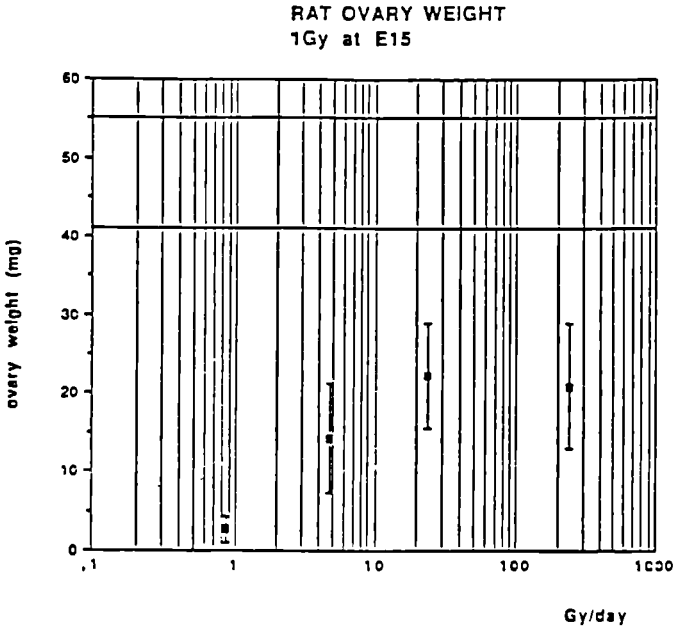
The rodent fetal gonads have been established as a more sensitive indicator of radiation damage than the fetal brain and levels of irradiation from incorporated radionuclides could be estimated up to 0.37 Gy day^{-1} protracted irradiation throughout gestation (or 0.6 Gy day^{-1} irradiation during the last third of gestation in mice). The limits of this model are animal survival for the studies of brain histology and germ cell survival for the effect on the gonads. With acute gamma irradiation of 1 Gy on day 15 of gestation, a dose-rate effect was observed in brain tissue at dose rates of 23.9 and 239 Gy day^{-1} but the greatest effect on the testes was observed with 0.87 Gy day^{-1}. However, the results obtained after acute irradiation are not applicable to protracted irradiations.

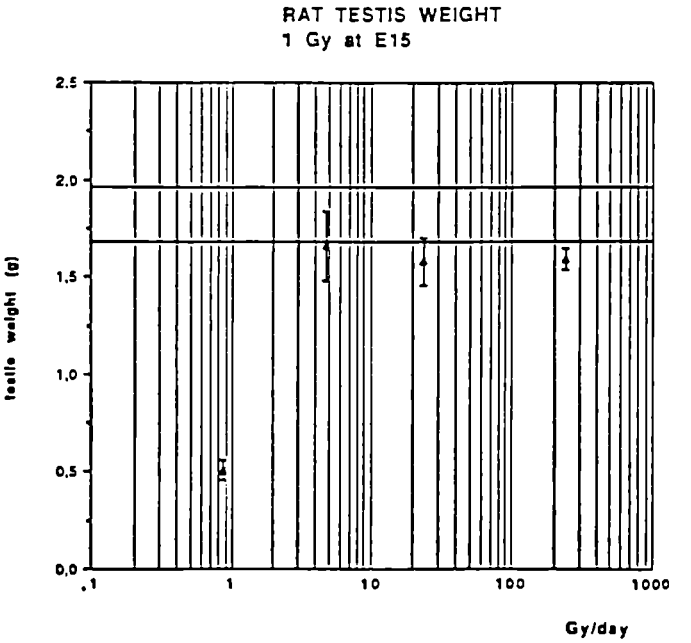
Protracted irradiation





Acute irradiation





Head of Project 3: Dr Henshaw

II. Objectives for the reporting period

The overall objective of the project is to provide human data for the distribution and retention of alpha-emitting radionuclides in fetal tissues at natural levels of exposure.

During the first period the aim was to collect and analyse human fetal tissue samples and placentae.

III. Objectives for next period

Further work will aim to determine the alpha-activity distribution throughout the fetus as a function of fetal age and to calculate the consequent alpha-dose to cells at risk with respect to their location within the fetus at various stages of development.

IV. Progress achieved including publications

A detailed investigation is underway of the distribution within the human fetus of natural alpha-radioactivity using autopsy samples obtained from various stages of fetal development from 18 weeks to stillborn. Where possible samples comprising placenta, cord, liver, spleen, thymus and spine have been obtained. These are loaded against TASTRAK (CR-39) alpha track detector and stored for at least one year. The technique allows total alpha-activity in a sample as small as one gram to be determined with a detection limit of approximately 0.01 Bq kg^{-1}. Of 45 cases currently under examination a completed analysis is available for 14 cases.

The alpha-activity in the placental/fetal unit appears to be dominated by ^{210}Po which is believed largely to be supported by ^{210}Pb. Total alpha-activity in the placenta is around 0.025 Bq kg^{-1} with levels in the liver, spleen and thymus ranging up to 0.05 Bq kg^{-1}. Values in the cord appear to be higher at around 0.1 Bq kg^{-1}. The highest activities in the fetus are in the spine where values up to 2 Bq kg^{-1} with an average of 0.75 Bq kg^{-1} are found. The activity in the spine is sufficiently high that it is possible to resolve the level of ^{226}Ra from the total alpha-activity present. Here the ratio of total alpha-activity to that from ^{226}Ra is around 40:1 in comparison with adult bone where the ratio is around 10:1. The reasons for this difference could be due to different transplacental transfer factors for ^{226}Ra and ^{210}Pb. A summary of current measurements is given in table 1.

Table 1 Average activities and dose rates to various fetal tissue organs (11 cases from 24 weeks to newborn) from long-lived α-radionuclides, preliminary data.

Tissue	Activity mBq kg^{-1}	Dose Rate µSv y^{-1}
Placenta	24	13
Umbilical Cord	97	52
Vertebrae outer	863	461
Vertebrae inner	663	354
Liver	37	20
Spleen	47	25
Thymus	17	9

III C

RISIKEN DER STRAHLENEXPOSITION UND IHRE BEWÄLTIGUNG

RISKS AND MANAGEMENT OF RADIATION PROTECTION

RISQUES ET GESTION DE L'EXPOSITION AUX RAYONNEMENTS

Progress Report

Contract: Bi6-213 **Sector: C11**
Title: The risks of radiation work: analysis of registry data

1 Stather NRPB

I. Summary of Project and Global Objectives

The UK's National Registry for Radiation Workers (NRRW) is a long-term epidemiological study of those exposed to ionising radiation in the course of their work and from whom radiation dose records are kept. Data from the NRRW are being analysed with the following aims.

(1) To determine whether there is any evidence of differences in the cause of and the age at death of workers exposed to different levels of radiation, and if any differences are found whether it seems likely that they can be attributed to radiation.

(2) If any differences are found which seem likely to be attributed to radiation, to estimate the magnitude of the risk.

(3) To estimate bounds to the possible risk for particular types of malignancy, such as leukaemia.

(4) To compare the mortality experience of radiation workers with national mortality data, and also that of other industrial groups for whom data exist.

Head of Project 1: Dr. Stather

II Objectives for the reporting period

To complete and validate the NRRW database prior to the first analysis.

To perform provisional analyses of the database.

III Objectives for next period

To publish the results of the first analysis of the NRRW.

To integrate further personal and dose information into the database.

To consider doses from internal emitters.

IV Progress achieved including publications

Considerable efforts have gone into the completion and validation of a database for the first analysis of the NRRW. Further personal and dose information has been integrated into the database. For those individuals who worked at more than one site, it has been necessary to correlate records from the different sites so as to produce consistent employment and dose histories. The first analysis of the NRRW is limited to a consideration of doses from external radiation. Doses from internal emitters are not yet available, although discussions with the participating organisations are in progress on this question. The validation exercises include cross-checks at the National Health Service Central Register (the principal source of follow-up data) and the Department of Social Security, and also data audits against the employers' central records, conducted at their sites.

The cohort of approximately 100,000 workers is predominantly (92%) male and has incurred a collective dose from external radiation in excess of 300 man Sv. Between six and seven thousand had died by the late 1980s. Provisional analyses have been carried out during the reporting period.

The analysis is in two parts. In the first part (the 'external' analysis), the mortality among radiation workers is compared with that expected on the basis of national mortality rates, taking age, sex and calendar year of death into account. The question of whether an additional correction for social class (or some surrogate of it) can be made is being examined. Standardised Mortality Ratios (SMRs) are calculated using the program PERSON YEARS, not only for individual causes of death but also - in the case of grouped causes - to look at trends with variables such as time since first exposure.

The interpretation of SMRs can be affected by the 'healthy worker effect', whereby working populations tend to be healthier than the general population. Consequently more weight is attached to the second part of the analysis (the 'internal' analysis), in which an examination is made of any trend in mortality with increasing dose, after adjusting for factors such as age, sex, calendar period and industrial classification, using the methodology of Darby and Reissland (1981). The internal analysis is carried out using the program ARFAR (At Risk For Any Reason) and ancillary software that were written at NRPB and that are being refined under the current CEC

contract BI6-347-UK(H). As a further validation exercise, the analysis programs are also being used to attempt to replicate the published results for those sub-groups of workers who have already been studied, ie. those working at the UKAEA in or before 1979 (Beral et al, 1985), at BNFL Sellafield in or before 1975 (Smith and Douglas, 1986), at AWE in or before 1982 (Beral et al, 1988), and at BNFL Chapelcross in or before 1975 (Binks et al, 1989).

Draft versions of the report describing details of the analysis have been distributed to the NRRW Data Management Group and the NRRW Advisory Committee. The full report of the first analysis will be published shortly.

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Progress Report

Contract: Bi7-053 Sector: C11
Title: Statistical results of the personal dosimetry service at the GSF

1 Regulla, Schraube GSF Neuherberg

I. Summary of Project and Global Objectives

The GSF Personal Dosimetry Service is the largest of the 5 official services in the FRG. Actually, up to 130.000 occupationally radiation exposed persons are monitored monthly by this service. The collected data basis is intended to be used for some studies on the dose distribution to the occupationally exposed population.

The first aim of the project is to indicate the trends in personal and collective doses on the available data base and to correlate it to the state structure (Bayern, Hessen, Baden-Württemberg and Schleswig-Holstein) and professional groups.

The second aim is to assess individual life time doses in view of the already implemented limits for the effective life time dose in the FRG.

Head of Project 1: Dr. Regulla, Dr.Schraube

II Objectives for the reporting period

For a ten years period, the dose data of the occupationally exposed people in 3 respectively 4 German states are analyzed with respect to the state where the workers are registered and with respect to the professional grouping. In order to support the feasibility of the 400 mSv life time dose limit, a retrospective 7 years survey is made for a subgroup of persons for which all personal data are available and which are uninterruptedly surveyed by the GSF service.

III Objectives for next period

The study will be supplemented by the year 1990 and a trial made to include data from years earlier than 1980, which are being compiled.

IV Progress achieved including publications

1. Trends in individual and collective dose

During the past 10 years the number of occuptionally exposed persons surveyed by the GSF personal dosimetry service increased steadily between 1980 to 1988 from 58.000 to 90.000 and in 1989 to 142.000 (in roughly 10.000 enterprises) because of the additional responsibility for a fourth German state. The relative portions of the main professional groups remained essentially constant.

It was observed that only between 15 and 20% of all persons received a dose of more than 0.1mSv at least once during one year. The resulting average annual dose in 1982 for these persons was for the workers in industry and research by a factor of 3 larger than for the workers in the medicine branch (fig. 1), but dropped to a factor 1.5 until 1989. Generally, the average dose decreased steadily since 1982 (with an exception in 1987) what may be attributed to an increasingly careful handling of radioactive sources and materials.

Among those working in the medicine branch, the nuclear medicine subgroup received the relatively highest average dose during the past ten years. But also here, a steadily decreasing radiation exposure is observed. In fig.2, the average annual doses over all professional groups are summarized with respect to the country in which the workers were registered. The dose distribution per country is influenced by the following

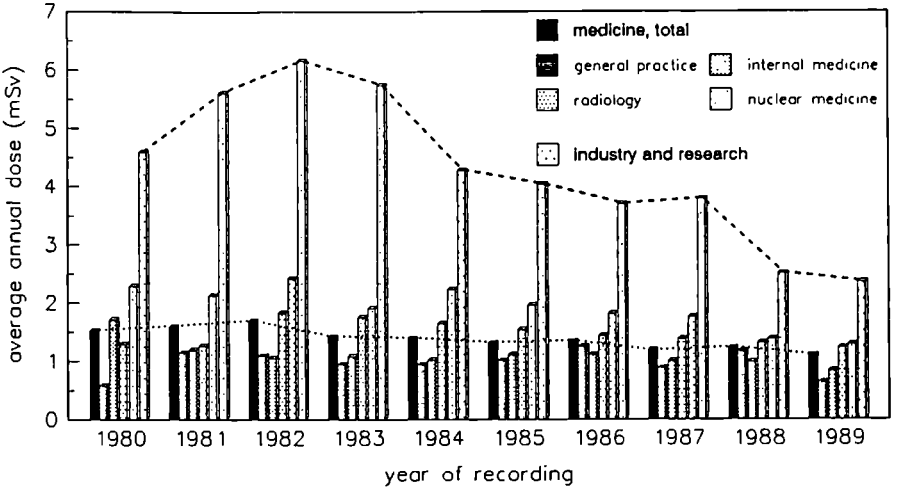

Fig.1: Average annual individual dose for all recorded values H>0.1mSv with respect to the main occupational groups "medicine" and "industry and research", and some subgroups.

circumstances: In Hessen, the comparably oldest power stations are situated and nuclear processing companies are present. In Bayern and Schleswig-Holstein there are a total of 8 power stations with a mean age of only 8 years, and no nuclear industry is present. It is observed that the pronounced differences between the countries in the early 1980s disappear in the late 1980s.

The collective doses in the German countries decreased slightly in the past 10 years and amounted in 1989 to approximately equally 15 manSv each in the countries Bayern, Hessen and Baden-Württemberg, respectively, and to 3 manSv in Schleswig-Holstein.

2. Individual life time dose

The individual life time dose in the FRG is limited to 400mSv, i.e., an average dose of 10mSv per year of occupation. The intention is to estimate whether this limit is followed taking into account the exposures in the past. The greatest problems are connected with the fact that i) by far not for all persons the date of birth is available, ii) a change of the place of occupation to another German country in which the GSF is not responsible, results in a loss of information to the GSF service on further doses received by the specific person.

Therefore, it is necessary to draw conclusions only from average statistical results during a limited observation time. A number of 18.000 persons could be identified for which dose records were available without any time gap during a

7 years time period. At 43% of the persons of this group, the total recorded 7 years dose was >=0.1mSv, at 1.1% it was >=70mSv. 76% out of the latter number came from the nuclear power and processing branch, 6% from conventional industry and

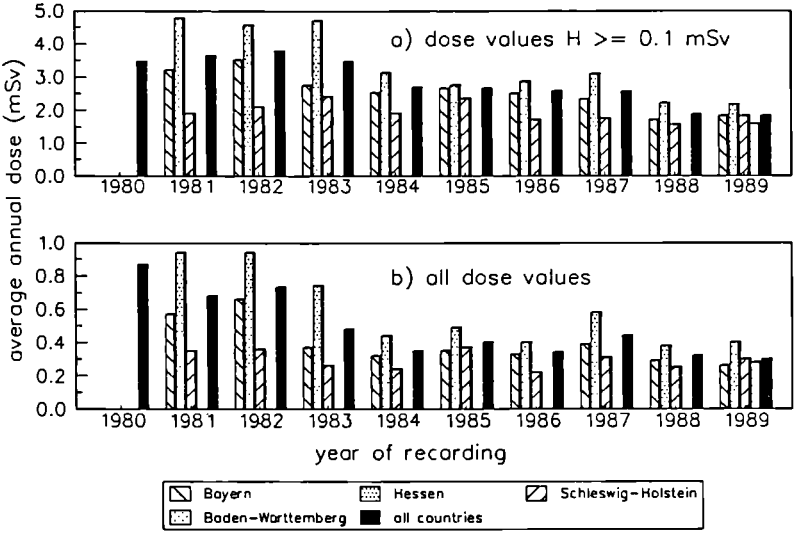

Fig.2: Average annual individual dose with respect to the country of registration

17% from medicine. An important observation was that only exceptionally the 70mSv were due to a single exposure, in the most cases, however, due to a continuous dose collection. This means that already in advance to the introduction of the life time dose limit of 400mSv, the extrapolation of the individual annual dose does not exceed this limit.

Another important observation was that over the past 10 years period the relative number of all people which received an annual dose of 10mSv and more, decreased from 2% in 1980 to 1% in 1989, where 90% were employed in industry and 10% in medicine.

Progress Report

Contract: Bi6-229 Sector: C14
Title: Health effects of chronic exposure to low dose ionizing radiation on workers of the Spanish Nuclear Energy Installations

l Artalejo CIEMAT

I. Summary of Project and Global Objectives

The objectives of the project are:

- To compare the mortality of JEN workers with the national rates
- If there were any evidence that suggested that the mortality at JEN is greater than the spanish rates, to establish whether this difference is related to exposure to ionizing radiation
- To suggest priorities for epidemiological research in the near future on the health of JEN workers

As stated in the 1989 report the project has obtained its objectives to a substantial degree. The following two sections summarize the methods employed and the results so far obtained:

Methods

The study design corresponds to a retrospective cohort study on 5303 JEN workers from 1954 to 1986. This cohort constitutes the 85% of the JEN labor force. Data collection for each worker comprises administrative and clinical data, exposure information (dosimetries) and cause specific mortality data. The statistical analysis has been carried out in two steps:

a) External comparison of the JEN mortality with the mortality of the spanish population, through standardized mortality ratios

b) Internal comparison of the cohort mortality by radiation exposure, through log-lineal models

Results

So far the results suggest that the mortality among JEN workers is generally lower than the national rates ("healthy worker effect"). However, it has been observed a significant increase in the mortality from cancer among the study cohort. This increase has not shown a radiation dose-effect relationship.

Mortality data are still lacked for a substantial part of the study cohort and it has not been possible to show, beyond reasonable doubt, a relation between excess cancer (specifically lung cancer) and radiation exposure among workers in the Spanish Nuclear Council (JEN). That is why our objectives for the next reporting periods are:

Head of Project 1: Dr. Artalejo
II Objectives for the reporting period

To develop and test a system of epidemiologic surveillance to:

a) increase the amount and quality of data available for the study cohort (reduce losses to follow-up). In this case the system will work retrospectively.

b) extend the follow-up of this cohort until December 1991. In this case the system will work prospectively.

III Objectives for the next period

The objectives for the next period will be to present the preliminary results of the analysis of the study cohort with its extended follow-up until December 1991. Also we aim to present the overall design of a cohort nested case-control study to further investigate the relationship between lung cancer and exposure to ionizing radiation and other risk factors among workers of JEN.

IV Progress achieved including publications

We are carrying out the implementation of a program of epidemiologic surveillance whose objective are:

a) to plan, implement and evaluate health interventions (primary, secondary and tertiary prevention activities) among the workers of JEN

b) to improve the amount and quality of the information on our study cohort

In particular this program of occupational surveillance identifies instances (cases) of illness, injury and/or "excessive" exposure to risk factors present in JEN. The identification of cases and the collection of information is carried out in the following manner:

a) Cases of illness and injury

The goal of the system is to obtain for every worker of JEN a history of specific medical symptoms and adverse health events (including mortality), and information on relevant demographic characteristics and personal habits that might act as independent risk factors, effect modifiers, or confounding factors in the development of occupational diseases or work- related disorders. In addition, this system will be used to obtain a relevant occupational history including current and past occupation and industry, and the potential for exposure to certain key identifiable risk work-place hazards.

a1) Primary collection of ad hoc information

We have adapted from NIOSH(1) a structured standard questionnaire to be used as part of periodic medical examinations (screening) of the JEN workers or as part of assessments of workers who participate in etiologic investigations, such as a future cohort

nested case-control study on the relationship between lung cancer and chronic low-level exposure to ionizing radiation in JEN.
The present questionnaire is a health personnel-administered questionnaire and has a modular construction that incorporates a set of core questions for use in all administrations and a set of modules to be selected and employed when needed (some modules to obtain a broad set of baseline data and some modules to be administered subsequently to elicit interval changes in status; some modules to be used to ascertain problems in specific organs; etc.)
The current questionnaire in not designed as a diagnostic tool for the clinical assessment of individual workers. The sensitivity and specificity required to make individual diagnoses exceeds that needed for an epidemiological instrument. As the questionnaire is administered by health personnel, it may (and will, in many instances)subsequently be combined with a deeper medical anamnesis and diagnostic tests (blood and urine specimens, pulmonary function tests, x-ray explorations, etc.) to confirm the presence of a health disorder.
The present modules are based on organ systems or on conditions associated with work related problems that occur relatively frequently and whose attributable risk proportion is high. This modules cover several areas: I)Demographics II)Occupational history III)Brief review of systems and part medical history IV)Personal risk factors and environmental history V) Conditions symptom-complex modules, including a)dermatologic conditions, b)mucosal irritation of the eye, nose and throat, c)respiratory disorders, d)hepatic conditions, e)renal conditions, f)musculoskeletal disorders, g)neurotoxic disorders, h)noise-induced hearing loss, i)adverse reproductive outcomes, j)work related injuries, k)work-related cardiovascular diseases and, l)work-related psychologic disorders.
The degree of development of these modules is variable. Our team has already adapted modules I to IV, and symptom modules devoted to respiratory disorders, mucosal irritation and hearing loss.
We are still evaluating different formats for the questionnaire which is already in the testing phase, being used in a pilot sample of JEN workers as part of their periodic medical examinations.

a2) Secondary collection of information from existing data sources
These sources include:

- vital statistics (mortality) compiled by the Institute Nacional de Estadística
- national statistics of work injuries and national statistics of occupational illnesses, compiled by the Ministry of Labor Relations and Social Security
- worker compensation files, collected by the Instituto Nacional de la Seguridad Social and INSALUD
- hospital discharge records in the Autonomous Region of Madrid
- the population based cancer registry of the Autonomous Region of Madrid (under development)

Presently we are still in the initial phases to match,in an individual basis, information from this data sources with identification characteristics of the workers from our study cohort.

b) Exposure to risk factors present in JEN

JEN has conducted a detailed assessment of the radiation exposure of all workers since the opening of the first installations in 1954. Results of these assessments are included in our previous report. Besides this system of information on hazards on the occupational environment and in addition to the data on risk factors for common diseases (tobacco, cholesterolemia, alcohol consumption, blood pressure, etc.) contained in the above questionnaire, it is planned to implement a program of surveillance of other hazards specific of the JEN. In particular this system should provide answers to the following questions(2):

- what exposure agents are found in the work-place?
- what is being done to control these exposures?
- which agents affect the most workers?
- where are exposed workers found?
- what health effects might these exposures produce?
- how are occupational exposures changing over time?

This project is still in its preparatory phase and we will report as it progresses.

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Progress Report

Contract: Bi6-111 Sector: C11

Title: Quantification of radiation risks, optimisation of procedures and analysis of occupational exposure

1 Jacobi GSF Neuherberg

I. Summary of Project and Global Objectives

The quantification of somatic radiation risks (i.e. the probabilities for the induction of cancer or leukemia by radiation) of low doses of ionizing radiation remains an important problem of scientific and practical interest. This is particularly true after the publication of the new results from the Radiation Effects Research Foundation in Japan, indicating significantly higher somatic radiation risks than hitherto assumed. The quantification problems are manily due to various uncertainties regarding the extrapolation of radio-epidemiological data presently available to

- future times (until all members of the collective have died),
- lower doses than 1 Gy,
- lower dose rates than acute irradiation,
- other populations than those from which risk factors were derived, and,
- for other types of radiation fields (e.g. neutrons and alpha particles).

In this project the data of the most important radio-epidemiological study, i.e. the Japanese "Life Span Study" of the atomic bomb survivors of Hiroshima and Nagasaki will be used in close co-operation with the Departments of Epidemiology and Statistics of RERF to establish estimates of the confidence regions for the somatic risk factors

- at low doses and low dose rates (by analysis of the shape of the dose-response curves),
- at future times (by employing different time extrapolation models), and
- for the contribution from neutrons (by comparing the responses in both cities).

This will be done by application of the stochastic simulation program for epidemiological data SIRIS, which has been developed by the GSF recently in the framework of the present CEC-Research Programme, in combination with the advanced statistical evaluation program AMFIT developed recently by Preston and Pierce at RERF, which is employed in the RERF-Analysis work and in the preparation of the BEIR-V-Report of the U.S. Academy of Science.

The original, individual data of the Life Span Study Data Base (differential in age at exposure, sex, city, organ doses due to photons and neutrons, and the local base line risk of mortality) will be used together with various dose-time response models to calculate with the Monte Carlo simulation-program SIRIS sets of late effects for the same population with the same radiation exposure. These "artificial" epidemiological data will be used to evaluate with AMFIT the statistical significant conclusions based on the original data, and to attempt own estimates of the regions of various somatic risk factors and their sensitivity on the underlying model assumptions.

Head of Project 1: Prof. Jacobi

II Objectives for the reporting period

To transport, implement and test in our fast Parallel-Computer the tools necessary for the study:
a) the voluminous RERF-Data set for the Life Span Study (LSS),
b) the simulation program for epidemiological data SIRIS, and
c) the statistical evaluation program AMFIT

III Objectives for next period

The two programes SIRIS and AMFIT will be used to quantify the uncertainties of analysis and inferences from the LSS with respect to various assumptions in the dose-time-effect relationships, e.g. shape at low doses, sensitivity on age at exposure and attained age, transfer of risk descriptions from the population in Hiroshima to that in Nagasaki and vice versa.

IV Progress achieved including publications

The RERF DS86 Cancer Mortality and Average Organ Transmission Factor Data Files were adapted to the parallel computing environment to be employed in this study. These files contain the cancer mortality data from 1950 to 1985 and the city- and age-at-exposure-specific transmission factors to be used for organ dose estimates. The data pertain to the same population of 75991 persons as the recent RERF Reports TR9-87, LSS 11/1 and 11/2, and have also been used for the BEIR V-Report. The data set is cross-tabulated over city, sex, age-at-exposure, time-since-exposure, and total DS-86 kerma. This input dat set is now available on the INTEL HYPERCUBE Computer to be used for modification by SIRIS.

The simulation program SIRIS has been extended to be capable to take into account differential base line risk values changing with calendar year which is an important and necessary improvement. SIRIS can now calculate stochastic epidemiological data sets for further analysis differential in sex, age-at-exposure, attained age, 25 natural and additional causes of death, using free selectable additional risk functions. The output routines have been adapted to the requirements of AMFIT.

The statistical analysis program AMFIT has been transferred to the HYPERCUBE under UNIX with the help of its author D. Preston, NIH. It is now ready for the production runs (ca. 10^4 will be necessary) to be carried out during the residual project period. Test runs have already been carried out to check the consistency of various "attained-age"-models with the original data set.

For graphical analysis of the multi-dimensional output data the program set PV-WAVE has been acquired and implemented on the SUN-Workstation used as Front-end computer for the fast Parallel-processor (32 processors INTEL860).

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SUMMARY OF CONTRACT BI6-116-UK

PROJECTS 1,2,3

Improvement of procedures to assess intakes of radionuclides from samples of airborne activity

Project 1: plate-out of radon daughter aerosols in domestic and mine environments

In large-scale surveys of radon daughter exposure in dwellings, radon gas exposure is normally measured rather than radon daughter exposure. This is because radon levels vary widely from day to day and month to month, so long-term measurements using passive detectors are required. There are no accurate passive techniques for assessing radon daughter exposure directly, so passive measurements of radon gas exposure are made, and lung doses inferred from the results. Similar passive measurements of radon gas are also made in mines, so conversion factors for mine environments are also required.

In order to convert from radon gas exposure to dose absorbed by bronchial epithelium, it is necessary to know the radon daughter equilibrium factor (F), and the unattached fraction of potential alpha energy (f_p). These parameters are affected by ventilation rates, aerosol concentration and size distribution and other factors. Under this contract a five-channel parallel diffusion battery was developed and calibrated to allow the size distributions of the radioactive aerosols to be studied. The performance of the equipment has been compared with instruments used in the USA, Belgium and Germany, and satisfactory agreement obtained.

Use of the equipment to measure the size distributions of radioactive aerosols in homes and mines is continuing. The results allow the unattached and attached fractions of the radon daughters to be distinguished. The results so far indicate that the unattached fraction of the potential alpha energy in homes is of the order of 15%, higher than has been assumed in some earlier work. The unattached fraction is affected by some domestic aerosols, in particular those due to cigarette smoking, which can significantly reduce f_p while increasing F.

Publication: Strong, J C, 1988. The size of attached and unattached radon daughters in air. Journal of Aerosol Science 19, 1327-1988.

Project 2: Deposition of aerosols in the upper respiratory tract

The lung dose delivered following inhalation of radioactive aerosols is significantly affected by deposition of aerosols in the nasal and oral airways. In the case of doses due to radon daughters, the deposition may be different depending on whether the daughters are attached to natural aerosols.

In order to estimate the deposition of unattached radon daughters in the nasal and oral airways, five plastic casts of human upper airways have been used. One was a cast from an adult at autopsy, and the others were fabricated from sheets of polymethyl methacrylate, the airway outlines having been obtained from nuclear magnetic resonance imaging (NMRI). Polonium-218 was generated by passing filtered air containing radon through a growth tube. Half of the air from the growth tube was passed through a reference filter, and half through a cast followed by an identical filter. The airflows were monitored using rotameters and vacuum gauges. Particle sizes were estimated by replacing the filters with a stainless steel mesh and filter, and measuring the deposition of particles on them. Comparison of the measured deposition with the expected deposition as a function of particle size allowed the polonium-218 aerosol size to be determined. The results of the measurements are shown in the table.

Cast	Flow, litre/m	Diameter, nm	Penetration, %
NMRI model, 2.5 year old, both nasal passages	4.9	1.25	25
	9	0.95	24
	18	0.85	28
NMRI model, 6 year old, both nasal passages	6.4	1.04	42
	9	0.95	37
NMRI model, adult A, both nasal passages	4	1.30	30
	10	0.96	37
	20	0.82	40
NMRI model, adult B, both nasal passages	4	1.30	30
	10	0.92	29
	20	0.80	29
Adult B, one passage	19	0.92	37
NMRI model, adult B, mouth	4	1.28	50
	10	0.92	31
	20	0.79	50
Moulded cast, one nasal passage	5	1.40	36
	11	1.10	35
	18	1.10	39

The data show substantial deposition of unattached radon daughters in the human nasal and oral airways, which need to be taken into account when estimating radon daughter doses to the lower regions of the respiratory tract.

Publication: J C Strong and D L Swift. Deposition of "unattached" radon daughters in models of human nasal and oral airways. Presented at the 29th Hanford Symposium, 1990, Richland, USA.

Project 3: The application of CR-39 etched-track detectors to low background counting and particle sizing of air samples

Workers exposed to long-lived alpha emitters such as plutonium or uranium are provided with personal air samplers (PAS) to monitor their intake. Activity on the filters is assessed using electronic counting equipment. The sensitivity of this technique is unsatisfactorily low, due to the low flow rate of the sampler, the short counting times used, and correction of the observed counts for counter background. The objective of this project is to assess the feasibility of an alternative technique of measuring the activity by use of alpha track registration on CR-39, which would allow very long counting times and low background levels.

A Cytoscan image analyser has been used to investigate the effects of clumping of activity in discrete particles and to plot the track density across autoradiographs. This image analyser had a disadvantage: it was very difficult to program, so that programming had to be contracted out to the analyser's manufacturers, causing severe delays when any changes were required. NRPB has now purchased a Quantimet 520 from Leica Cambridge specifically for counting etched tracks in CR-39. This image analyser provides facilities for measuring the x-y coordinates of tracks, the area, circumference, roundness, angle and various other parameters. In addition, the analyser can be programmed in a version of BASIC, so that changes can be easily and rapidly implemented by NRPB staff. The Quantimet has been used to identify tracks due to long-lived alpha emitters and plot their locations, and its ability to measure the angle of the major axis of tracks has been demonstrated. The use of this facility in identifying tracks which originated from the same active particle will continue to be explored.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-F-344-F

Commissariat à l'Energie Atomique
29-33 rue de la Fédération
F-75015 Paris

Head(s) of research team(s) [name(s) and address(es)]:

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DPT/SPIN/SEIP
Commissariat à l'Energie Atomique
Bâtiment 389
F-91191 Gif-sur-Yvette Cédex

Telephone number: (1) 6908.29.13

Title of the research contract:

Conception et réalisation d'un banc d'étalonnage de radon 222 et de ses produits de filiation à vie courte dans l'air.

List of projects:

1. Conception et réalisation d'un banc d'étalonnage de radon 222 et de ses produits de filiation à vie courte dans l'air.

Title of the project no.:

Developing a standard test bench for radon 222 and its airborne short-life decay products

Head(s) of project:

Jean CHARUAU

Scientific staff:

J. CHARUAU - M. AMMERICH - L. DREZET

I. Objectives of the project:

The aim of the project is to adapt an existing radioactive aerosol calibration installation "ICARE" at the Saclay Nuclear Research Centre (CEA), in order to provide precise activity concentrations of ^{222}Rn and its short-life decay products that may be used to calibrate measuring instruments.

ICARE is currently being used for the certification of instruments employed in measurement of artificial radioactive particulate airborne contamination. ICARE is essentially a wind tunnel in which aerosols calibrated in size and labelled with ^{137}Cs or ^{239}Pu are injected upstream of the test section.

To extend ICARE's field of application to the case of instruments employed in measurement of natural radioactivity, a new line of injection has been designed during 1989 including three standard sources of ^{222}Rn and a reference device for the activity concentration measurements of this gas.

II. Objectives for the reporting period:

The supplementary test bench equipment used to produce and measure ^{222}Rn decay products, and the instrument test chamber, are subject to a second CCE contract in 1990.

The new system will be designed for :

- producing aerosols, size and number concentration calibrated, to carry a fraction of radon daughters ; the AMAD of this fraction being representative of environmental conditions,

- measuring the three daughters volume activities, their potential alpha energies, the percentage of attached particles ; and the equilibrium factor, using a computing system and a new program,

- allowing the calibration tests of instruments either inside a chamber for the smallest ones or outside for the biggest ones, the velocity of the chamber air flow being adjustable up to 5 cm/s and the volume activity of ^{222}Rn from 4 to 4,000 Bq/m^3.

III. Progress achieved:

III.1. New ^{222}Rn injection line

The new radon gas injection line, designed under the 1989 CEC Contract (n° B16-F-332-F), includes three standard solid sources of ^{222}Rn and a container for reference measurement of radon volume activity. Hereafter is shortly described this line.

Standard solid sources of ^{222}Rn

The system uses a new patented radon source, which consists of a homogeneous solid deposit of ^{226}Ra in an acrylic felt disk impregnated with manganese oxides. The deposit is obtained from a standard solution whose atoms are fixed by the manganese oxides (via ion exchange).

Each of the three radioactive felts (^{226}Ra activities: 70, 670 and 5400 kBq) is mounted in a source-holder device. A regulated supply of filtered air flows through it continuously. All the radon produced by the radium is transferred to the sweeping air if the humidity ratio is higher than 50%. The emission factor, therefore, is 100% and constant.

Container for reference measurement of radon volume activity

The volume activity of ^{222}Rn is measured by a new patented reference device. A standard container (500 cm^3), generally used for reference measurement of radioactive gases emitting gamma rays, is inserted into the air sweeping circuit downstream from the radon sources. After the sampling process, the container is disconnected. Three hours later radon daughters are in equilibrium with ^{222}Rn.

Internally, the container is equipped with an arrangement of special metal grids creating thousands of cells on which radon daughters are deposited by the Brownian diffusion mechanism. The ^{214}Pb and ^{214}Bi activities measured by gamma spectrometry are therefore representative of the ^{222}Rn gas activity, the container and the spectrometer being calibrated with standard ^{85}Kr and ^{127}Xe gases.

Radon gas circuit and ageing unit

Figure 1 below represents the new ^{222}Rn source air circuit connected to the ageing unit. The three felts containing ^{226}Ra are swept by regulated airflowrates of 1 l/min, with a humidity ratio comprised between 80 and 95%.

The ^{222}Rn volume activities come to 8.5, 81 or 650 Bq/m^3. A system for partially discharging from 0 to 0.9 l/min of the air allows injection into the ageing volume of from 10 to 100% of the activity provided by the sources.

Therefore, this system enables the entire desired range of ^{222}Rn volume activity (about from 4 to 4,000 Bq/m^3) to be provided in the test chamber (see III.4).

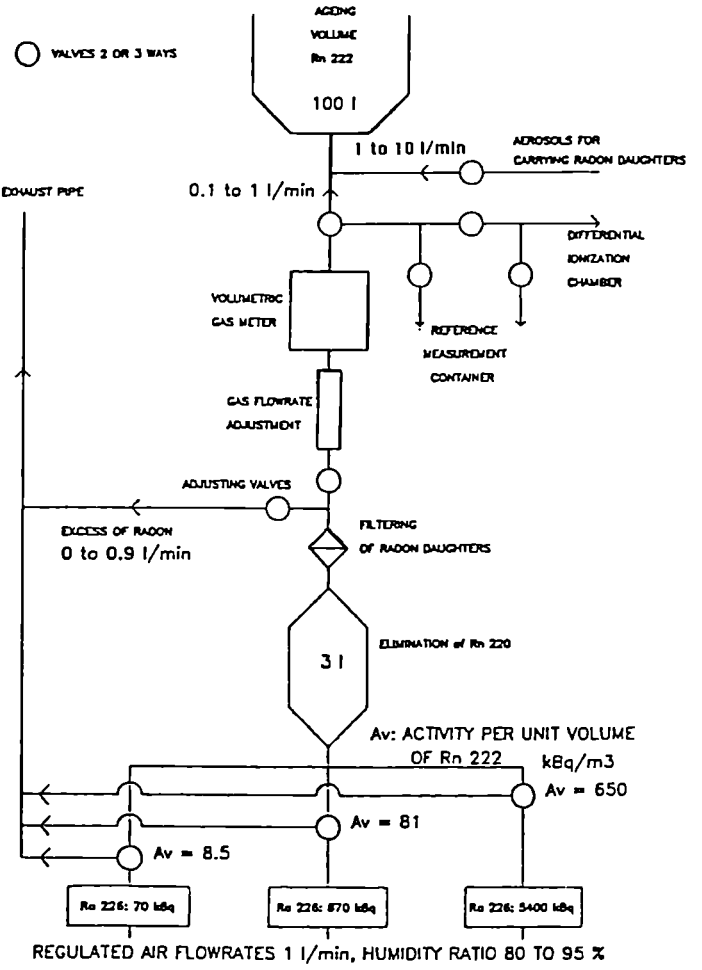

Figure 1 - The ^{222}Rn sources air circuit

III.2. Radon daughters carrier aerosol generation and measurement system

Radon daughters carrier aerosol size distribution

A pneumatic Collison type nebulizer generates CsCl particles to carry a part of the radon daughters in the ageing volume. To obtain this dry aerosol, the droplets nebulized from an aqueous solution of this salt (1.3 g/l) are passing through a diffusion drier. The heart of this device is two concentric cylinders formed by a wire screen (inner tube) and a plexiglass tube (outer tube). Silica gel fills the annulus and maintains a dry atmosphere into the inner tube ; the wet particles flowing through this tube do not come into contact with the silica gel, thus avoiding particle loss.

By brownian diffusion mechanism, a part of ^{218}Po, ^{214}Pb and ^{214}Bi atoms, formed during the residence time into the ageing volume, will be attached on CsCl particles surface. As shown on the figure 2, the attachment coefficient (β) of atoms depends on the particles diameter. Bigger is a particle, higher is the value of β. Consequently, the size distribution in activity of the carrier aerosol after attachment of radon daughters atoms is not the same that the number size distribution of inactive carrier aerosol. The Number Median Aerodynamic Diameter (NMAD) measured by a Differential Mobility Particle Sizer (DMPS manufactured by TSI) is 0.11 μm, and the geometric standard deviation is 2.0. The value of the Activity Median Aerodynamic Diameter (AMAD) is higher: the calculated value from attachment coefficient curve is 0.21 μm whereas the experimental value is 0.23 μm, with a geometric standard deviation of 1.9. If necessary, this size could be modified: it depends on the concentration of the CsCl salt in the nebulized aqueous solution. The size distribution in activity of the radon daughter's attached fraction is representative of indoor conditions /1/ and /2/.

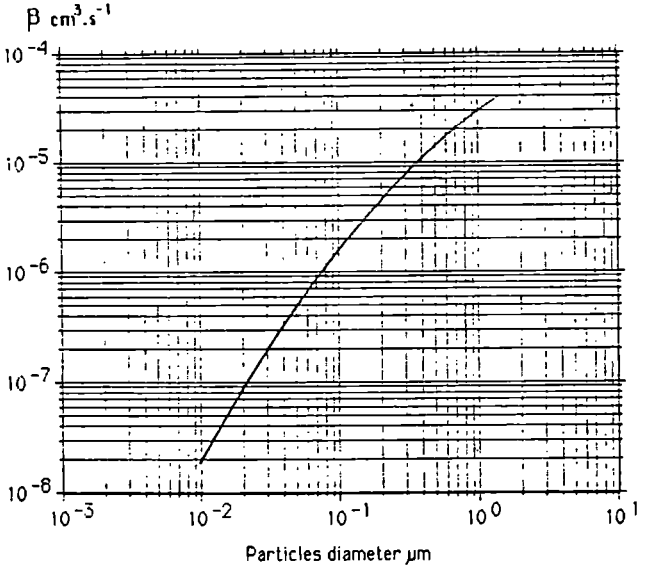

Figure 2 - Attachment coefficient β vs diameter of particles

System for adjusting aerosol concentration

As shown on figure 3 the particles concentration of the dry aerosol can be adjusted by a system filtering a part of particles in the airflow. The percentage of cleaning depends on the relative values between the pressure drop of the capillary tube through of which particles have to pass and the pressure drop of the adjustable valve and HEPA filter retaining particles contained in the derived airflow. So, the ratio between downstream and usptream aerosol concentration varies as the percentage of the airflowrate passing through the capillary tube.

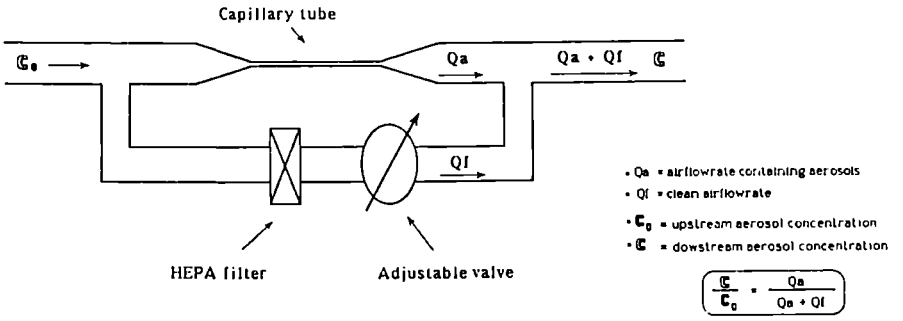

Figure 3 - System for adjusting aerosol concentration

Two identical systems are placed in series in order to obtain the required range of aerosol concentration. More, an adjustable exhaust allows the airflowrate to be varied from 1 to 10 l/min. So, the residence time of radon, its daughters and the carrier aerosol in the ageing volume (100 liters) can vary. This condition allows the equilibrium factor between radon and daughters (see III.3) to be adjusted from 0.1 to 0.6.

The number concentration of particles entering into the ageing volume is measured by a Condensation Nucleus Counter (CNC manufactured by TSI) considered as a reference device for fine particles counting. The concentration of the carrier aerosol ranges from about 10^2 to a few 10^4 particles by cm^3 of air. This range allows the radon daughters attached fraction to be adjusted from 5% to 95%.

III.3. Radon daughters radioactive characteristics computing system

Two radon daughters samplers are located between the outlet of the ageing unit and the inlet of the test chamber (see III.4), as shown on the view of radon and radon daughters unit of ICARE test bench (figure 4). The particles are simultaneously collected on Millipore membranous (0.8 μm AAWP type) during a specified time, the two airflowrates being equal. One of the filter holders is fitted with a system retaining the very fine particles (unattached fraction) of radon daughters. A lot of very small diameter tubes are included into a cylinder preceding the filter holder. The deposition rate of the unattached fraction calculated from brownian diffusion laws /3/ is more than 99% ; this very high value is due to the important developed surface. The diffusion coefficient used for the unattached fraction particles is 5.4 10^{-6} $m^2.s^{-1}$.

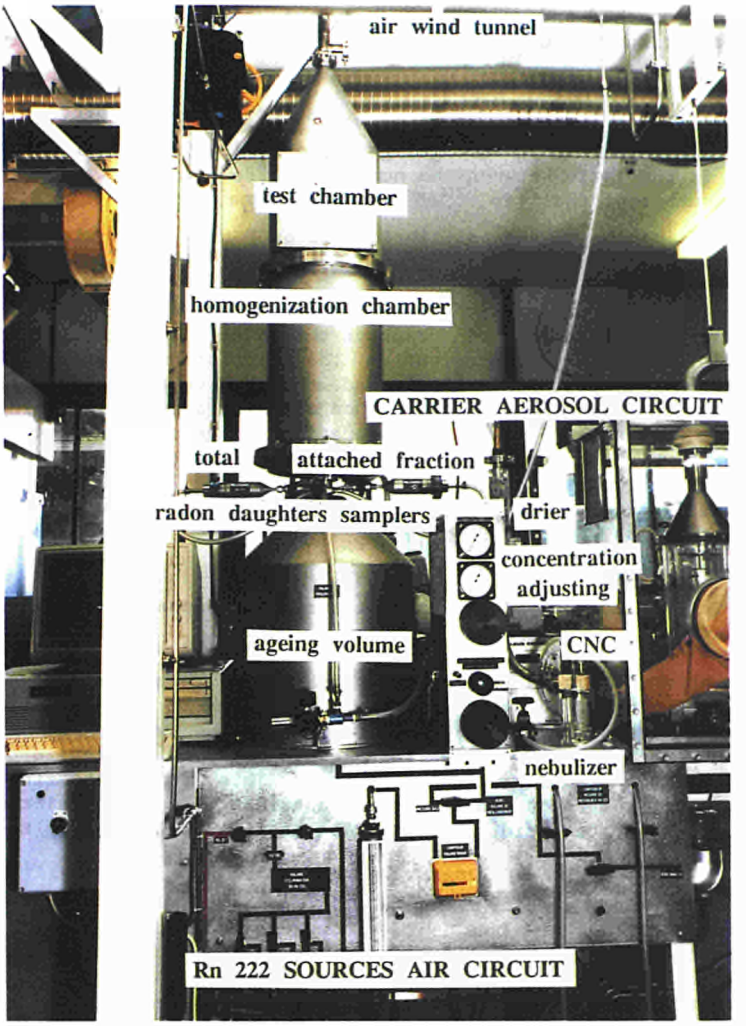

Figure 4 - View of radon and radon daughters unit of ICARE

After aerosols sampling, the deposited activities of radon daughters on the two membranous are measured simultaneously by two alpha counters (fig. 6). The both signals are analyzed by a new computing system /4/ and /5/ called DECADE (DEconvolution du Comptage Alpha des DEscendants du radon). Pulses coming out the counters are added up every second and the computer analyzed every minute their numbers related with their occuring time. These informations are compared to the theoretical variation laws of ^{218}Po, ^{214}Pb and ^{214}Bi activities. At the end of the counting time, an algorithm for data reduction gives the volume activity, potential alpha energy concentration and relative uncertainty of these values for each decay product, the equilibrium factor and the percentage of attached radon daughters.

From attached and unattached decay products volume activities, DECADE algorithm calculates the equilibrium factor by the following procedure:

- calculation of the number of atoms for each decay product,
- determination of the ageing time of radon from R value /6/ which is the ratio between the number of ^{218}Po atoms and the total number of the ^{218}Po, ^{214}Pb and ^{214}Bi atoms,
- estimation of the equilibrium factor F from the ageing time /6/ ; a polynomial function between R and F is used.

During the counting time, an estimation with its relative uncertainty is displayed every minute about the total volume activity and potential alpha energy concentration. The calculation is stopped when the wished relative uncertainty is reached. Compared to the Thomas method using fixed periods of time, our method allowing adjustable timing of sampling, waiting and calculation operations gives some advantages: adaptability to various operational conditions, better measurement sensitivity, and estimation of the relative uncertainty. Using conditions of the Thomas method we have checked that the results are the same with the both algorithms.

III.4. Test chamber of instruments measuring atmospheric natural radioactivity

As shown on fig. 4 and fig. 5, ^{222}Rn and its decay products created in the ageing unit are injected into a chamber where they are diluted by a clean air from 6 to 12 m^3/h. The dilution air is coming all around the injection tube from six calibrated holes in order to homogenize the mixing by the convection effect of circular jets. Before entering in the test chamber, the radon and daughters containing air grows quiet through a special perforated grid. The retention of fine particles due to this grid is less than 5 %.

The 0.3 m diameter cylindrical test chamber is 0.4 m height. Measurement of the volume activities homogeneity in this chamber, by 27 solid track detectors (KODAK LR115 type) shows that the values in the median and upper plans are equal even near the wall, taking into account the uncertainty of this type of detector (10%). However, in the lower plan near the grid, we have observed a mean value 15% below. The air velocity in the chamber varies from 2.5 to 5 cm/s with the chosen dilution airflowrate. For these conditions, the radon volume activity ranges between 4 and 4000 Bq/m^3. Nevertheless, to obtain the lowest values it is necessary to fit a radon adsorber device in the dilution air circuit in order to avoid any contribution of the fluctuating radon volume activity coming from the environment. A system using cooled active charcoal has been already studied in our laboratory /8/.

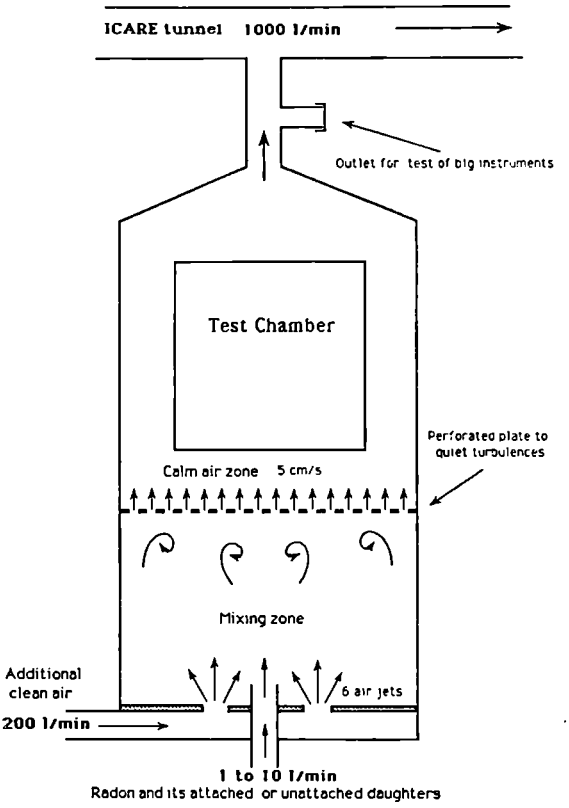

Figure 5 - Test chamber of instruments measuring ^{222}Rn and its decay products

DISCUSSION

The objectives of the project are reached. A lot of sequential and continuous measurements of radon by an ionization chamber, connected to the test chamber outlet, confirm a very good stability and reproducibility of the concentration.

Some improvements have to be done concerning the measurement of radon daughters directly into the test chamber, and the adding of a radon adsorber system in the dilution air circuit.

Figure 6 shows the new general view of the standard test bench "ICARE".

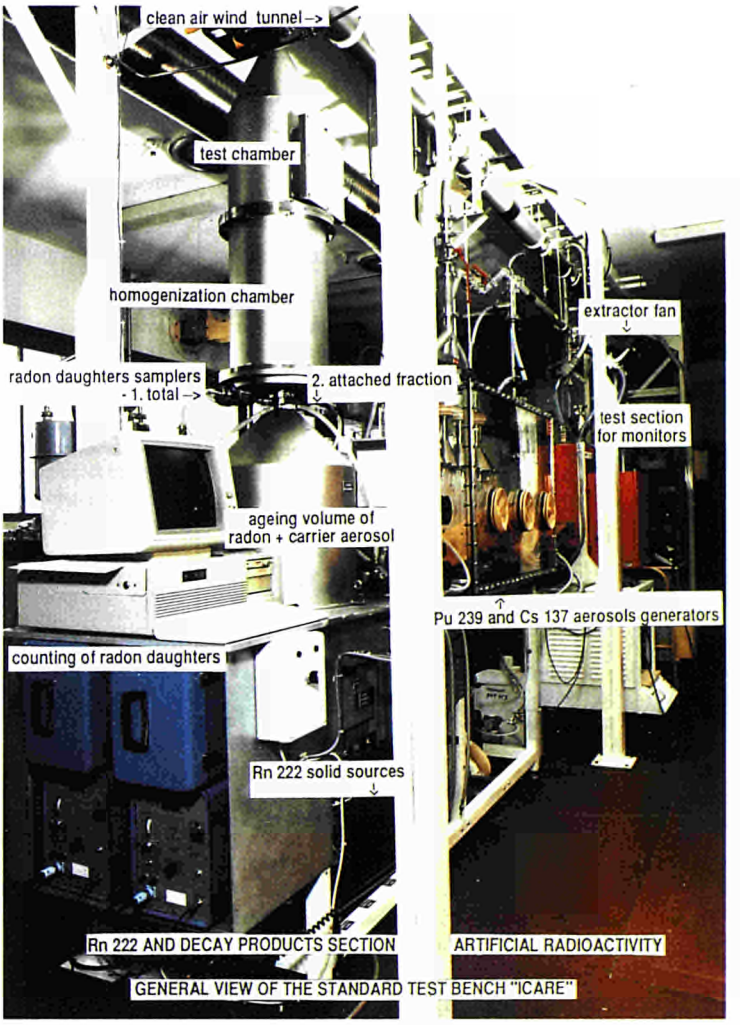

Figure 6 - General view of the standard test bench ICARE

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Etude du dépôt des particules dans les conduits. Optimisation des tubes de prélèvement des aérosols radioactifs.
Rapport CEA-R-5158, 1982

/4/ J. LE GAC
Détermination de l'activité des descendants du radon 222 à vie courte par une méthode de déconvolution temporelle.
Communication personnelle, oct. 1988

/5/ B.M. HARTLEY and M.I. HARTLEY
A new method for the determination of the activity of short half life descendants of radon.
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/6/ Séminaire sur la radioprotection des travailleurs dans les mines et usines de traitement des minerais d'uranium. Recueil des exposés, tomes I et II, Niamey, CEA/IPSN/DPT/CRPM, nov. 1986

/7/ L. DREZET
Qualification d'une nouvelle méthode de détermination de l'activité alpha des descendants du radon 222 à vie courte dans l'air.
Rapport interne CEA/IPSN/DPT/SEIP, juin 1989

/8/ L. DREZET
Contribution à l'étude et à la mise au point d'un équipement d'étalonnage des appareils de mesure du radon 222 et de ses descendants à vie courte dans l'air.
Université de Franche-Comté. UFR Sciences et Techniques.
Rapport DEA n° 1251, juin 1990

Progress Report

Contract: Bi6-347f Sector: C12

Title: Radon sources and models (NRPB Association)

1	O'Riordan	NRPB
2	De Meijer	Univ. Groningen
3	Damkjaer	Univ. Denmark - Tech.
4	Majborn	Risø National Laboratory
5	De Mets	CSTC
6	De Jong	TNO - Den Haag
7	Ball	NERC
8	Enflo	Nat. Inst. of Rad. Protec.
9	Proukakis	Univ. Athens - School of Medicine

I. Summary of Project and Global Objectives

The work of mapping radon sources on a large scale, by BGS and NRPB (UK), has been making good progress, with large numbers of measurements of radon in soil gas made and mapped, and interpretation of the results has begun. In order to characterise radon sources on individual sites, the Technical University of Denmark has been developing drilling and sampling equipment and a radon emanation measurement system.

The availability and movement of radon is being studied both by modelling and measurement. This work was assisted by a meeting held at KVI (NL) attended by all the contractors concerned in this area. The meeting allowed the contractors to discuss their work and to increase the level of cooperation. KVI (NL) has designed and is constructing laboratory equipment to allow the measurement of movement of radon in soil. A prove to measure radon, permeability and water content of soil has been designed by KVI in collaboration with the Technical University of Denmark and a prototype constructed. Riso (DK) has been testing field sites in preparation for the construction of a test structure. A site has been chosen and the structure is being designed in the light of results from modelling the movement of radon in soil. SSI (S) has instrumented two houses for continuous collection of data on radon, temperatures and pressures, and has a zone model which can be used to predict radon levels for comparison with the measurements. The model is being developed to improve its handling or air inputs and driving mechanisms. TNO (NE) has been developing and testing a model to estimate dose equivalent to the public due to soils and buildings, as a function of various soil and building parameters. The University of Athens (GR) has been

setting up facilities for radon and other measurements, training staff and starting radon measurements in homes.

CSTC (BE) has carried out trial calculations of the effectiveness of various remedial measures using a finite difference model. Measurements have also been made in a school with high radon levels, and remedial measures recommended. NRPB (UK) have measured radon levels in homes where various radon preventive or remedial measures have been installed. These have shown that some measures are unsuitable for general use, while others appear to be robust and effective.

Head of Project 1: Mr O'Riordan

II. Objectives for the reporting period

Provision of data on radon in homes for BGS. Measurement of radon in new and existing homes with various anti-radon measures.

III. Objectives for next period

Data on radon in homes will be transferred to BGS. The radon potential map developed by BGS will be tested by making further measurements in homes, and conclusions drawn. Tests of a wide range of anti-radon measures in existing and new homes will continue, with homes being retested to determine the durability of the measures.

IV. Progress achieved including publications

A method has been developed for transferring data on radon in homes in a given area to BGS in a way that preserves the detailed geographical information (for correlation with geology) without compromising the confidentiality of the radon results. To do this, a computerised list of all the grid references at which NRPB have measured radon in homes is transferred to BGS. BGS attaches a code relating to the geology of each grid reference, and returns the file. NRPB then attaches the results of radon measurements in homes for each grid reference, and delete the grid references. The file is returned to BGS with only geological codes and radon results, sorted in such a way that no individual home can be identified. This procedure has been agreed and will be implemented shortly.

About thirty homes on three building sites in a radon affected area are under investigation. The homes all have suspended concrete floors, some incorporating radon preventive measures. The measures on trial are similar to those recommended by the UK Department of Environment in areas where there is a significant risk of radon problems: a continuous membrane across the whole area of the floor, and provision for sub-floor ventilation in case this is required. Implementation of these measures has proved relatively expensive, at about £1000 per house, because of material costs and the need to use skilled labour to ensure proper installation. Measurements have shown low radon levels in homes both with and without preventive measures, due to the warm summer. Measurements are continuing through the winter to determine the effectiveness of the preventive measures.

Tests have been carried out in various homes and other buildings where radon remedial measures have been installed. In buildings with solid concrete floors, sub-floor sumps attached to appropriate extract fans have been shown to be both effective and durable within the limited time the trials have been running. Remedial measures on suspended timber floors have proved to be more problematical. Sealing the top surface of the floors has proved to be difficult and disruptive for the householder. Two types of mechanical underfloor ventilation have been tried: underpressuring the sub-floor space, and provision of a continuous flow of air across the space. The first of these has proved to be less effective in reducing radon levels and more likely to cause problems with

dampness of the floor. The second technique is providing promising results. Tests in buildings where the natural underfloor ventilation has been increased by the provision of extra vents have shown this to be unreliable in reducing radon levels.

Head of Project 2: Dr De Meijer

II. Objectives for the reporting period

Searching and hiring of personnel, designing and ordering of the radon vessel and measurement devices, to enable measurement work to commence.

III. Objectives for next period

- Calibration of the shape factor for the permeability probe;
- Introducing a pressure difference between the perforated box near the bottom of the vessel and the outside. Measuring the pressure field along the axis of the vessel and comparing the results with model calculations;
- Measuring radon concentrations in the soil as function of depth in the soil, water content and permeability of the soil and the height between the soil and lid.

The data will be analysed with models that will partly have to be developed next year. Collaboration is planned with Lyngby on the permeability probe and with BRRI in Limelette and Risö on the pressure field.

IV. Progress achieved including publications

The first step in the present programme was to design and construct equipment to measure the transport of radon in soil as function of various soil parameters. Since such experiments can not be executed under controlled conditions *in situ* it was opted to carry out the experiment in the laboratory with an optimal knowledge of parameters. As soil parameters are thought: porosity, permeability, grain size distribution, radium content and water content.

For this purpose a stainless steel vessel was designed. The dimensions of the vessel are on the one hand limited by handling and on the other hand by the properties of the soil. For example the diffusion length of radon in soil may amount to 1 m, indicating that the dimensions of the vessel should be at least 2 m to avoid boundary problems. Water content in the soil may be regulated by controlling the water level and is also influenced by the properties of the soil: eg, the capillary suction indicated by the pF-value.

The vessel is supposed to represent also a scale model of a crawl space and is therefore equipped with a lid which height can be adjusted. The lid will have ports for measuring the radon concentration and to ventilate the pseudo-crawl space.

All requirements and boundary conditions resulted in a cylindrical vessel with an inner diameter of 2 m and a height of 2 m. Around the upper one meter there will be a ring filled with water to close of the air under the lid from the air in the laboratory. In the wall of the vessel twelve ports for entering probes are foreseen. The design of the vessel has been completed, the vessel is presently under construction and will be delivered in January 1991.

To measure the value of the parameters a multi-functional probe was designed in collaboration with the Technical University of Lyngby, Denmark. This probe should enable us to measure radon concentration in the soil gas, permeability of the soil and the water content of the soil. The main problem in the beginning phase will be the determination of the shape factor of the probe. This shape factor has to be measured to derive values for the permeability. For this purpose a perforated box has been designed to be placed near the bottom of the vessel, and covering almost the complete cross section of the vessel. The vessel is filled with dry sand through which an airflow is induced via the perforated box. Using this simple geometry the permeability of the sand can be measured straight forward (without the probes). Afterwards the shape factor can be deduced by measuring the permeability with the probes. A prototype of the probe has been assembled and will be tested.

Presently the following quantities for various samples of commercially available sand are being measured: radium content, radon emanation factor, grain size distribution and pF-curve. From the values of these quantities the most suitable type of sand for the first investigation will be selected. Moreover a start has been made to prepare input for model calculations. Here a coordination has been discussed with BBRI in Limelette, Belgium and Risö, Denmark.

Head of Project 3: Dr Damkjaer

II. Objectives for the reporting period

Planning of the work. Design and testing of the small diameter drilling equipment and accessories. Design and testing of laboratory equipment for radon emanation measurements of small samples. Design and preliminary testing of permeability probe.

III. Objectives for next period

The technique for measurement of radon emanation from small soil samples (50 gram) must be reconsidered. Track etch detectors may be useful, especially when a large number of samples have to be measured.

A laboratory procedure for the calibration of the permeability probe will be developed. Field tests of the packing technique for the borehole casing will be performed and a site-mapping of the permeability will be made.

A radon detector for soil gas measurements will be developed. The detector is planned to measure radon in soil gas at the bottom of the borehole. A site-mapping of the radon concentration in soil gas will be made.

IV. Progress achieved including publications

A set of drilling tools have been made. This includes four 20 mm coarse drills with extension rods for depths down to 5 metres, and three 12 mm drills for sampling and for preparation of probe positioning. Also two soil samplers, capable of removing 25 cm^3 samples of soft soil from depths down to 4 metres have been made. In addition accessories like a 2.5 metre long chisel and a hauling tool have been made. The drills are operated with a hand held electrical machine supplied from a portable generator. Experience with the drilling tools and a borehole casing technique has been gained during the period.

A radon emanation measurement system for small samples (50 gram) of soil has been assembled and tested. The system is based on scintillation cells with renewable scintillation foils. The air volume of the total system is kept as low as 150 cm^3 in order not to dilute the radon concentration. The results achieved so far indicates that the background count rate, even with fresh scintillation foils, is too high.

A probe for gas permeability measurements in soil has been made. The probe, which has a diameter of 16 mm, is led through the borehole casing tube to the measurement position. A packing procedure designed to prevent gas leakage along the casing has been tested in the laboratory.

The necessary field instrument for the permeability probe has been assembled and preliminary laboratory and field measurements have been performed.

Head of Project 4: Dr Majborn

II. Objectives for the reporting period

During the first year of the contract time has to be spent on the preparation work required for the development of a test structure and other experimental equipment. It was necessary to select the appropriate site and also to carry out model developments required for the project.

III. Objectives for next period

During the next year the test structure will be established. Initially, the set-up will be used to study the entry of soil gas and radon into the cylinder for a series of steady-state depressurizations. The experimental results will be compared with the results of model calculations, based on a detailed mapping of the site. The latter will include permeability measurements performed by the Department of Electrophysics, the Technical University of Denmark.

IV. Progress achieved including publications

Preparatory work needed for the realization of a simple test structure at a field site has been carried out. The test structure will consist of a 40 litre, stainless-steel cylinder placed in a circular excavation of a depth of about 0.5 m and diameter of about 2.5 m. The excavation will be lined with an airtight membrane kept in position by backfill material. Soil gas can enter the cylinder through an adjustable interface in the bottom. Basically, the test structure simulates a small slab-on-grade house with a dominant crack in the centre of the floor. A small hut on top of the set-up will provide shelter for the instrumentation. The depressurization and ventilation rate of the cylinder will be electronically controlled by a mass-flow controller, thereby limiting the influence of natural driving forces. Pressures, temperatures and radon concentrations will be measured continuously in the cylinder and in a few selected locations in the soil. In addition, the barometric pressure and outdoor temperature will be measured.

Soil-gas radon concentrations at a depth of 0.5 m have been measured at a number of potential site locations at Risø National Laboratory. Finally, an area has been selected, where the test structure will be established. The soil in the area is mainly moraine clay (ie, glacial deposits composed mainly of sand, silt, clay and stones). This type of soil is typical for a large part of Denmark. Initial soil investigations in the area have been carried out by the Department of Electrophysics, the Technical University of Denmark. In addition, we have measured soil-gas radon concentrations at six locations and permeability at one location close to the planned site at depths of 0.5 - 0.7 m. The soil-gas radon concentrations were about 40 kBq/m^3 at most of the locations, and the permeability was about 5 x 10^{-12} m^2. Further permeability measurements will be made in the area.

Our initial modelling efforts have been directed towards the design of the experimental site. Analytical expressions for a linear, cylindrical, and spherical geometry have been used for estimating the effective soil-gas resistance, the depletion of soil-gas radon, and the radon entry, as functions of geometrical parameters and soil permeability. A two-dimensional, finite-difference model for

studying soil-gas transport has been developed. It has been used to examine the influence of a high-permeability layer at the interface between soil and test structure. It was found, that the incorporation of such a layer (say, 10 cm of gravel) will make the experimental situation less sensitive to possible soil in homogeneities close to the entry point.

Head of Project 5: Dr De Mets

II. Objectives for the reporting period

The aims for the reporting period were:

a) to study the possibility of using a particular three-dimensional finite difference code (usually used for solving heat transfer problems) in the framework of the radon problem, in order to design and to evaluate remedial actions against radon in buildings. This includes the following objectives:

- to set the physical hypothesis and justifications of the model,
- to search for relevant data concerning soils and materials permeabilities, leakage characteristics of floor slabs,...
- and to perform some realistic sample calculations.

b) to start field measurements allowing to achieve a better understanding of radon transport in buildings, the role of air-tightness and ventilation and to suggest and test remedial strategies. It was planned that these measurements included tracer gas and pressurization techniques, together with radon concentration measurements.

c) to better understand the expected contribution from the other participants, and to be attentive to possible collaborations with them.

III. Objectives for next period

a) The study of the possibilities of the code TRISCO in the framework of the radon problem will be continued. Special questions concerning, for example, the calculation of the gas travelling time through the soil, and some interesting consequences of the implicit linear model assumption should be analysed in detail.

We plan to perform more calculations in order to study the sensitivity of the results on factors like, for example, the soil permeabilities and/or the design and operation of the mitigation system. In particular, 2-D calculations adapted to the configurations of some of the other contractors (KVI, RISO and/or Tech. Univ. of Denmark) will be performed.

b) In relation with the on-site measurements in ITE-Libramont additional measurements will be made in order to evaluate the effects of the proposed countermeasures. Concerning the radon concentrations, a protocol for the evaluation measurements should be established: it is an open question whether these evaluation measurements should be made solely during the occupation period of the rooms or during a longer period (24 hours measurements for example). It is also planned to make detailed pressurization measurements in all the rooms. If necessary, complementary remedial actions will be proposed and evaluated.

c) A program of field measurements in different kind of buildings will probably start at the end of next year. In this context, a number of mitigation strategies will be proposed and evaluated, and calculations with the code TRISCO should be made.

d) We hope to start (end of 1991) a study concerning the possibility of using, in the framework of radon movement in buildings, a particular computer program which models indoor interzone airflows as well as pollutant concentrations.

IV. Progress achieved including publications

a) Use of the code TRISCO for the prediction of the performance of remedial actions against radon in buildings

In order to reduce the convective flow which is the principal responsible for the high indoor ^{222}Rn concentrations, several remedial actions are being developed and used in many countries. Since they don't always respond as expected, there is a need of tools helping in their design and their evaluation.

During the reporting period, the possibility of using the three-dimensional finite-difference code TRISCO for the evaluation of ^{222}Rn mitigation strategies in dwellings was considered. We have well delimited the domain of validity of the model and we have justified the following main hypothesis:

- the radon transport from soil into a dwelling occurs mainly by pressure-driven air flow
- the air flow under the slab is supposed to be laminar
- the steady-state condition is adopted.

The program we use calculates the pressure fields under the floor slab, therefore allowing the control of the ability of mitigation systems to avoid the penetration of the soil-gas into the house.

Sample calculations were done during the reporting period. These calculations concern a house without basement, with an entry route for soil-gas: the floor-wall joint. They take into account the indoor-outdoor pressure differences and the soil and materials permeabilities. A particular subslab depressurisation system was included in the calculations. Any other configuration may be defined. Because one can take into account all the details of the configuration in a very simple way, and because of the simple and useful graphical outputs (2 and 3 dimensions), the code appears to be particularly well suited for calculating pressure fields in the soil, therefore allowing practical evaluations of mitigation systems performances.

b) On-site measurements

We are doing measurements in a school (ITE-Libramont, Belgium) well known for its

high radon concentrations, as a case study. A measurement campaign was made from 13 to 28 August 1990. Data analysis was performed during the last few months.

The degree of permeability to air of the studied zone of the building was measured using the pressurization method. The air-tightness of the inner walls of the various rooms was also evaluated. Measurements of tracer gas (N_2O injected in the basement) concentrations in different rooms for different experimental conditions were made, in function of time, together with radon concentration measurements.

This first study allowed us to present some conclusions concerning the radon entry paths and the influence of a number of factors on the measured concentrations. As a consequence of these conclusions we have suggested some remedial actions to the school authorities, mainly the sealing of the cracks and joints between the floor and the walls, and the installation of mechanical ventilation systems producing an over-pressure in the rooms and improving the global ventilation. The possibility of subfloor ventilation is not considered for the moment, because of practical reasons.

c) CEC contractor's coordination meeting (KVI Groningen)

The contractor's coordinating meeting at KVI Groningen (25-27 November 1990) was fruittul concerning the understanding of the contribution of the other participants and the potential collaborations.

As a consequence of the discussions and exchange of informations with some of the other contractors, CSTC will make calculations (partly similar to those presented in a)) adapted to their experimental configurations.

Publications covering work of reporting period

a) P Cohilis, P Wouters, D L'Heureux, "Prediction of the performance of various strategies of subfloor ventilation as remedial action for radon problems", 11th AIVC-Conference, "Ventilation System Performance", Belgirate, Lake Maggoire, Italy, 18-21 September 1990.

b) P Cohilis, P Wouters, J Verheyden, L Vandaele, R Bossicard, D L'Heureux, P Voordecker, "A case-study concerning radon problems in schools: the ITE in Libramont-Belgium (13-28 August campaign)", presented at the CEC Contractor's Coordination Meeting at KVI Groningen, The Netherlands, 25-27 November 1990.

Head of Project 6: Dr De Jong

II. Objectives for the reporting period

To carry out modelling of radon entry indoors into dwellings by taking account of a number of varying identified factors.

III. Objectives for next period

The set up of the test structure has been delayed some what due to a reorganization within TNO. The coming period will be used to perform the validation measurements and to determine the effect of countermeasures.

IV. Progress achieved including publications

During the reporting period, a model was developed which estimates the indoors dose equivalent due to inhalation and external irradiation. The model takes the following radon sources into account:

- pressure driven radon transport via the soil into the underfloor space and dwelling
- diffusion of soil gas into the underfloor space and successive transport into the dwelling
- exhalation of radon from building material

To calculate the respective contributions, the numeric values of a number of input parameters are fed into the PC. These parameters relate to:

- soil (ao porosity, emanation factor, Ra-226 content)
- weather (temperature gradient inside/outside, wind direction and strength)
- location (watertable, geometry factors)
- building materials (ao surfaces, thickness, emanation factor)
- inhabitants (ventilation, residence time)

Performing the calculation, using typical parameter values encountered in the Netherlands, radiation doses are obtained which are in the expected ranges. There are plans for a further validation.

Head of Project 7: Dr Ball

II. Objectives for the reporting period

Development of Radon Potential Mapping Techniques. Investigations of the use that existing geological and geochemical data bases; new measurements of soil and bedrock radioelement concentrations; and the determination of soil gas radon levels, may have to contribute to the efficient recognition of areas of high radon values in houses.

The first field area is of some 400 square kilometres (the Chapel an le Frith sheet 99 of the British Geological Survey). Using the techniques outlined above a field survey of the radioelement geochemistry of the area was to be undertaken and a provisional map showing the distribution of the zones of high radioelement concentrations produced.

III. Objectives for next period

Starting from April 1991, it is proposed to undertake a similar survey of an area in south west England of contrasting geology. Here the area is underlain by uraniferous granite intruded into volcanic rocks and mudstones. There are also fault bound basins filled with conglomerates and sandstones. The area is structurally more complex with very large faults which intersect most rock types and there are uranium (pitchblende) veins.

IV. Progress achieved including publications

The field investigations have been carried out. The area is underlain by Carboniferous rocks ranging from limestones (the oldest) through to mudstones and sandstones. Intrusions of basic igneous rocks also occur which are mainly in the limestones. Uranium mineralisation in the limestone was known, and exists as collophane (phosphatic) modules, but also occurs as both collophane and uraninits in some of the mineral veins. Further uranium concentrations have been identified which would give rise to high radon emanation. These include disseminated uranium minerals in the limestone (not yet identified but probably collophane), and which give wholerock uranium concentrations of about 5-25 ppm. Other occurrences result in moderate uranium levels in karst infill pockets, both pre-dating and contemporaneous with glaciation. High uranium concentration in mudstones, especially in marine incursions, give rise to only moderate radon emanations owing to the lower permeabilities in mudstones compared to limestones.

Survey methods has involved the measurements of soil gas radon and thoron, γ spectrometric determinations of Bi-214, Tl-208 and K-40 photopeak intensity in soils and rocks and have included the determination of total U and labile Ra. Analysis of the data shows that most of the variation in total γ activity can be attributed to variation in uranium concentration in rocks and soils. Statistical analysis indicates that there is a highly significant difference in the radon values in soil gas, in relation to rock types, so that the geological map provides a useful first indication of radon in soil gas distributions. This work was undertaken in the early part of the year. During the remaining period attention has concentrated on establishing the validity of some of the tentative conclusions reached. Repeated traverses covering an area of contrasting geology, land use and

rock permeability shows an identify in radon distribution patterns for different seasons. Laboratory studies for the determination of emanation coefficients of soils are underway and data will be compared with studies of carrier gas concentrations already undertaken.

A provisional soil gas Radon Concentration map has been produced. Early data indicates that this provides a good indication of areas in which houses with radon values above the action level may be found.

Head of Project 8: Dr Enflo

II. Objectives for the reporting period

The initial, two-year phase of this project will concentrate on data collected in Sweden. During the reporting period we have addressed the following objectives. 1) Begin data collection in Sweden by finding and securing use of two test homes, prepare and install instrument packages and begin continuous monitoring of radon and other environmental parameters. 2) Begin experiments using tracer gases to determine radon source strengths into the homes and airflows within the homes. 3) Begin development of simple and approximate theoretical models of radon entry which treat wind and temperature effects as mechanisms driving radon entry separately.

III. Objectives for next period

a) Continue collecting, cataloguing, and analyzing data using the two test homes.
b) Develop a model for indoor interzone airflows to be implemented in the radon flow model.
c) Continue experiments with tracer gases to determine radon source strengths into the homes and airflows within the homes, similar to those mentioned above, during a different season and weather conditions.
d) Continue model development and application, including collaboration with other contractors in the CEC/NRPB association agreement.

IV. Progress achieved including publications

Our research focuses on understanding the behaviour of some basic parameters associated with radon entry and movement indoors. The quantity of main interest is the amount of air infiltrating a dwelling from the radon-containing soil gas versus the relatively radon-free outdoor air. We hope to understand how the amount of air infiltrating a dwelling from these two different pathways changes with relation to each other, with time, and with environmental driving forces such as temperatures inside and outside the dwelling and the wind. We are using both theoretical modelling and measurements in real houses to obtain a better understanding of these processes. The following explains briefly our progress since February, 1990.

During the past year we have instrumented two houses for collecting continuous data, which includes environmental temperatures in a variety of locations indoors and outdoors, pressure differences across the building shell in a variety of locations, and radon gas concentrations in different indoor or subfloor zones. The data are recorded electronically and hourly averaged data are stored on a computer located in the house. The two houses both have indoor radon concentrations which average between 100-200 Bq/m^3 in the living level and the source of the radon is the soil. The first house, (labelled 901), was instrumented last March and data collection began at that time. The substructure of this home consists of a basement with two attached crawlspaces, and a single floor living level above the substructure. This house will most likely be the more difficult to understand of the two, because of its more complicated substructure. The second house, (labelled 902), was instrumented in October and data collection began Nov 1. The structure of 902 is rather straightforward, consisting of a rectangular two-storied house on top of a

small crawlspace on top of the ground. We hope the simplicity of the structure will be useful in our modelling efforts, which are described next.

We currently have a working radon flow model which takes as input time-varying measured airflows and predicts the time-varying radon concentrations in each zone specified indoors. Our modelling efforts have been concentrated in two areas. The first has been to develop a simple formulism for air infiltration into a dwelling which calculates the air infiltrating from the soil gas separately from the air infiltrating from the outdoor air. Also, the mechanisms driving air infiltration are limited to temperature differences between the indoors and the outdoors and to wind, and these mechanisms are treated as separately as possible. The goal is to see how simple we can make an infiltration model and retain enough of the physics to learn something from the model. The simple formulism will be checked using data collected at house 902, and using the more complicated flow model. The second effort has been to begin to develop or obtain a procedure for calculating airflows between zones for use in the radon flow model. In previous research the airflows have been measured using multi-tracer gas techniques which are expensive and not always easily available or necessarily reliable. We have completed some preliminary tracer gas measurements in house 901 which give total infiltration into different zones, but not interzone flows.

Publications covering work of reporting period

Radon Dynamics in Swedish Dwellings: A Status Report. The paper is due at the end of January 1991, and is due to be presented at the USEPA 1991 International Symposium on Radon and Radon Reduction Technology in April, 1991.

Head of Project 9: Prof Proukakis

II. Objectives for the reporting period

1. Design and plan the experimental programme which will lead to the relationship between radon sources in the substrate soil and indoor radon concentration in Greece.

2. Technology transfer as regards the techniques of alpha track detector plastics. Training of a physicist in such techniques.

3. Establish an alpha track detector measuring capability in Greece.

4. Carry-out the radon measurements and analyse the results.

5. Gamma spectroscopic analysis of surface soil samples, employing the high resolution Ge detector set-ups of NTUA.

III. Objectives for next period

1. Bring to completion the establishment of the alpha track detector measuring capability in Greece. Quality assurance of the radon data by intercalibrations and intercomparisons between the collaborating laboratories.

2. Carry-out the radon measurements and analyse the results.

3. Gamma spectroscopic analysis of surface soil samples, employing the high resolution Ge detector set-ups of NTUA.

4. Evaluate the results and investigate the relationship between ^{226}Ra concentration in the substrate soil and indoor radon concentration.

IV. Progress achieved including publications

1. Following a thorough survey of:

   - the available published literature,
   - the above mentioned pilot survey, and
   - unpublished results of a research about the natural radioactivity content of greek soils, conducted by the Nuclear Engineering Section of NTUA at 717 sampling locations over the country, which will be concluded after the examination of another 750 locations,

   ten sampling sites of particular interest (highly above or lower than average expected radon concentration) have been primarily selected to start this research. The sampling procedure and analysis of both track detectors and soil samples has been determined so that to limit

as far as possible random errors. In some of the dwellings to be monitored greek and american (Terradex) radon detectors will be installed together in order to make field intercomparisons of both types and ascertain the method's quality assurance.

2. A young physicist, Mrs X Efstratiou, has spent fifteen days at the Natural Radiation Laboratory of the University College Dublin, in order to be trained in techniques based on alpha track plastics.

3. After coming back to Greece Mrs Efstratiou started setting-up the relevant experimental facility, assisted by Dipl. Eng. Mr M Anagnostakis who is also employed in the project. Until now two CR-39 plastic sheets, sufficient for the production of about 300 22 mm square detectors, have been ordered from England and have just arrived in Greece. It is expected that in two months time the first greek detectors will be ready to be deployed. Furthermore, the chemical etching procedure has been developed and successfully tested. Finally, appropriate orders have been put abroad for the supply of a trinocular eyepiece tube, an interface unit and a video-camera which will allow an available microscope to properly analyse the alpha-particle tracks of the chemically etched plastic detectors. The delay with which equipment ordered abroad arrives in Greece, due to currency and customs constraints, must be pointed-out.

4. At one of the sites with particular interest (Arnaia in Northern Greece) 10 Terradex detectors have already been deployed. Greek detectors will be immediately installed, as soon as they will be available, simultaneously at the majority of the sites selected.

5. Twenty-five soil samples collected at the surrounding of the monitored sites in Arnaia, have already been prepared for their gamma spectrographic analysis. Moreover, 15 of them have already been analysed and they all present ^{226}Ra contents significantly higher (> 40 Bq/kg) than the average value of greek soils (mean value 26 Bq/kg, std. dev. 19 Bq/kg, sample size 717) according to the NTUA results.

Progress report

Contract: Bi6-114 Sector: C12

Title: ASSESSMENT AND MANAGEMENT OF RADIATION EXPOSURE AND RISKS FROM NATURAL AND MAN-MADE RADIATION SOURCES

1. Summary of Project and Global Objectives

This is a continuation of a former contract, with E.C. support only to the project concerning natural radioactivity and emphasis to the indoor radon problems and related studies. Therefore, the original title does not represent exactly the current activities.

The general target of the Project is to improve the knowledge on the internal exposure of the Greek population to the decay products of ^{222}Rn in air and to provide methodological support to the Greek authorities in the implementation of any future measures related to the control and reduction of this exposure.

A basic objective related to this target is the introduction of the track-etch detector technology in the Environmental Radioactivity Laboratory for determination of the integrated exposure to ^{222}Rn indoors. This refers to the whole circle of the method, in order to enable fully independent indoor radon studies and surveys.

A second objective is to improve the knowledge about the sources of radon in Greece, which includes:

1. Continuation of the survey of natural radioactivity in the Greek soils. This will provide information on the main source of radon - ^{226}Ra in the soil, and will point out the regions, where most probably enhanced indoor radon concentrations could occur. In addition, this survey will result in external dose rate mapping of the country, by use of the complete set of analysis data, which include a sufficient number of radionuclides of the ^{238}U and ^{232}Th series, as well as ^{40}K.

2. Development of methodologies and construction of devices for field and laboratory measurements of the exhalation rate of radon (from soil and from building materials respectively).

3. Development of methodology and construction of device for field determination of the "radon availability" in the soil, which can be defined as the product of the soil-gas concentration of radon and the soil permeability. This type of measurements is expected to provide more reliable indication of the potentially "hot spot" indoor radon regions, as compared with the data on ^{226}Ra in soil.

Head of Project: Dr. P. Kritidis

II. Objectives for the reporting period

1. To complete the methodology work related to the introduction of the track-etch detector technology for determination of the integrated concentration of radon in air and to prepare a pilot indoor radon survey in the region of Athens.

2. To complete the first phase of the survey of natural radioactivity in the Greek soils.

3. To complete the construction and calibration of a laboratory device for determination of the exhalation rate of radon from samples of building materials.

III. Objectives for next period

1. To complete the pilot indoor radon survey in the region of Athens and to utilise the experience related to its implementation for the organisation of larger survey, covering the whole country.

2. To analyse the results of the soil radioactivity survey with respect to the identification of regions of interest for indoor radon survey, as well as with respect to the external dose rate mapping of the country.

3. To start a study of the natural radioactivity and the radon exhalation potential of the building materials used in the 3.5 millions Athens region.

4. To start methodological and experimental work related to the determination of the "radon availability" in the soil.

IV. Progress achieved

1. The work related to the introduction of the track-etch detector methodology included:

1.1. The development of a user-friendly method for manual determination of the track density, based on the magnified projection of the etched surface (LR-115 type) on a 40x40 cm semi-transparent screen, after suitable blue-colour filtering and the "quasi-linear" track counting in 1x40 cm randomly selected bands, which minimises the double-count, skip-count and subjective decision effects.

1.2. The study of the personal variations of the "counting efficiency" and the introduction of personal "operator efficiency" coefficients to correct for these variations.

1.3. The construction of a small calibration chamber, suitable for simultaneous exposure of 8 measuring pots or several tens of unprotected films.

1.4. The determination of optimal etching conditions (duration, etching solution density and temperature etc.) in order to minimise the low limit of detection and to achieve maximum repeatability of the results. The sensitivity achieved is 1.5 counted tracks/cm^2 for exposure of 1 Bq month m^{-3}, while the low limit of detection (2σ of the background) is below 10 Bq month m^{-3}.

1.5. The selection of suitable, light-tight metal pots for housing the detector films, to avoid the effect of inhomogeneous deposition of the short-lived decay products due to static electricity and the contrast degradation in the case of prolonged film exposure to light.

The results of this methodology work have been reported in (1), and also in two radon contractors meetings organised during 1990 by the Radiation Protection Program of the E.C.

2. The first phase of the survey of the natural radioactivity of the Greek soils has been completed. About 600 samples from the whole country have been analysed, after appropriate treatment, by use of high-resolution gamma-spectrometry. The specific activities of the major gamma-emitting radionuclides of the uranium and thorium series, as well as of ^{40}K have been determined.

Paired measurements of the external exposition rate and the soil radioactivity have been made in several tens of selected areas with homogeneous soil content and "2π" geometry of the surface, in order to test the validity of the theoretical relations used to calculate the external dose rate as a function of the specific activities of the soil radionuclides.

A statistical analysis of the results is in progress, in order to determine the regional averages and variations, as well as the country area- and population- weighted averages.

The results of these studies have been reported in (2-4) and constitute the basic material of the Thesis (5). A part of these results has been submitted to the NRPB group preparing the "Natural Radiation Atlas of Europe".

3. A new version of laboratory device for determination of the exhalation rate of radon has been developed and calibrated. Determination of exhalation rates down to 10^{-5} Bq s^{-1} is possible from samples of volume up to 4 l. The device can also be used as alternative calibration chamber for track-etch detectors. The sensitivity of the system allows to test various materials, pots etc. for radon leakages. This is especially important for the correct gamma-spectrometry determination of ^{226}Ra by use of the radon decay products photopeaks.

Publications

1. Kamenopoulou V. and Kritidis P., "A methodology for determination of radon concentrations in the air of Greek houses with track detectors". Proc. I Nation. Congr. of Radiation Protection, Athens, 24-26.10.1990 (in press, in Greek).

2. Probonas M. and Kritidis P., "A study of the natural radioactivity of the Greek soils". Proc. I Nation. Congr. of Radiation Protection, Athens, 24-26.10.1990 (in press, in Greek).

3. Kritidis P., "Ionising radiations in the environment - quantities and variations". Proc. I Nation. Congr. of Radiation Protection, Athens, 24-26.10.1990 (in press, in Greek).

4. Florou H. and Kritidis P., "Natural radioactivity in environmental samples from an island of volcanic origin". "Marine Pollution Bulletin" (in press).

5. Probonas M. "The exposure of the Greek population to gamma-rays of terrestrial origin", Thesis (submitted to the Medical School of Athens University).

Contract: Bi6-314 **Sector: C12**
Title: Natural exposure from radon and radon progeny in Spanish houses. II Part.

1 Quindós Poncela Universidad de Santander

I. Summary of Project and Global Objectives

The measurement of radon levels in Spanish houses started in October, 1988 within the CEC Contract BI6-0314-E entitled "Natural exposure from radon and radon progeny in Spanish houses" and was prolonged as II Part, until December,1991.The global objectives of the Project are:

a.- Determination of radon gas levels in Spain to evaluate the actual status relative to this field.

b.- Selection of areas of Spain with high radon levels in houses.

c.- Measurement of radioactivity in soils and building materials in order to correlate them with the presence of radon in houses.

d.- Study of the relationship between the measurements of passive dosemeters and an instantaneous method employing modified Lucas cells developed in our laboratory.

e.- Evaluation of the external dose from the radium, thorium and potasium content in soils and building materials.

f.- Study of the relationship between the external dose derived from the radioactivity of soils and building materials and the experimental results from a portable monitor.

g.- Correlate the geographical distribution of lung cancer in Spain with the presence of radon in the houses.

h.- Study of sources of radon in houses in selected areas with special emphasis in soils, building materials and water through the measurement of exhalation rate from soils and building materials and the evaluation of radon in water.

i.- Measurement of radon progeny using a grab sampling method, the WL and the equilibrium factor in a group of houses of selected areas in Spain.

Head of Project 1: Dr. Quindós Poncela

II Objectives for the reporting period

d.- Study of the relationship between the measurements of radon using passive dosemeters and an instantaneous method employing modified Lucas cells developed in our laboratory.

e.- Evaluation of the external dose from the radium, thorium and potasium content in soils and building materials.

f.- Study of the relationship between the external dose derived from the radioactivity of soils and building materials and the experimental results from a portable monitor.

g.- Correlate the geographical distribution of lung cancer in Spain with the presence of radon in the houses.

III Objectives for next period

h.- Study of sources of radon in houses in selected areas with special emphasis in soils, building materials and water through the measurement of exhalation rate from soils and building materials and the evaluation of radon in water.

i.- Measurement of radon progeny using a grab sampling method, the WL and the equilibrium factor in a group of houses of selected areas in Spain.

IV Progress achieved including publications

At the present moment we have just finished satisfactory the a), b) and c) objectives covering more than 2,300 individual measurements of radon in spanish houses. A description of the results obtained from these measurements has been reported in a previous work and are included in the publication section.

In this progress report we have developed the c), d) and e) objectives.Taking into account the seasonal and diurnal variation of the radon concentration and in order to approach our instantaneous measurements with the cells to time averaged values we have developed our survey during the winter period and collected the sample in the first hours in the morning, that means under restrictions.In these conditions comparing the data derived from instantaneous and integrated measurements, the latter carried out employing passive etched tracks detectors from Terradex, USA and NRPB, UK in the same houses, no significant differences were found between the results obtained by both two techniques.

In a first phase of the survey, we have identified the areas in the country with high radon levels in the houses, preferently located at the west and northwest of Spain.In a second phase, during the winter of 1989 we have placed in 94 houses of these areas passive integrating detectors from the

two above manufacturers in order to evaluate indoor radon time averaged concentrations.
After an exposure period of 3-4 months, the detectors were recovered and shipped to the manufacturer for analysis. At the time of placing and removal ofthe detectors, we have also taken grab samples using the technique described above. From these last measurements we have derived the average of the two values found as result to be compared with the obtained from the integrated measurements.
The three distributions of indoor radon concentrations obtained by using the grab sampling method and the two types of passive integrating radon detectors mentioned above are approximately log-normal distributed and,then, geometric means and standard deviations were used to compare surveys.Although the geometric mean concentrations derived from the grab-sampling measurements are slightly higher than the integrated values, results of t-test and correlations between data for the three distributions indicate no significant differences between them ($p<0.05$).
We have also studied the percentages of houses for different intervals of indoor radon concentration for the three distributions, taking as limits of these intervals the recommended action levels published by several countries.The conclusion was that the percentages of houses in which remedial action is necessary are the same for the three distributions, what emphasize the coincidence of the results from the three techniques.Nevertheless, the size of the interval for the lowest indoor radon concentrations (0-55 $Bq.m^{-3}$) obtained by using the grab sampling method is appreciably smaller than for the passive integrating detectors.This fact, of course, is related with the more sensitivity in the radon detection of these latter detectors as compared with the instantaneous measurements from the cells.A more complete description is shown in a paper to be published in Health Physics.
Concerning the e) objective, more than 700 soil samples were collected along the total surface of Spain have been measured by gamma spectrometry.In order to validate our results we are now coordinating an international exercise of intercomparison which includes 15 laboratories, some of them included in the CEC Radiation Protection Programme.The results of this exercise are now in processing and will be published in a close future. We have available then a map of radioactive contents in Spanish soils.This map with the inclusion of more than 1,000 individual measurements of external gamma radiation employing a portable monitor is part of our collaboration with Dr. B.M.R.Green from NRPB,UK, in the the Atlas of ionising radiation from natural sources in Europe.Specific data about the region of Cantabria have been accepted to be published and an

internal report is ready in order show the relationship between the external dose derived from the radioactivity of soils and the experimental results from the portable monitor measuring in the same place in developing of the objective f).
The objective g) is almost finished from a general point of view.A paper included to be published next summer entitled Radon and lung cancer in Spain, shows in details the progress achieved in this field.
During the extension of our Contract until December, 1991 the objectives h) and i) will focus our research interest. We have available now only a few amount of data related to the characterization of radon sources and their influence on the presence of radon in houses and little more related to the measurement of radon progeny and equilibrium factor.In both cases, more information is absolutely required.

PUBLICATIONS

"Radon, principal fuente de radiacion natural".L.S.Quindos. J.Soto, P.L.Fernandez,G.Newton, J.J.Peña, J.Arteche, E.Villar (in spanish).Revista Española de Fisica, vol 3, nº 2 oo 22-27.1989.
"Atmospheric pressure effects on improved Lucas cells". L.S.Quindos, G.Newton. ITRI Report LMF-126.December.1989.
"Terrestrial gamma radiation levels outdoors in Cantabria,Spain". L.S.Quindos, P.L.Fernandez,J.Soto,C.Rodenas. (to be published in Journal of Radiological Protection).
"National survey on indoor radon in Spain". L.S.Quindos,P.L.Fernandez,J.Soto. (to be published in Environment International).
"A modified Lucas cell for leakage measurement from encapsulated radium sources". L.S.Quindos, P.L.Fernandez,J.Soto, G.Newton.(to be published in Applied radiation and isotopes).
"Medida de la concentracion de radon en el interior de viviendas españolas".L.S.Quindos,P.L.Fernandez, J.Soto.(to be published in Revista Española de Fisica)(in spanish).
"Short versus long term indoor radon measurements". L.S.Quindos, P.L.Fernandez,J.Soto.(to be published in Health Physcis).
"Radon and lung cancer". L.S.Quindos, J.Gomez, P.L.Fernandez, J.Arteche, G.Romero, J.Soto, C.Rodenas.(to be published in Radiation Protection Dosimetry).
"Evolucion de la mortalidad por cancer de pulmon en España (1981-1985)"L.S.Quindos,J.Madrid. (submitted to be published in Revista de Sanidad e Higiene Publica)(in spanish).

Progress Report

Contract: BI6-208 **Sector: C12**
Title: Evalution of the population exposure to radon in the vicinity of uranium mining facilities.

1 Galvão

LNETI

I. Summary of Project and Global Objectives

To assess the exposure of the portuguese population due to the indoor radon inhalation.

In a first step to develop a regional survey in regions of technologically enhanced natural radioactivity.

In a second step to implement a indoor radon national survey.

To determine the equilibrium factors in some regions of Portugal.

To study the outdoor radon concentrations and to evaluate its contribution to the indoor radon concentration in contaminated zones.

Head of Project 1: Dr. Galvão

II. Objectives for the reporting period

To assess the distribution of the radon concentrations in the dwellings of the country, chosen on a statistical basis.

To study the radon daughters equilibrium factors in selected houses.

III. Objectives for next period

To accomplish the indoor radon national survey.

To evaluate equilibrium factors in more dwellings, in order to have a mean value from the different regions of the country.

Finally to assess the doses to the portuguese population due to the inhalation of radon.

IV. Progress achieved

1.1 - Indoor radon

The indoor radon national survey was pursued with measurements in the 276 counties (the administrative region considered) of the country.

The results obtained during this period concern about 3560 surveyed houses.

The total recovery of the distributed dosemeters up to now was about 55%.

The observed individual concentrations range from 6 $Bq.m^{-3}$ (the detection limit) to 2.7×10^{3} $Bq.m^{-3}$ and the geometric mean for each county range from 7 $Bq.m^{-3}$ (Beja) to 200 $Bq.m^{-3}$ (Guarda).

The results obtained (table 1) were grouped by districts and the corresponding arithmetic means were calculated. In brackets we present the range of the arithmetic means obtained in the different counties of each district.

Districts	Indoor radon concentration ($Bq.m^{-3}$) Arithmetic mean	Range
Aveiro	43	(21-141)
Beja	32	(7- 61)
Braga	101	(33-210)
Bragança	41	(17- 96)
Castelo Branco	86	(12-223)
Coimbra	55	(12-165)
Évora	33	(13-158)
Faro	38	(14-162)
Guarda	134	(28-258)
Leiria	33	(8- 88)
Lisboa	25	(7- 66)
Portalegre	66	(15-207)
Porto	67	(13-143)
Santarém	30	(11- 65)
Setúbal	28	(12- 37)
Viana do Castelo	89	(50-160)
Vila Real	90	(42-154)
Viseu	122	(23-202)

Table 1 - Indoor radon in Portugal

The indoor radon arithmetic means obtained in the different counties until final December 1990, were sent to NRBP in order to contribute for the European Atlas of Natural Radiation.These results are presented in annex.

The frequency distribution of the indoor radon, observed up to now is presented in Fig.1. As it can be seen in the histogram, concentrations until 50 $Bq.m^{-3}$ are the most significative (58.5 %) but there are still 9.4 % of the measured values higher than 200 $Bq.m^{-3}$.

1.2 - Equilibrium factors

To evaluate the equilibrium factors, measurements were performed in some selected dwellings.

The active measurements of radon and radon daughters were made with active dosemeters which are left in dwellings for 3-4 weeks integration period. In each dwelling both passive and active dosemeters were used together, in order to estimate the equilibrium factor. However, only a few values are available, due to some difficulties in the active measurements.

The equilibrium factors obtained up to now in dwellings from different districts are:

Dwellings	Equilibrium factor (F)
dwelling 1 (Faro)	0.55
dwelling 2 (Guarda)	0.64
dwelling 3 (Lisboa)	0.57
dwelling 4 (Lisboa)	0.68
dwelling 5 (Coimbra)	0.41
dwelling 6 (Loures)	0.66
dwelling 7 (Évora)	0.72

1.3 - Outdoor radon

Concerning the evaluatiuon of outdoor radon, measurements in the vicinity of the uranium mining facilities were also performed, in 1990, to study the horizontal and vertical dispersion of the radon emanated from the tailings.

This programme was made with the assistence of the KFR/FRG. kfK dosemeters were used and placed in a grid of 100m x 100m whitin an area of 500m x 500m. Some dosemeters were also exposed at different heights (from 2m to 12m), in order to know the vertical radon dispersion.

The dosemeters were exposed during three months and processed at the Karlsruche Nuclear Research Center.

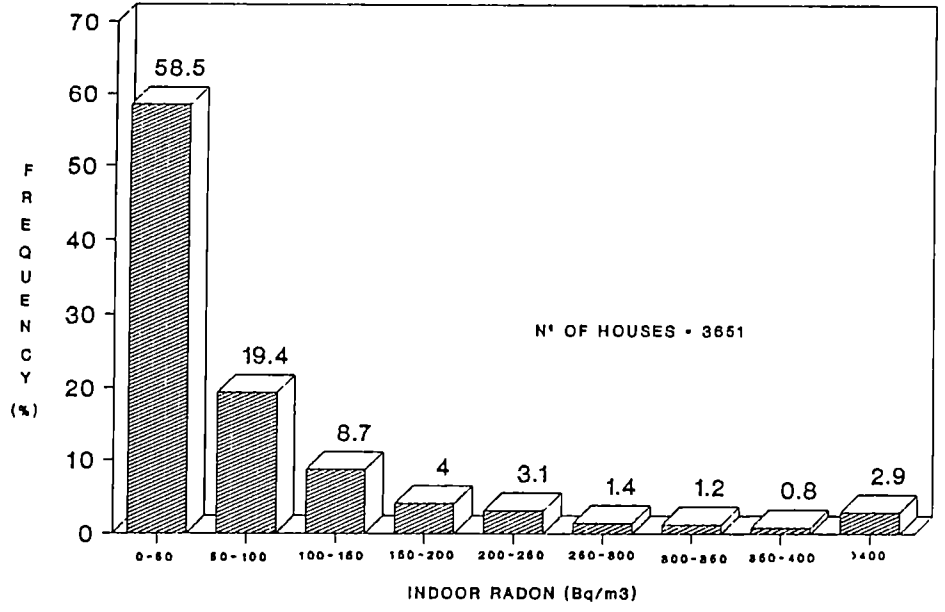
In these measurements, a mean radon concentration of (71 $Bq.m^{-3}$) lower than in the previous year was found (110 $Bq.m^{-3}$). This might be due to higher temperatures, lower soil humidity and lower wind speed during at the first period (1989).

Concerning the vertical dispersion, values from 95 $Bq.m^{-3}$ to 78 $Bq.m^{-3}$ were obtained for heights increasing from 2 to 12 m.

1.4 - Publications covering work of reporting period

Faísca, M.C. e Ribau Teixeira, M.M. - "Níveis de ^{222}Rn medidos em habitações portuguesas" - 7ª Conferência Nacional de Física, 24-27 Setembro 1990, Lisboa.

FIG. 1 - FREQUENCY DISTRIBUTION OF INDOOR RADON CONCENTRATION



FEBRUARY 1991

ANNEX

INDOOR RADON (Bq m-3)

District	County	Mean		Range	Obs
		Geometric	Arithmetic		
Aveiro	Agueda	41	46	(18-123)	F
	Albergaria-a-Velha	18	21	(9-40)	F
*43Bq m-3	Anadia	38	43	(21-81)	F
	Arouca				NA
	Aveiro	25	31	(6-111)	F
	Castelo de Paiva	33	44	(10-106)	F
	Espinho	51	62	(21-158)	F
	Estarreja	46	54	(19-127)	F
	Feira	12	12	(6-23)	F
	Ilhavo	29	34	(16-56)	F
	Mealhada	122	141	(54-186)	F
	Murtosa	23	34	(8-105)	F
	Oliveira de Azemeis	37	40	(20-64)	P
	Oliveira do Bairro	29	39	(9-128)	F
	Ovar	15	22	(6-93)	P
	Sao Joao da Madeira	19	20	(11-35)	P
	Sever do Vouga				NA
	Vagos	23	27	(11-53)	F
	Vale de Cambra	41	46	(22-109)	P
Beja	Aljustrel	18	22	(10-54)	P
	Almodovar	37	49	(6-183)	F
*32Bq m-3	Alvito	14	15	(8-22)	P
	Barrancos				NA
	Beja	7	7	(6-11)	F
	Castro Verde	43	46	(19-68)	F
	Cuba	6	7	(6-9)	P
	Ferreira do Alentejo	27	32	(12-81)	F
	Mertola	39	55	(9-164)	F
	Moura	14	20	(6-64)	F
	Odemira	32	37	(20-78)	P
	Ourique	45	61	(6-143)	P
	Serpa	8	9	(6-26)	F
	Vidigueira	46	57	(6-93)	F
Braga	Amares	164	210	(93-846)	F
	Barcelos	47	61	(6-309)	F
*101Bq m-3	Braga	58	74	(6-254)	F
	Cabeceiras de Basto				NA
	Celorico de Basto				NA
	Esposende	26	33	(8-145)	F
	Fafe	52	119	(6-1574)	F
	Guimaraes	102	135	(21-561)	F
	Povoa de Lanhoso	115	134	(51-255)	F
	Terras de Bouro	41	54	(9-220)	F
	Vieira do Minho	23	41	(6-301)	F
	Vila Nova de Famalicao				NA
	Vila Verde	105	153	(19-1000)	F

District	County	Mean		Range	Obs
		Geometric	Arithmetic		
Braganca	Alfandega da Fe				NA
	Braganca	14	16	(6-39)	F
*41Bq m-3	Carrazeda de Ansiaes	65	92	(24-279)	F
	Freixo de Espada a Cinta	28	39	(6-125)	F
	Macedo de Cavaleiros	26	31	(6-54)	F
	Miranda do Douro	38	96	(17-369)	F
	Mirandela	41	53	(6-114)	F
	Mogadouro	14	17	(6-45)	F
	Torre de Moncorvo	17	21	(8-49)	F
	Vila Flor	21	30	(7-114)	F
	Vimioso	14	17	(6-40)	F
	Vinhais				NA
Castelo	Belmonte	95	125	(21-237)	F
Branco	Castelo Branco	121	223	(13-1030)	F
	Covilha	108	180	(21-788)	F
*86Bq m-3	Fundao	128	193	(68-1450)	F
	Idanha-a-Nova				NA
	Oleiros	26	28	(10-50)	F
	Penamacor	29	50	(6-171)	F
	Proenca-a-Nova	15	21	(6-59)	F
	Serta	17	19	(6-43)	F
	Vila de Rei	10	12	(6-37)	F
	Vila Velha de Rodao	18	33	(6-162)	F
Coimbra	Arganil	75	107	(10-386)	P
	Cantanhede	13	15	(6-44)	F
*55Bq m-3	Coimbra	33	45	(7-258)	F
	Condeixa-a-Nova	54	65	(15-125)	P
	Figueira da Foz	29	34	(14-102)	F
	Gois				NA
	Lousa	67	99	(21-336)	F
	Miranda do Corvo	48	51	(21-85)	F
	Montemor-o-Velho	15	20	(6-67)	F
	Oliveira do Hospital	118	165	(25-587)	F
	Pampilhosa da Serra	12	23	(6-136)	F
	Penacova				NA
	Penela	28	40	(6-92)	F
	Soure	27	38	(12-129)	F
	Tabua				NA
	Vila Nova de Poiares	43	57	(8-107)	F
	Mira	10	12	(6-22)	F

District	County	Mean		Range	Obs
		Geometric	Arithmetic		
Evora	Alandroal	135	158	(55-320)	P
	Arraiolos	13	16	(6-41)	F
*33Bq m-3	Borba	27	34	(6-127)	F
	Estremoz	35	39	(15-62)	P
	Evora	71	86	(17-271)	P
	Montemor-o-Novo	32	35	(10-52)	P
	Mora	12	13	(8-30)	F
	Mourao	24	32	(15-110)	P
	Portel	14	16	(6-27)	P
	Redondo	21	27	(6-56)	F
	Reguengos de Monsaraz				NA
	Vendas Novas				NA
	Viana do Alentejo				NA
	Vila Vicosa	17	27	(6-81)	P
Faro	Albufeira	35	37	(22-57)	P
	Alcoutim	23	28	(7-66)	F
*38Bq m-3	Aljezur				NA
	Castro Marim	34	37	(24-72)	F
	Faro	23	27	(6-58)	P
	Lagoa	20	22	(12-32)	P
	Lagos	27	36	(7-95)	F
	Loule	12	16	(6-56)	F
	Monchique	74	162	(7-1080)	F
	Olhao	30	52	(6-134)	P
	Portimao	37	51	(6-227)	F
	S. Bartolomeu de Messines	19	23	(6-55)	F
	S. Bras de Alportel	17	24	(6-61)	F
	Silves	28	59	(6-314)	F
	Tavira	12	20	(6-98)	F
	Vila do Bispo	13	15	(6-20)	F
	Vila Real de Sto. Antonio	11	14	(6-41)	F
Guarda	Aguiar da Beira	63	74	(18-152)	F
	Almeida	44	61	(16-209)	F
*134Bq m-3	Celorico da Beira	126	199	(6-892)	F
	Figueira Castelo Rodrigo				NA
	Fornos de Algodres	109	116	(62-154)	F
	Gouveia	155	175	(39-363)	F
	Guarda	200	258	(28-1344)	F
	Manteigas	49	64	(12-136)	F
	Meda	27	28	(15-33)	P
	Pinhel	193	259	(32-616)	F
	Sabugal	75	137	(10-711)	F
	Seia	153	175	(65-332)	F
	Trancoso	50	64	(21-173)	P
	Vila Nova de Foz Coa				NA

District	County	Mean		Range	Obs
		Geometric	Arithmetic		
Leiria	Alcobaca	17	26	(8-108)	F
	Alvaiazere				NA
*33Bq m-3	Ansiao	42	48	(21-95)	F
	Batalha	31	36	(15-62)	F
	Bombarral	8	8	(6-11)	P
	Caldas da Rainha	16	20	(6-60)	F
	Castanheira de Pera	22	29	(6-82)	F
	Figueiro dos Vinhos				NA
	Leiria	35	41	(6-108)	F
	Marinha Grande	18	20	(9-43)	F
	Nazare	23	27	(7-65)	F
	Obidos	38	50	(15-138)	F
	Pedrogao Grande	69	88	(28-265)	F
	Peniche	10	13	(6-51)	P
	Pombal	28	40	(6-251)	F
	Porto de Mos	15	19	(6-49)	F
Lisboa	Alenquer				NA
	Amadora	27	30	(9-58)	P
*25Bq m-3	Arruda dos Vinhos	24	28	(10-65)	P
	Azambuja	19	23	(6-55)	P
	Cadaval	7	7	(6-14)	P
	Cascais	61	66	(41-102)	P
	Lisboa	25	29	(6-70)	P
	Loures	26	28	(6-45)	P
	Lourinha	13	17	(6-47)	F
	Mafra	9	10	(6-32)	F
	Oeiras				NA
	Sintra	25	32	(10-76)	P
	Sobral Monte Agraco	10	12	(6-23)	P
	Torres Vedras	10	14	(6-64)	P
	Vila Franca de Xira	19	23	(6-58)	P

District	County	Mean		Range	Obs
		Geometric	Arithmetic		
Portalegre	Alter do Chao	23	27	(8-49)	F
	Arronches	68	85	(36-262)	F
*66Bq m-3	Avis	22	26	(9-57)	F
	Campo Maior	24	32	(6-103)	F
	Castelo de Vide	47	78	(6-647)	F
	Crato	116	169	(23-878)	F
	Elvas	18	21	(6-58)	F
	Fronteira	56	68	(28-148)	P
	Gaviao	13	15	(9-31)	P
	Marvao				NA
	Monforte	26	36	(8-70)	P
	Nisa	160	207	(47-498)	F
	Ponte de Sor	26	33	(11-84)	F
	Portalegre	35	60	(6-293)	F
	Sousel				NA
Porto	Amarante	77	91	(31-314)	F
	Baiao	77	100	(21-178)	F
*67Bq m-3	Felgueiras	99	101	(29-313)	F
	Gondomar	30	49	(6-212)	F
	Lousada				NA
	Maia	30	37	(6-125)	F
	Marco de Canaveses	95	118	(22-842)	P
	Matosinhos	41	51	(10-128)	F
	Pacos de Ferreira	11	16	(6-63)	F
	Paredes	84	108	(32-326)	P
	Penafiel				NA
	Porto	29	49	(9-141)	P
	Povoa do Varzim	12	13	(6-37)	P
	Santo Tirso	127	143	(53-338)	P
	Valongo	27	35	(6-125)	F
	Vila do Conde	24	32	(6-66)	F
	Vila Nova de Gaia	48	65	(6-288)	F

District	County	Mean		Range	Obs
		Geometric	Arithmetic		
Santarem	Abrantes	31	39	(10-138)	F
	Alcanena	20	45	(7-201)	F
+30Bq m-3	Almeirim	22	29	(6-77)	F
	Alpiarca	10	11	(6-26)	F
	Benavente	13	15	(6-26)	P
	Cartaxo				NA
	Chamusca	20	24	(9-65)	F
	Constancia	18	21	(8-43)	F
	Coruche	27	37	(8-93)	P
	Entroncamento				NA
	Ferreira do Zezere	13	16	(6-32)	F
	Golega	18	22	(8-44)	F
	Macao				NA
	Rio Maior	52	65	(19-206)	F
	Salvaterra de Magos				NA
	Santarem				NA
	Sardoal	20	30	(6-98)	F
	Tomar	26	30	(11-42)	P
	Torres Novas	18	23	(6-76)	F
	Vila Nova Barquinha	13	44	(6-349)	F
	Vila Nova de Ourem	30	34	(9-58)	F
Setubal	Alcacer do Sal	11	12	(6-26)	F
	Alcochete				NA
+28Bq m-3	Almada				NA
	Barreiro				NA
	Grandola	13	18	(6-31)	F
	Moita	23	27	(6-56)	P
	Montijo				NA
	Palmela				NA
	Santiago do Cacem	24	33	(6-103)	F
	Seixal	35	37	(28-40)	P
	Sesimbra	29	32	(11-52)	P
	Setubal				NA
	Sines	30	34	(17-80)	F
Viana do	Arcos de Valdevez	114	128	(31-309)	F
Castelo	Caminha	57	73	(17-200)	P
	Melgaco				NA
+89Bq m-3	Monsao	54	71	(6-216)	F
	Paredes de Coura	55	97	(6-515)	F
	Ponte de Lima	54	85	(17-433)	F
	Valenca	66	81	(29-201)	P
	Ponte da Barca	137	160	(56-429)	F
	Viana do Castelo	49	50	(29-61)	F
	Vila Nova Cerveira	53	57	(35-104)	P

District	County	Mean		Range	Obs
		Geometric	Arithmetic		
Vila Real	Alijo	56	73	(6-180)	F
	Boticas	58	68	(63-73)	F
+90Bq m-3	Chaves	152	154	(142-183)	P
	Mesao Frio				NA
	Mondim de Basto	57	76	(14-247)	F
	Montalegre				NA
	Murca	42	48	(7-112)	F
	Peso da Regua	29	42	(15-239)	F
	Ribeira da Pena	126	145	(63-239)	F
	Sabrosa	75	96	(18-327)	F
	Santa Marta Penaguiao	55	69	(27-171)	F
	Valpacos	42	46	(23-106)	F
	Vila Pouca de Aguiar	104	114	(67-150)	P
	Vila Real	110	144	(34-759)	F
Viseu	Armamar	53	70	(14-135)	P
	Carregal do Sal	118	272	(23-755)	P
+122Bq m-3	Castro d'Aire	140	181	(47-296)	F
	Cinfaes	49	67	(11-190)	P
	Lamego	92	155	(21-1219)	F
	Mangualde	173	240	(47-1006)	F
	Moimenta da Beira	32	45	(6-138)	P
	Mortagua	25	30	(7-55)	F
	Nelas	177	267	(38-2686)	F
	Oliveira de Frades				NA
	Penalva do Castelo	49	56	(15-103)	F
	Penedono	63	78	(17-208)	P
	Resende	62	76	(14-130)	P
	Santa Comba Dao	60	70	(26-169)	P
	S. Joao da Pesqueira	14	23	(6-56)	P
	S. Pedro do Sul	156	171	(70-338)	F
	Satao	122	146	(30-291)	P
	Sernancelhe	83	121	(20-426)	F
	Tabuaco	51	80	(15-243)	P
	Tarouca	38	46	(17-114)	F
	Tondela	149	202	(54-617)	F
	Vila Nova de Paiva	96	117	(44-308)	P
	Viseu	113	127	(44-312)	F
	Vouzela	117	157	(13-423)	F

F - Final results
P - Provisional results
NA - Results not yet available
\+ - Arithmetic mean of the District

Contract: Bi7-013 **Sector: C12**
Title: Retrospective assessment of radon exposure from long-lived decay products.

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I. Summary of Project and Global Objectives

The discovery that the indoor surface activity of Po-210 (a long-lived decay product of Rn-222) can be used as an indicator of long-term exposure to airborne radon daughters is the starting point for this project. The objectives of the project are to study the chain of processes which in the indoor environment leads from airborne radon to embedded long-lived daughters and to reveal those exposure conditions in which the surface activity concentration of the long-lived radon decay products is a useful estimate of lung cancer risk.

The study of the embedded long-lived decay products of radon-222 is the only presently known method for "measuring" accumulated radon daughter concentration levels from the past. The results from this investigation will be therefore of importance when assessing past and future indoor exposures to radon and its decay products and will also improve our understanding of the fate and behavior of radon daughters in the indoor environment. The major potential application area of the long-lived radon daughter method is radon epidemiology and as a number of these studies are now underway the development of this technique is very timely and appropriate.

Specifically detection methods for both short- and long-lived radon decay products deposited on indoor surfaces under realistic conditions will be improved. An instrument based on the track-etching technique for real-time plate-out measurements of short-lived radon daughters will be developed and the feasibility of auto radiographic track-etch methods for measuring Po-210 embedded in surfaces will be investigated. Alpha spectrometry using pulse ionization chambers will be a reference method for the Po-210 analysis of large area samples and an open-flow transportable ionization chamber will be developed for non-destructive measurements.

The deposition of short-lived radon daughters onto surfaces is a critical process in the transformation chain from airborne to embedded activity. The long-term and positional variability in the plate-out process will be studied under controlled laboratory, as well as realistic indoor conditions. The experimental results from laboratory and dwelling exercises will be compared with model predictions and special cleaning studies, in order to differentiate between adsorbed and absorbed surface activity, will be performed.

The two-year goal of the project is to reveal the usefulness of the polonium-in-glass method, or any other similar technique involving long-lived decay products, and to identify areas in which further studies are warranted.

Head of Project 1: Dr. Samuelsson

II Objectives for the reporting period

To investigate and improve the pulse ionizing chamber as a reference detector for the analysis of Po-210 on Rn-222 (radon)-exposed surfaces. To study the variability of surface Po-210 in houses and within houses.

III Objectives for next period

To identify suitable substrate objects in homes and clarify under which conditions surface Po-210 activity can be used as a radon exposure and lung cancer risk estimator.

IV Progress achieved including publications

The main emphasis of the study has been placed on the development of the pulse ionization detector for the analysis of Po-210 on large-area samples and on investigations of plate-out and Po-210 levels in authentic indoor environments. Suitable glass samples can be found in most dwellings and different types of plane glass sheets have served as test objects for plate-out activity.

DETECTOR DEVELOPMENTS

The surface activity of long-lived decay products encountered in dwellings is low, typically less than a few becquerels per square metre. In order to detect radon exposures below 1 kBq y m^{-3} a detector sensitivity better than 0.5-1 Bq m^{-2} is required. Such a low surface activity, corresponding to less than one decay per square centimetre per hour, discouraged us from the direct determination of the (almost) pure β-emitters Pb-210 and Bi-210. The very weak and superficially embedded activity advocates alpha spectrometry (Po-210, E_α=5.3 MeV) and the use of large-areasamples.

Low-background solid state devices (SSD) are useful for alpha spectrometry of Po-210, but are limited in area. The present problem of Po-210 adsorbed onto glass surfaces, would require impracticably long analysis times, of the order of days or more, and we have therefore chosen to use pulse ionization chambers (PIC). The net alpha count rate of our closed PIC is about 100 times that obtained with a 300 mm^2 SSD. The closed PIC, a gridded chamber with plane-parallel electrodes, is essentially a stationary device, accommodating samples with diameters of less than 180 mm. Larger samples must be cut down in size in order to be analysed. When taking samples from private dwellings, such a destructive procedure is naturally disadvantageous and it was decided to develop a new type of PIC with the following characteristics:

1) Alpha counting efficiency and energy resolution comparable to the closed PIC.
2) Non-destructive measurement of semi-infinite samples
3) Mobile detector applicable as a reference detector during field and *in situ* studies of Po-210.

The resulting detector, in its latest version constructed with the technical support of the Laboratory of Applied Physics I, Technical University of Denmark, is a lightweight construction of the open-flow type where the sample/surface to be measured covers the opening of the chamber. Utilizing high-quality electronics, the new open PIC works excellently giving a net count rate of about 0.9 min^{-1} for a surface activity of 1 Bq m^{-2} Po-210. The alpha energy resolution is typically 0.7 % (rel. FWHM) for extended glass sheet sources. Vertically orientated surfaces can be analysed *in situ* with no significant deterioration in performance. The open-flow PIC has been described in more detail by Johansson et al. (1991).

PLATE- OUT HOMOGENEITY IN DWELLINGS

Little is known about the inter-room, intra-room, and temporal variations of the radon daughter plate-out under realistic indoor conditions. Knowledge in this field must be improved before the applicability of the retrospective radon (daughter) method, based on the superficially absorbed Po-210, can be assessed.

Several windows from a private house were given to us following renovation and four of them have been investigated for Po-210 inhomogeneity by placing the open PIC at different positions on the window panes. Relative to the centre value, there is a significant, but only moderately enhanced surface activity of Po-210 in the upper part (Figure 1). It is too early to draw definite conclusions concerning the intra-window variations from the single case presented in Figure 1, but if the trend holds after more systematic investigations, the positioning of the detector on a single window pane surface should not be too critical.

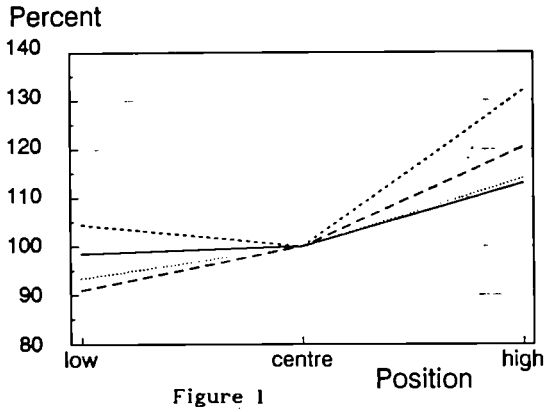

Figure 1

In an effort to investigate the in-house variability of Po-210 on window surfaces and at the same time test the open PIC under field conditions, the necessary equipment was installed in a van and parked outside the dwelling in question. The house is a self-draught ventilating, single-family house constructed from alum-shale-based

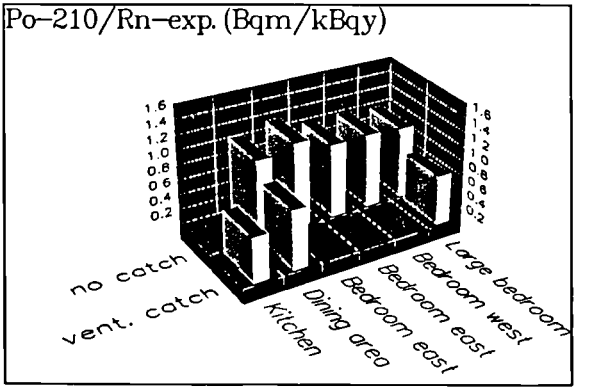

Figure 2

lightweight concrete. The surface Po-210 activity (Bq m^{-2}) relative to the estimated radon exposure (kBq y m^{-3}) is given in Figure 2. Except for the kitchen, radiators are situated beneath the windows. Three of the windows are equipped with special window catches for ventilation purposes. These three windows exhibit a low Po-210/radon, ratio indicating that the room radon measurement is not relevant close to windows frequently used for airing. Excluding the windows used for ventilation, the spread in the ratios in Figure 2 is surprisingly low, only ± 10%.

THE CORRELATION OF RADON AND Po-210 IN DIFFERENT DWELLINGS

The surface activity of glass samples taken from 22 detached houses constructed partly of alum-based lightweight concrete is compared the estimated radon exposure in Figure 3. (The quantity "radon exposure" is chosen simply for the reason that

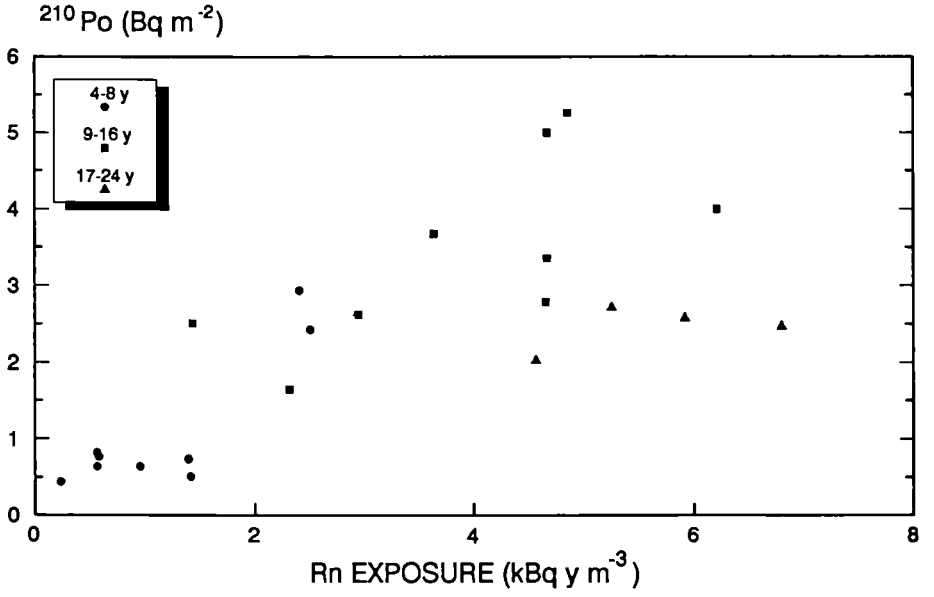

Figure 3

the present-day values of radon can be easily measured. It would have been preferrable, of course, to have compared the Po-210 values with a quantity more closely related to the lung cancer risk experienced by peopel living in the houses). The radon concentrations were measured by means of CR-39 cups, supplied and analysed by National Institute of Radiation Protection (SSI), Stockholm, placed in the room from which the glass sample was taken. The exposure period covered two months and the radon exposure was estimated simply by multiplying the film cup radon value by the glass sheet exposure time, (<24 years) as given by the home-owner. All the glass samples in Figure 3 were carefully cleaned of any loose activity before being analysed. The coefficient of correlation (r^2) in Figure 3 is 0.54. The spread in the values is due to several reasons, inaccurate knowledge of the true radon exposure, inter-house differences in equilibrium factors and plate-out conditions

at the same radon concentration, and different cleaning practices of the households. Dust and grease can build up on a glass surface over a period of years and the alpha recoils will increasingly be trapped in the contaminant and not in the glass matrix.

PUBLICATIONS

C. Samuelsson: Recoil-Deposited Po-210 in Radon-Exposed Dwellings. Twenty-Ninth Hanford Symposium on Health and the Environment. October 15-19, 1990, Richland, Washington U.S.A.

L. Johansson, B. Roos and C. Samuelsson: Alpha-Particle Spectrometry of Large-Area Samples Using an Open-Flow Pulse Ionization Chamber. ICRM Symposium on Low-Level Measuring Techniques and Alpha Particle Spectrometry, Monaco, June 4-7, 1991.

Head of Project 2: Dr. Jonassen

II Objectives for the reporting period

A.

Preliminary study of the influence of electric fields, spurious or controlled, on the plate-out of short–lived radon daughters on room surfaces.

B.

Manufacture of pulse ionization chambers.

III Objectives for next period

In the next period plateout of short–lived radon daughters will be studied at different aerosol conditions under the influence of electric fields, of such magnitude which are likely to be encountered in normal living and working rooms and also technically enhanced field strengths of both polarities.

IV Progress achieved including publications

A

The plateout experiments have been carried out in a laboratory room with a volume of approximately 120 m^3.

The radon concentration in the room can be monitored to levels up to approximately 5000 $Bq \cdot m^{-3}$. The individual daughter concentrations and the unattached fractions can be measured by alpha spectroscopy and the use of multiple screens. The aerosol concentration is measured by a condensation nucleus counter.

During the plateout experiments CR--39 track etch detectors were placed on nine selected positions of the walls, floor and ceiling.

Because of the extensive delays of the project the actaul measurements did not start til the beginning af January 1991, and up till mid May 1991 it has only been possible to conduct three regular plateout experiments, since the laboratory room also had to be used for other purposes.

The three series of measurements had the following characteristics: 1) low aerosol concentration (< 10.000 aerosol particles per cm^3) and no electric fields, 2) low aerosol concentration and electric fields from a high voltage ionizer mounted in the ceiling, and 3) high aerosol concentration (about 100.000 aerosol particles per cm^3) and no electric fields.

Unfortunately something went wrong with the processing of the detectors from experiment no. 2, so at present we only have the results from experiments 1 and 3 only differing in aerosol concentration.
These experiments have given us the general plateout pattern for the room in its (electrically) undisturbed condition and the results also confirms that the plateout rate increases with decreasing aerosol concentration.

B.
Our laboratory has also supplied technical assistance in manufacturing three pulse–ionization chambers, of which two are presently in use in Lund and one in Potsdam, NY (Phil Hopke).
For further details concerning the ionization chambers is referred to the report from the coordinator.

Head of Project 3:Dr. Falk

II Objectives for the reporting period

To investigate autoradiographic track-etch techniques for measurement of Po-210 embedded in the surface of glas. To study different techniques to discriminate Po-210 tracks from background tracks. Track shape and size together with absorption layers between the CR-39 detector and the glass surface are the parameters to be studied to optimize the sensitivity of the method.

III Objectives for next period

To select the techniques on the criteria that it should be a practical method with adequate sensitivity. To study the reproducibility and limitations of the methods chosen. To measure a number of glasses exposed in dwellings.

IV Progress achieved including publications

USING CR-39 DETECTORS TO MONITOR Po-210 ACTIVITY EMBEDDED IN GLASS SURFACES.

Surface activity of Po-210 on glass exposed in homes can be a measure of long term exposure to radon daughters. The objective of our work is to find a practical method to monitor the activity by using CR-39 in autoradiographic mode.

The CR-39 track-etch technique is, at our institute, presently utilized in radon monitoring using plastic holders for the detectors into which the radon gas diffuses. The detectors are chemically etched and analyzed in an image analyzer (Cambridge Instruments Quantimet Q520). The analysis system facilitates programming capabilities which makes it useful also for different tasks like the one described in this progress report.

Description of glasses used

The experiments so far have been performed using the following glasses:

1. Two glasses exposed to radon and radon daughters in the SSI radon chamber. Estimated Po-210 surface activities are 6 and 12 Bq/m^2.
2. One domestic glass with a measured Po-210 surface activity of 4.5 Bq/m^2.
3. One background glass.
4. One perspex glass.

Method

The CR-39 detectors used are of SSI standard size and shape (13x37 mm) with a thickness of 1.2 mm. The material is delivered in 700 cm^2 sheets by TASL in Bristol, UK. The individual pieces are cut and numbered by an engraving machine. In order to minimize background caused by exposure during manufacturing special care is taken to minimize handling time and the material is antistatically treated immediately following unpacking.

The detectors are positioned on the glass surface with or without an absorber and fixed using frames and tape. Each glass surface has been covered

by several separate detectors to increase the analyzed surface area and to facilitate evaluation of activity inhomogeneties. Exposure time has been around three weeks. Immediately after dissembling, the detectors are etched according our standard procedure in 20.5 % 90°C NaOH for 2 hours. Typical alpha tracks areas are 400-600 μm^2.

Analysis

The analysis of all detectors has been performed in different ways:

1. Standard evaluation low magnification (4 objective lens) standard selection of "true" tracks using limits on size and shape.
2. Special evaluation using a specifically developed reading program which for all detected "features" stores 10 parameters (i.e. size and shape). This has been done using both low and high magnification (4 and 10 objective lens). For each detector a file is saved containing data on all features. In the subsequent analysis different acceptance criteria can be tested to optimize analysis.

For each detector 0.2-1.2 cm^2 (depending on method) has been analyzed. Typical track densities varies between 20 (background) and 1000 per cm^2.

Experiments

The objective of our study is to find a practical method with adequate sensitivity and signal/background ratio to facilitate estimation of long term exposure to radon daughters. The major problem is the background activity of the glass which, when measured using autoradiography, is both significant in magnitude and varying from one glass to the other. The primary task, as we see it, is therefore to minimize the background tracks without lowering the signal from surface activity beyond usefulness.

A number of experiments/exposures have been performed during autumn 1990 and spring 1991. These are:

1. Exposures using mylar folio (1.8 mg/cm^2) in layers of 1-3.
2. Exposures without absorbers on all types of glasses.

Results

A total of 80-100 detectors were exposed in each of four experiment series. The last two series comprise 164 detectors. The results of glass 2 (see above) are presented below in table 1 and 2 based on the analysis of 40 detectors. The four different cases represent different analysis techniques with or without absorber.

Case 1 is the standard technique used for radon exposed detectors. Case 2 is similar but an absorber has been used during exposure.

Case 3 and 4 represents efforts to optimize the analysis technique given the two exposure situations without or with absorber. In the first case there is a mixture of tracks of all shapes. The elongated tracks have hit the detector surface at an angle and tend to originate at or near the glass surface. Thus by exclusively studying the elongated tracks (with a high shape factor) many of the α-particles originating within the glass (background) can be excluded. In the second case when using an absorber the α-particles that reach the detector must have sufficent energy to penetrate the absorber. By this a part of the background α-particles originating from

a depth in the glass will be discriminated. The tracks obtaind using an absorber tend to have circular shape. These two cases thus represents different approaches to minimize the background influence.

Case	Obj lens	Accept size interval μm^2	Accept shape interval	Absorber thickness mg/cm^2	Front: Number of tracks	Back: Number of tracks
1	4	225-900	1.00-1.50	0	1911	793
2	4	225-900	1.00-1.50	1.8	1232	374
3	10	300-700	1.25-1.70	0	597	123
4	10	300-700	1.00-1.35	1.8	785	150

Table 1. Analysis of CR-39 detectors exposed in autoradiograph mode.

Notes to table 1:
Objective lens 4 typically gives 28 pixels per track while lens 10 gives 173. The shape factor is defined by $(perimeter)^2/(4 \cdot \pi \cdot area)$. A perfectly round object with an infinite number of pixels gives shape=1.0. The area analyzed varies but numbers refer to 1 cm^2. Exposure time varies but numbers refer to 100 days.

The relative standard deviation of the net "counts" can be calculated as $[(S+B)/(S-B)]^{\frac{1}{2}}$ where S = front side track density and B = back side track density. The detection limit (MDA) can also be estimated using the results above. All errors are, however, supposed to be caused by counting statistics alone. This is not the case but the experiments carried out so far does not give enough information on the true errors. The MDA is calculated as three times the standard variation of the background.

Case	Ratio (S-B)/B	Sensitivity	Rel. error(%)	MDA Bq/m^3
1	1.4	248	4.7	0.34
2	2.3	191	4.7	0.30
3	3.9	105	5.7	0.32
4	4.2	141	4.8	0.26

Table 2. Results

Notes to table 2:
The sensitivity is the number of tracks above background per Bq/m^2 and cm^2 and assuming a 100 days exposure time. The relative error is the standard deviation of the net "count" as defined above.

The most frequent radon level in Swedish homes is 60 Bq/m^3. According to preliminary results on plate-out rates this would result in a build-up of Po-210 of .06 Bq/m^2 per year on a glass surface. Using a typical MDA above (0.3 at 1 cm^2) implies that, at that radon level, exposure time must exceed 5 years to facilitate detection.

The true variations are probably significantly higher which means that the MDA:s will be higher. This may be compensated by analyzing larger areas of the CR-39 detector (10-100 cm^2).

Publications: None

Head of Project 5 : Dr. Vanmarcke - Head of Project 4 : Dr. Poffijn

II Objectives for the reporting period

Joined project between SCK/CEN (project 5) and RUG (project 4).

To determine the fraction of absorbed Po-214 which reappears at the glass surface due to the recoil energy.

To determine the influence of the different parameters of the room model on the fraction of the activity absorbed in the glass.

To study the effect of household cleaning on the deposited activity.

III Objectives for next period

To study both experimentally and theoretically the uncertainties associated with the estimation of the long term radon exposure from the α-activity of Po-210 of vitreous glass.

A tentative test will be carried out in the scope of the Ardennes epidemiological study.

IV Progress achieved including publications

The theory of Lindhard (1968) provides a framework to determine the range of low energy heavy ions in amorphous media. Two recoil nuclei have to be considered, Pb-214 with a recoil energy of 112 keV and Pb-210 with a recoil energy of 146 keV. The details of the calculations are given by Landsheere (1989).

The depth distributions of Pb-214 and Pb-210 are shown in figure 1. The full line and the dot and dash line are calculated from Pb-214 and Pb-210 nuclei deposited on the surface of vitreous glass and recoiling into the glass.

The broken line is the depth distribution of Pb-210 from Po-214 absorbed in the glass. The diffusion of the radon decay products in glass is negligible so that Pb-214 and Po-214 have the same distribution just as Pb-210 and Po-210. The depth distribution of Po-210 will always be a mixture of the two Pb-210 lines. The contribution of each line depends on the values of the transfer probabilities of the room model.

The probability for recoiling Pb-210 to reappear at the surface of the glass is calculated from the depth distribution of absorbed Pb-214. The resulting probability is 29.8%.

The absorbed decay products are found in a thin layer of less than 100 nm, see figure 1. It should be investigated if decades of household cleaning doesn't remove this layer.
Another problem arises when the vitreous glass is not regularly cleaned. Dust will cover the glass so that a fraction of the recoil nuclei will be stopped in the dust and will be wiped away when the glass is eventually cleaned. These considerations indicate the need for some tedious experimental work.

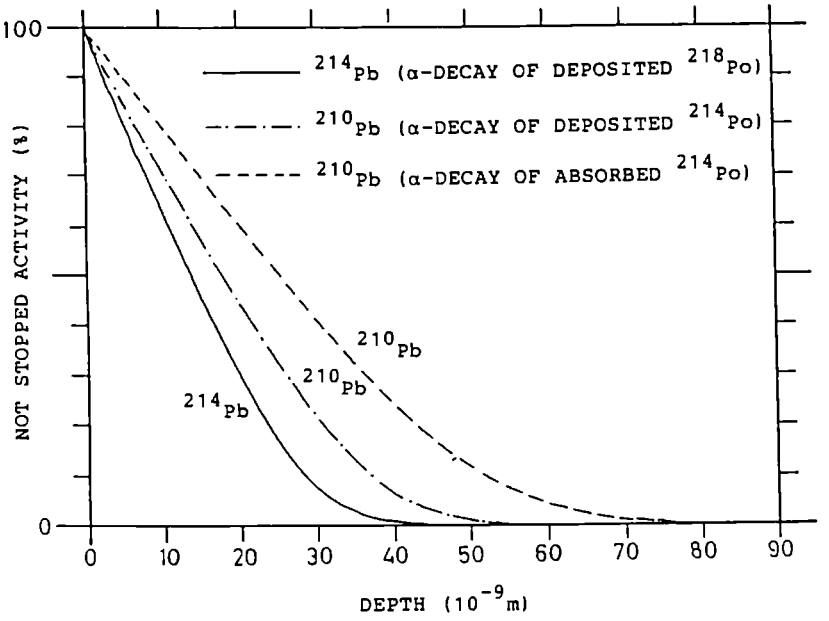

FIGURE 1. The penetration depth distributions of Pb-214 from decaying Po-218 deposited on the surface and of Pb-210 from deposited Po-214 and from absorbed Po-214.

Experimental investigations (Cornelis, 1990a; Cornelis, 1990b; Cornelis, 1991) indicate that 15% of the deposited activity remains on the surface of vitreous glass when cleaned once with a cloth containing alcohol. This may be due to radon decay products forming chemical bonds to the glass or to deposition of the decay products into microcracks present on the surface of glass.

The fraction of the Po-210 activity remaining on vitreous glass depends on the values of the parameters of the room model. Most of the variability is due to the deposition constant of the unattached decay products and due to the attachment rate. The surface activity of Po-210 is given in table 1 assuming a radon air activity of 1 Bq/m^3 during 50 years. During this period the following conditions are assumed to be present on an average.

- Ventilation rate 1.0 1/h.
- Surface to volume ratio 3 1/m (a typical value for a furnished room).
- 15% of the deposited activity is not cleaned away.
- Deposition constant of the unattached decay products 10 1/h or 20 1/h or 30 1/h. The same value is taken for all of the decay products.
- Deposition constant of the attached decay products is 1/100 of the deposition constant of the unattached decay products.
- Attachment rate 20 1/h or 40 1/h or 100 1/h.

TABLE 1. The deposited and absorbed surface activities of Po-214 and Po-210 assuming a radon air concentration of 1 Bq/m^3 during 50 years

X	λ_u	Without cleaning				With regular Cleaning
	λ_d	Deposited Po-214	Absorbed Po-214	Deposited Po-210	Absorbed Po-210	Absorbed + 15% deposited Po-210
l/h	l/h	Bq/m^2	Bq/m^2	Bq/m^2	Bq/m^2	Bq/m^2
20	10	0.12	0.04	0.07	0.07	0.08
20	20	0.16	0.06	0.09	0.10	0.11
20	30	0.18	0.08	0.10	0.11	0.13
40	10	0.08	0.03	0.05	0.05	0.06
40	20	0.13	0.05	0.08	0.08	0.09
40	30	0.15	0.06	0.09	0.09	0.10
100	10	0.05	0.01	0.04	0.03	0.03
100	20	0.09	0.03	0.06	0.05	0.06
100	30	0.11	0.04	0.07	0.06	0.07

The surface activity of Po-210 is only 3 to 13% of the radon air activity. The attachment rate and the deposition constant are about equally important. The lower and the higher values of the attachment rate are typical for rooms without and with aerosol sources. The surface activity is about a factor of two lower if aerosol sources are present in the room. Turbulence influences the deposition constant. The presence of a convection heater near the vitreous glass, for instance, will enhance the surface deposition.

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Cornelis, J., Vanmarcke, H., Landsheere, C., and Poffijn, J. (1991). Estimating radon levels from Po-210 in glass. Proceedings of the 1991 symposium on radon and radon reduction technology. EPA, Philadelphia.

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Lindhard, J., Nielsen, V., and Scharff, M. (1968). Approximation method in classical scattering by screened coulomb fields. Mat. Fys. Medd. Dan. Vid. Selsk. 36 : 10.

Head of Project 6: Dr. McLaughlin

II Objectives for the reporting period

The principal objective was the construction and testing of a radon decay product plateometer. This new type of device uses the alpha track plastic CR-39 to measure in real time alpha particles arising from the decay of the short lived radon decay products Polonium-218, Lead 214 and Bismuth-214/Polonium-214 which plateout from the air onto a glass surface. From measurements of the distribution of alpha tracks on the CR-39 using an image analysis system it is intended to demonstrate the ability of the plateometer to make determinations of the steady state surface activities of the decay products on the glass.

III Objectives for next period

The plateometer will be used under controlled laboratory conditions to investigate the effects of air flow over surfaces and aerosol characteristics on the plateout behaviour of both unattached and attached radon decay products. In collaboration with other contractors it is intended to use plateometers to investigate the variation of plateout in some high radon dwellings. Improvements in the basic instrument and associated procedures will also take place in particular in the area of image analysis software and alpha track energy resolution.

IV Progress achieved including publications

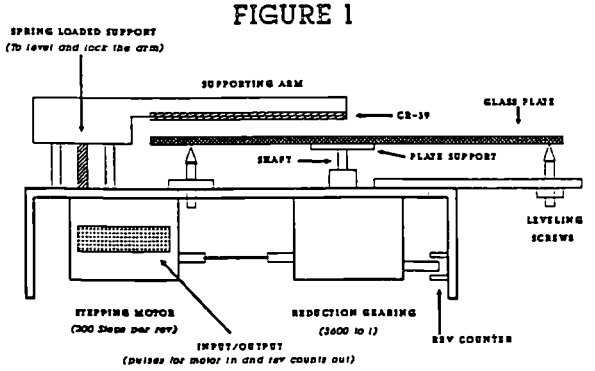
An instrument has been developed to measure in real time the specific activity of short lived radon decay products deposited or plated out on surfaces. A schematic diagram of essential features of the device and its use are as follows: A 15 cm radius circular glass plate is rotated beneath a stationary arm carrying a 90^0 sector of the alpha track detector material CR-39. It is planned also to use other suitable materials such as polished stainless steel as plateout surfaces. In order to achieve the degree of smoothness and stability of motion necessary for accurate measurement a high precision stepping motor and reduction gearing were used. The motion is monitored and controlled by an electronic control unit. The device as constructed allows the gap between the CR-39 and the glass to be maintained parallel at separations as small as 0.2 mm which is essential to good time resolution of the alpha track distribution obtained. A plateout measurement in a radon environment is carried out by rotating the glass surface, on which the plateout radon decay products have reached steady state, beneath the CR-39 alpha detector. As the steady state activity on any part of the glass enters the shadow of the CR-39 it is no longer supported by plateout and it decays. The emitted alpha particles are recorded by the CR-39 in a geometry close to 2π. The exact geometry factor at a given separation is empirically determined. The speed of rotation (which may be varied) is normally set at one revolution per six hours so that the 90^0 CR-39 sector records the alpha decay of the plateout activity on the glass over a period of 1.5 hours which approximately equals two half lives for the plateout activity. Each 1^0 sector on the CR-39 thus in effect is a decay time zone of duration 1 minute on which the integrated alpha activity is recorded.

For a point on the glass emerging from the shadow of the CR-39 plateout will re-commence and the surface activities at that point will re-grow

towards steady state. The point on the glass will continue to be subjected to plateout for 4.5 hours before re-entering the shadow of the CR-39 which is more than ample time for steady state to be re-gained. Thus the activity on the glass as it enters under the leading edge of the CR-39 is always in steady state.

On completion of an exposure the CR-39 is removed, etched and the distribution of alpha tracks on its surface is measured using an image analysis system (Quantimet Q 520). Because of the non-zero or finite size of the separation between the glass and the CR-39 it has been found that "edge effects" are present with tracks arising from decays taking place outside the geometric shadow of the CR-39. It has been empirically found that excluding alpha tracks within a 2mm strip around the edge of the CR-39 removes any edge effect interference. At the leading edge of the CR-39 this means in effect that track information in the first one minute time zone is not usable. This is analogous to the case of post sampling counting of decay product airborne activity collected on membrane filters where counts cannot usually be made during the 1 to 2 min period of transfer time of the filter from holder to counting system. The similarity between the way in which plateometer and filter alpha data are used is worth noting. In particular the approach of counting alpha tracks on the plastic in time zones (such a 1 min or any other interval) is analogous to the post sampling counting of alpha activity from filter deposits in different time intervals. Calculating the surface activities from the track data at present is by means of gross alpha procedures such as those due to Jonassen, Knutson and others. If adequate energy resolution of the alpha tracks can be achieved then the inherently more accurate alpha spectroscopy analysis procedures maybe used.

FIGURE 1



At present for the purposes of calculating the steady state activities of the decay products on the glass alpha tracks in a representative part of each chosen time zone on the plastic are counted. Considerable effort has been and will continue to be devoted to the development of software for the image analysis system such that ultimately every individual track on the plastic maybe given a set of location and time coordinates. This approach while intrinsically appropriate does give rise to practical problems in respect to the time required to locate each individual track and also subsequently in the data handling. For the present device 17500 fields of view each containing up to 100 tracks may need to be scanned. This means that as many as 2 million tracks may need to be located and

measured. Image analysis counting times up to 8 hours may be needed for this and the subsequent processing of the data. It is hoped to minimise such problems by the use of efficient algorithms and by selective scanning of the CR-39.

The plateometer essentially measures the decay of surface deposited radon decay products. Thus for a given exposure time track counting statistics improve in conditions which give rise to high surface activities. As the various room models show clearly the level of surface activity obtained in a room is very dependent on the relevant amounts of unattached and attached activities as well as air flow patterns. It is thus impossible to decide on a single operational exposure time for the instrument and in the next phase of the work it will be tested under different conditions both in the laboratory and in dwellings. As an indicator of its performance it has been found that at an indoor radon concentration of 400 Bq/m^3 in laboratory air an exposure time of about two weeks is needed to make accurate measurements of surface activities. Improvements in image analysis software are expected to reduce this time somewhat.

Contract: Bi7-047 **Sector:** C12
Title: Characteristics of radon- and thoron daughters aerosols.

1	Porstendörfer	Georg-August-Universität
2	Poffijn	Univ. Gent
3	Vanmarcke	CEN - SCK
4	Akselsson	Univ. Lund
5	Tymen	Univ. Brest
6	Falk	Nat. Inst. of Rad. Protection
7	Ortega	Univ. Politécnica de Catalunya

I. Summary of Project and Global Objectives

In all dosimetric models the particle size of the aerosol-attached activities and of the "unattached" activities (ultrafine cluster mode) are important parameters for the estimation of the radiation exposure.

The physical and chemical interaction (particle growth, cluster formation, plate-out rates) of the unattached radon and thoron decay products with trace gases (SO_2, NO_x, humidity) and other aerosol particles will be studied in radon chambers under controlled conditions. Besides this chamber experiments the exact shape of the unattached and aerosol-attached activities will be measured under realistic living conditions and the results will be compared with model calculations. Measurements of the size dependence of the regional deposition in the human respiratory tract will also be performed.

For these investigations the sensitivity and efficiency of different experimental techniques for measurements of the size distributions (active and inactive) have to be improved and modified techniques will be developed (e.g. diffusion batteries, impactors, electrostatic classifiers). These techniques have to be calibrated with monodisperse aerosol particles. The different methods including different data evaluation methods have to be compared during joint exercises.

Head of Project 1: Dr. Porstendörfer

II Objectives for the reporting period

Design of a radon chamber to study the influence of humidity, trace gases, aerosol particles and radon gas concentration on the cluster formation and growth processes of the short-lived radon decay products.

Improvement and calibration of size fractionating methods (e.g. impactors, diffusion screens) with the purpose to measure accurate size distributions in the diameter size range less than 20 nm (unattached region) and to investigate differences between the size distributions of the different short-lived radon daughters.

Continuation of measurement of size distributions of the thoron decay poducts in indoor and outdoor atmospheres.

III Objectives for next period

The construction of the radon chamber including classifier and rotating screen disk will be finished and first measurements will be performed.

Size distribution measurements of the radon decay products RaA-RaC are planned with the on-line-alpha-impactor after the end of the calibration procedure. The size distribution measurements of the thoron decay products will be continued.

Other parts of the project deal with the influence of air cleaners on the change of the size distributions and on the amount of the unattached fraction and with the particle growth in atmospheres with high humidity.

IV Progress achieved including publications

A radon chamber of 0.05 m^3 was designed to study the dynamics of cluster formation processes of radon progeny in controlled atmospheres. The major parts of this chamber are an electrostatic classifier and a rotating screen diffusion disk for measuring radioactive particles in the diameter size range between 0.5-10 nm. Especially the dimension and the inlet of the cassifier was designed to minimize diffusion losses of small particles.

It is planned to measure the inactive number size distribution by a commercial available condensation particle counter (TSI, CPC Model 3025) in connection with the screen diffusion technique and the classifier. The capacity of this CPC was tested with monodisperse NaCl and Ag particles. A 50% registration efficiency of 2.9-3.1 nm was found (Fig. 1) and a slight decrease of the efficiency from 1 to 0.96 in the particle concentration region above 60,000 cm^{-3}. The influence of the chemical substance of the aerosol particles on the detection efficiency (hygroscopic or nonhygroscopic) can be neglected.

Activity size distributions of RaB and RaC are usually determined by conventional impactors using gamma-spectroscopic methods after air sampling. However, more detailed investigations require the measurement of the size distributions of the aerosol-attached fraction of all nuclides RaA-RaC. For this purpose a low level cascade impactor was designed and constructed where the size fractionated activities can be measured during air sampling by alpha-spectroscopy. This impactor consists of nine

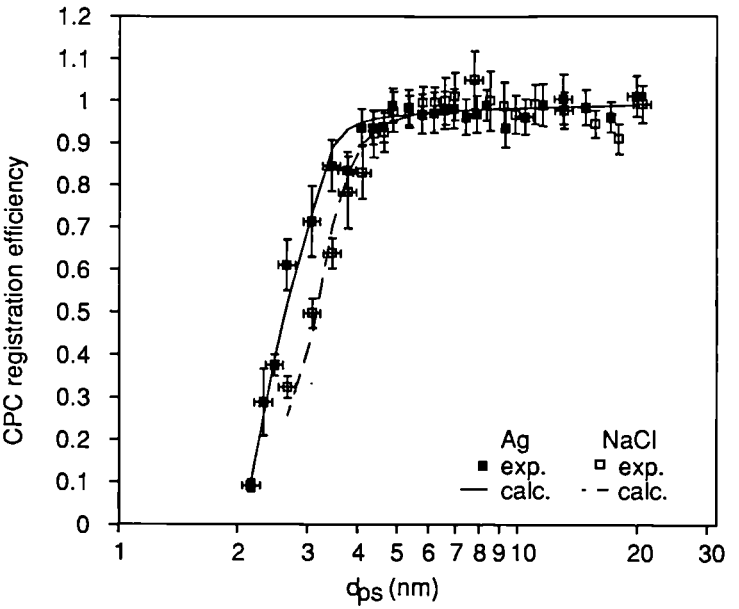


Fig. 1: Experimental and calculated registration efficiency of the TSI model 3025 CPC.

stages and a back-up filter and works at a flow rate of 5 m^3/h. The aerosol-attached activities of each stage are deposited on surface barrier detectors with large areas (2000 mm^2) covered with thin foils (4 µm). The energy resolution is sufficient to determine all size distributions of RaA-RaC from the emitted alpha particles. Rotating detector foils yield sampling times of some weeks for measurements in the human environment at low activity concentrations. The calibration of this impactor is in progress.

Size distribution measurements of the thoron decay product ThB (^{212}Pb) were continued in indoor and outdoor environments using a low pressure impactor (type BERNER). More than 80% of the activity is associated to aerosol particles in the accumulation mode with average diameters of 217 nm indoors and 330 nm outdoors, respectively (see table 1). From these measurements it can be estimated that about 10% of the activity is attached to particles in the nucleation region with median diameters less than 82 nm. The ThB size distributions are similar to the size distributions of the short-lived radon progeny.

Instrumentations to measure the airborne radon daughters and the unattached fraction has been intercompared with the Swedish Rad. Prot. Institut (R. Falk) and the University of Brest (G. Tymen) during a CEC workshop in Göttingen.

Table 1: Average ThB size distributions measured in indoor (without additional aerosol sources) and outdoor atmospheres.

comment/no. of meas.	$AMAD_2$ (nm)	$sigma_2$	f_2	$AMAD_3$ (nm)	$sigma_3$	f_3	$AMAD_4$ (nm)	$sigma_4$	f_4
indoor/10	< 82	?	0.14	217	1.8	0.86	-	-	-
outdoor/44	< 82	?	0.11	330	2.0	0.87	4240	1.6	0.02

Publications

Kesten, J.; Reineking, A.; Porstendörfer, J. Calibration of the TSI model 3025 ultrafine condensation particle counter. to be published in Aerosol Science and Technology 15 (1991).

Reineking, A.; Butterweck, G.; Kesten, J.; Porstendörfer, J. Unattached fraction and size distribution of aerosol-attached Rn and Tn daughters in realistic living atmospheres and their influence on radiation dose. Proceedings of the 29th Hanford symposium on health and the environment. Indoor radon and lung cancer: reality or myth. October 1990, Richland, WA, USA.

Head of Project 3 : Dr. Vanmarcke - Head of Project 2 : Dr. Poffijn

II Objectives for the reporting period

Joined project between SCK/CEN (project 3) and RUG (project 2).

To determine the influence of turbulence on the deposition rate constant of the unattached decay products of radon. In particular it will be investigated if there is a difference in deposition rate between the different unattached decay products.

To design a measurement system to estimate directly the deposition of the decay products of radon in the nasal cavity and in the tracheobronchial region.

III Objectives for next period

To build a measurement system based on wire screen penetration theory to provide estimates for the activities deposited in the nose and in the bronchia. The system will allow to test different sets of screens and to be operated at different face velocities. The three sampling heads will be calibrated in laboratory conditions and during a joint exercise.

IV Progress achieved including publications

The deposition rate constants of unattached Po-218 and Pb-214 were evaluated from the measured radon and decay product concentrations in a 1 m^3 chamber as a function of the degree of turbulence. The turbulence is induced by ventilation and by generating heat. The results are shown in figure 1 and figure 2. The progress of the deposition rate constants is the same for both decay products. The value for Pb-214 is however significantly lower than that of Po-218. The details of the measurements and of the calculations are published in Vanmarcke et al. (1991).

The theory of Crump and Seinfeld was fitted to our deposition data and the coefficient, k_e, and the exponent, n, in the definition of the eddy diffusion coefficient was examined.
k_e was found to be proportional to λ_V^3 (ventilation) and $W^{3/2}$ (generated heat), which agrees with the value reported by Lin et al. (1953) and disagrees with the one reported by Crump et al. (1983). With our data we are not able to discriminate between the values of the exponent, n, reported in the literature.

Figure 1 and figure 2 indicate a clear difference between the deposition rate constants of Po-218 and Pb-214. The corresponding difference in diffusion coefficient is about a factor of three, which means that the diameter of the unattached Pb-214 particle is twice as large as the diameter of the unattached Po-218 particle. The difference could be due to the physical and chemical properties of the two elements.

The models currently used for predicting the concentrations of the decay products in the indoor environment assume equal deposition rate constants for Po-218 and Pb-214. However a significantly lower deposition rate constant for Pb-214 would explain some of the difficulties we found in fitting the model to the data we collected during our case studies in

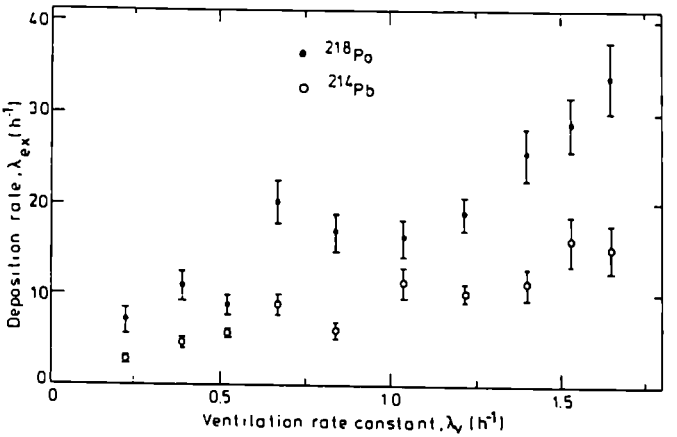


FIGURE 1. The deposition rate constants of unattached Po-218 and Pb-214 as a function of the ventilation rate.

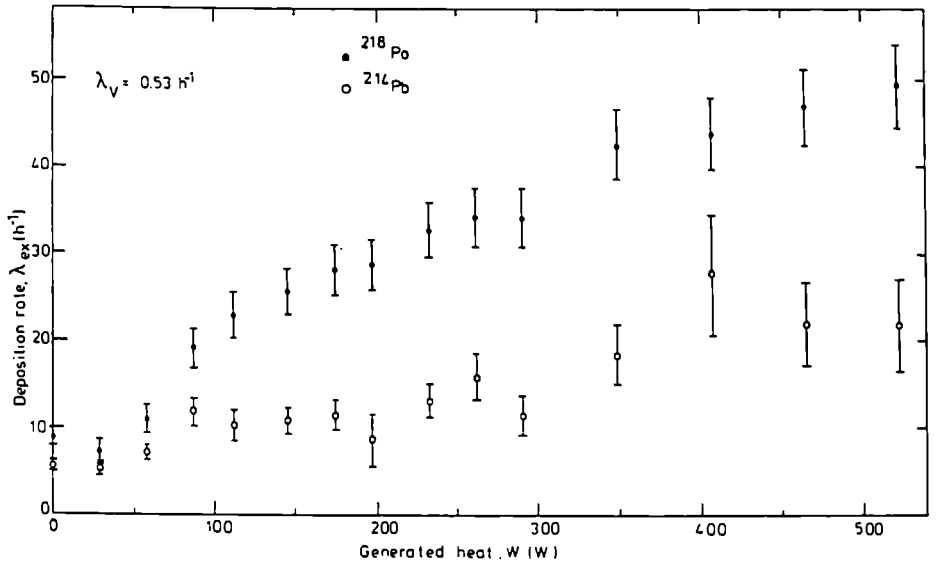


FIGURE 2. The deposition rate constants of unattached Po-218 and Pb-214 as a function of the generated heat.

houses. Indeed, when we apply the deposition rate constant of the unattached Pb-214 to the unattached Po-218, we systematically underestimate the measured radon concentrations.

According to Hopke et al. (1990) it is possible to design a measurement system based on wire screen penetration theory to simulate the deposition of the decay products in the nasal cavity and in the tracheobroncial region. The measurement system consists of three sampling heads. An open-faced filter giving the total airborne activity, a screen covering the filter yielding the nasal deposition and five screens covering the filter yielding nasal and bronchial deposition. The sampler is under construction. The three simultaneous measurements must be comparable within 1% in order to give meaningful results. Limiting orifices and a special design of the sampling head have been applied to meet this goal.

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Crump, J.G., Flagan, R.C., and Seinfeld, J.H. (1983). Aerosol Sci. Technol. 2:303-309.

Hopke, P.K., and Ramamurthi, M. (1990). Health Phys. 58:291-295.

Lin, C.S., Moulton, R.W., and Putnam, G.L. (1953). Ind. Eng. Chem. 45:640.

Vanmarcke, H., Landsheere, C., Van Dingenen, R., and Poffijn, A. (1991). Aerosol Science Technol. 14:257-265.

Head of Project 4: Prof. Akselsson

II Objectives for the reporting period

The objectives for the reporting period were a) to further develop the experimental facility for radon - aerosol studies, b) to develop alfa-spectrometry using detection over large areas, c) to develop a multi-jet impactor, which combines a low cut-off diameter with a high flow rate and d) to perform introductory controlled studies on the interaction between radon daughters and aerosol particles.

III Objectives for next period

The objectives for next period are to design a five-stage multi-orifice impactor for activity distribution studies, to further develop the technique to measure the size distribution of the activity by using a differential mobility analyzer and c) to apply the techniques for well-controlled conditions and for a limited number of realistic environments.

IV Progress achieved including publications

During the reporting period the experimental facility for radon studies at Lund Institute of Technology has been further developed and introductory studies regarding radon daughter - aerosol particle interaction have been performed. A multi-orifice impactor stage has been constructed and tested.

Figure 1 shows a schematic view of the experimental facility. The room itself (volume = 19.8 m^3) is housed in a trailer. The walls are made of urethane, covered with glass-fibre, plastic and plastic paint. Inside, there are 12 supply and 12 extract terminals along the long-sides of the room.

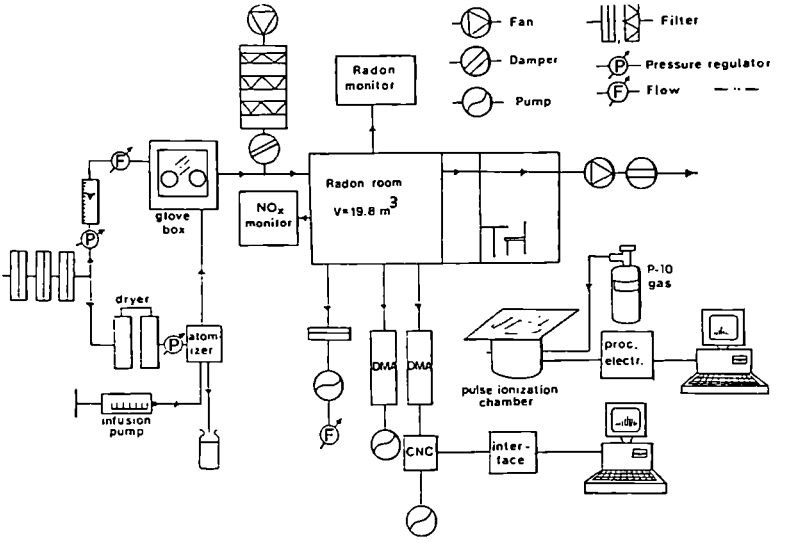


Fig.1 Schematic view of the experimental facility.
Abbr. DMA=Differential Mobility Analyzer.
CNC=Condensation Nucleus Counter

The room has its own ventilation system: Pressurized air from a compressor is filtered and dried and then supplied to the room. Various aerosols can be added and mixed in the glove-box. The instruments for monitoring the concentrations of radon, radon-progeny and NO_x, are located outside the room. The size-distribution of the passive and the active aerosol is also measured. Plate-out is measured with a large-area pulse-ionization chamber. The unattached fraction is measured with the single screen/filter technique.

Measurements show that the room can provide an atmosphere with an unattached fraction ranging from 0 to 100 % (fig. 2) and an equilibrium factor ranging from 0.1 to 0.7 (fig. 3) when the room is ventilated at a rate of 0.5 h^{-1}.

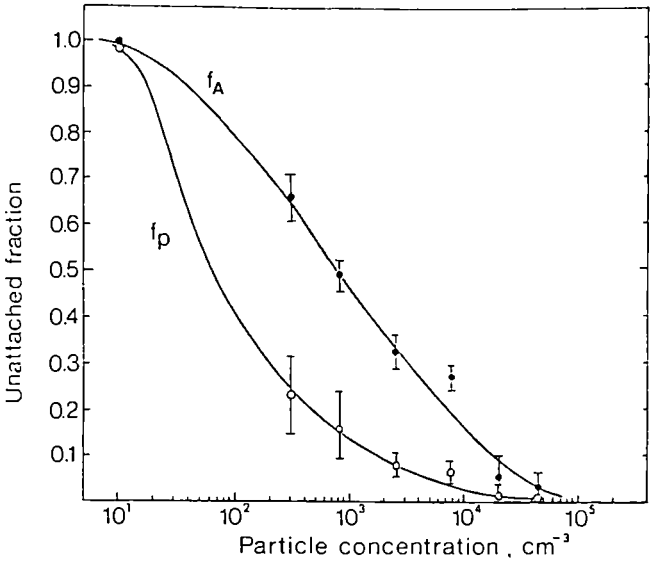


Figure 2. Unattached fraction, fp, and ratio of unattached to total activity of RaA, f_A at various particle concentrations. Ventilation rate = 0.5 h^{-1}. NaCl aerosol: geometric mean diameter = 100 nm, activity, mean diameter = 250 nm, geometric standard deviation regarding diameter = 2.0

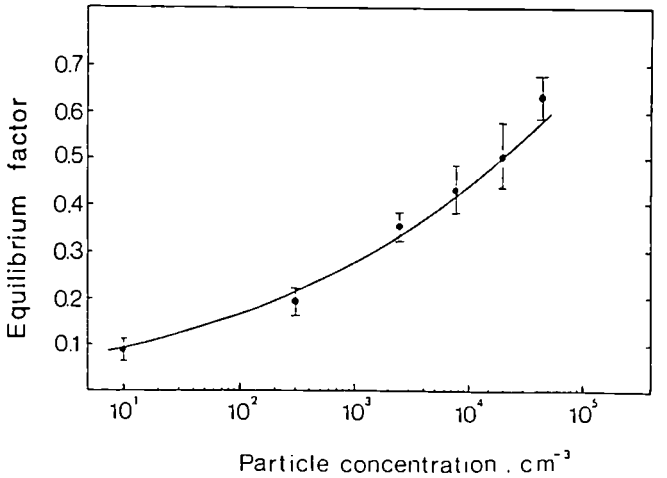


Figure 3. Equilibrium factor at various particle concentrations. Ventilation rate = 0.5 h^{-1}. NaCl aerosol: geometric mean diameter = 100 nm, activity mean diameter = 250 nm, geometric standard deviation regarding diameter = 2.0

An impactor stage has been constructed and an experimental set-up for testing and calibrating impactors for sub-micron particles has been designed.

In order to optimize the impactor design, the collection characteristics have been studied as a function of jet-to-plate distance and of the Reynold's number of the jet. The current impactor stage consists of 2700 laser-drilled orifices. The diameter of the orifice plate is 26 mm. The orifice diameter is 50 µm. A cut-off diameter between 50 and 100 nm can be achieved. The air flow is about 30 l/min. and the pressure drop over the stage is about 20 kPa.

Resulting Publications

An Experimental Facility to Simulate Radon-Progeny Behavior in Dwellings
Proceedings of the 29th Hanford Symposium on Health & the Environment - "Indoor radon and lung cancer - reality or myth?", Richland, Wa, USA, October 1990
Eklund P, Bohgard M

Multi-Jet Impactor with 50 Micrometer Diameter Nozzles for Uniform Deposition of Submicron Particles
Abstract, Proceedings of the Symposium of the Nordic Society for Aerosol Research, Gothenburg, Sweden, November 1990
Gudmundsson A, Bohgard M, Hansson H-C

A Full-Scale Experimental Set-Up for Determining Relevant Parameters for Radon Daughter Behaviour in Dwellings
Abstract, Proceedings of the Symposium of the Nordic Society for Aerosol Research, Gothenburg, Sweden, November 1990
Eklund P, Bohgard M

Head of Project 5: Dr. Tymen

II Objectives for the reporting period

In some houses of Brittany having high radon levels, it has been planned to carry out two simultaneous studies : firstly search radon sources by using classical investigation method for measuring radon, PAEC, γ rate and radon emanation from ground and walls an secondly, study behavior of Rn D size distribution in connection with ambient particle size distribution, whereas unattached fraction was also examinated.

III Objectives for next period

Priority will be given to the improvement of activity data obtained from the SDI 2001 sampling device particularly by using α-spectrometry, or if not possible by trying to optimize the determination of RnD concentrations on samples by following continuously global α-activity decay as a function of time, in order to achieve a better estimate of individual RnD size distribution. Parallely, size distribution of unattached fraction will be also investigated. Otherwise tentative will be made to examine, in field, influence of air cleaners on RnD behavior.

IV Progress achieved including publications

In order to identify sources of radon-222 and to caracterize simultaneously indoor radon daughters (Potential α energy, equilibrium factor, size distribution, unattached fraction) in houses with high radon level, specifical study have been carried out in 5 houses of Brittany, selected on following criteria : Rn concentration > 400 $Bq.m^{-3}$, PAEC > 1 $\mu J.m^{-3}$, TLD γ dose > 100 mrad/year. Corresponding values were obtained during field compaign of 1985-1989 in the living part of these houses.

Table 1 resumes different technical procedure used in this study.

From Measurements of Radon emanation rates through walls and ground, in conjunction with PAEC and γ level measurements, we have been able to locate sources on mud floor constituion the basement of those houses.

Indeed, Radon emanation rate were measured in the $(5\text{-}200).10^4.at.m^{-2}sec^{-1}$ range which is 5 to 10 times more than the usually encountered value of 10^4 $at.m^{-2}sec^{-1}$

In those same houses, RnD size distributions have also been investigated and compared with natural particle size distribution.

An exemple of such a comparison is given on fig. 1. Table 2 summarizes overall results obtained in theses investigated houses.

Concerning fp and Activity Mean Diameter mesurements, values we have measured seems to be in agreement with those of PORSTENDÖRFER-REINEKING group . However AMD of individual RnD appear to be divergent. This can be explained by the fact that, because statistical errors of α counting have a significative incidence on RnD concentration calculated on each sample by the Tsivoglou method, data treatment becomes more inconsistent. It is clear that for our concern, an immediate effort has to be spent on improvement of our counting and analysis procedure of data.

	Measured parameters	Sampling or technical process	Analysis
indoor radon daughter study	Instantaneous PAE ($\mu J/m^3$)	Filter (MIMIL)	ROLLE Method
	Time integrated PAE ($\mu J/m^3$)	Special air-sampling equipment (active CEA dosemeter)	α-track counting
	Unattached fraction	Wire-screen + filter	THOMAS Method
	Radon daughter size distribution	Andersen impactor+6 channels granular bed diffusion battery (SDI2000)	Non-linear TWOMEY Algorithm method
	Particle size distribution	TSI 3040 + CPC	Non-linear TWOMEY Algorithm method
Identification of sources	Rn-222 activity concentration (Bq/m^3)(indoor and outdoor)	Continuous air sampling	ionization chamber
	Rn-2222 emission flux (At/m^3)(indoor and outdoor)	Accumulation	LUCAS flask
	Rn-222 activity in soils	Sampling in 0.6 m depth	LUCAS flask
	τ radiation (cps/sec)		τ counting (SPP2)
	Ventilation	Helium injection	Mass spectrometry

Table 1. Technical processes in operation for a complete analysis of a radon problem in houses (cooperation LPARA-CEA).

Unattached fraction

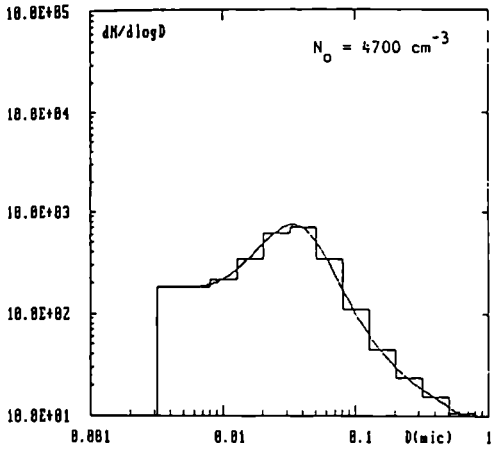
$1.1\ \% < f_p < 31\ \%$
Mean value 10.3 %

Attached fraction

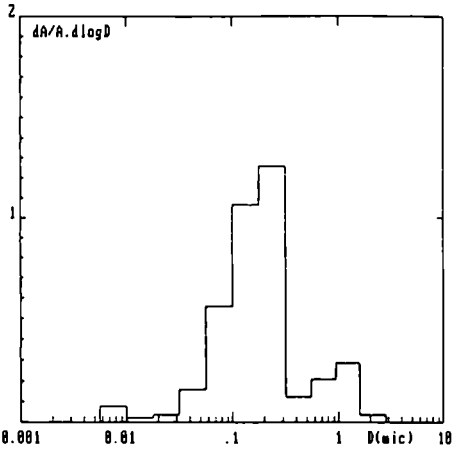
0.11 μm < A.M.D. Global < 0.2 μm
0.02 μm < A.M.D. Po218* < 0.08 μm
0.07 μm < A.M.D. Pb214 < 0.13 μm
0.1 μm < A.M.D. Bi214 < 0.23 μm

*unreliable

Table 2



Particle size distribution (living room house n° 4, no aerosol source)



α-activity size distribution (unattached fraction f_p = 16 % not reported here).

Fig. 1

Head of Project 6:Dr. Falk

II Objectives for the reporting period

Experimental studies on radon daughter deposition in the human respiratory tract during nasal and mouth breathing is performed. The dose from inhaled radon daughter in indoor atmosphere is to a substantial part due to the "unnattached" part. An attemt to assess the fraction of radon daughters penetrating through the nasal cavity and deposited in the bronchial and lung region is made for both the unattached and the attached radon daughters. Assembling the instruments for the characterization of the exposure environment as well as the instruments for measurement of regional deposited radon daughter.

III Objectives for next period

Repeated measurements of the deposition of radon daughters in the respiratory tract is planned with special attention to the deposition pattern of the unattached fraction. Size distribution of the unattached fraction will be measured. Our goal is that the experimentally found data on the size and location of the deposited radon daughters will have a quality good enough to improve the dose calculation of inhaled radon daughters.

IV Progress achieved including publications

RESPIRATORY TRACT DEPOSITION OF RADON DAUGHTERS IN HUMAN AIRWAY

Experimental studies of the fraction of inhaled radon daughters deposited in the human airways have been carried out with a combination of two different techniques. In parallel with the exposure of the subject, measurements of the air borne radon daughters has been performed with a system of fine mesh wire screens that simulates the penetration and deposition in nasal and tracheobronchial region.

Exposure conditions

A radon-chamber having a volume of 30 m^3 is used for the exposure. The concentration of Radon-222 and Radon-222 decay products, temperature, aerosol concentration, ventilation and humidity is continuously recorded during the exposures. Two extreme exposure conditions have been chosen during this first stage of experiments, namely high aerosol concentration giving virtually no airborne unattached fraction and low aerosol concentration when the unattached fraction dominates.

The high aerosol concentration is achieved by burning organic material and running a condensation aerosol generator. Before exposure the aerosol is allowed to "age" for at least 1 hour to achieve stable condition during exposure. During exposure the aerosol concentration was more than $50 \cdot 10^3$ p/cm^3 and the aerosol size distribution can be assumed to have a single mode with a mean diameter in the range of 0.1-0.3µm.

The low aerosol concentration is achieved by closing the ventilation system of the radon chamber and running an air cleaner inside the room. The air cleaner was switch off at least 1 hour before exposure to allow stable conditions during exposure. An aerosol concentration less than 300 p/m^3 could be maintained in this way.

Assessment of the unattached fraction is performed using fine mesh wire screens for collection and subsequent α-spectrometric measurements of the screens and back-up filter.

Simultaneous determination of the radon daughter concentration is performed in both inhaled and exhaled air to assess the fraction of inhaled radon daughter deposited in the whole respiratory tract, including the nasal and bronchial region,(Method A). See FIG 1.

The assessment of the regional deposition on inhaled radon daughters is done with external γ-measurement of the subject a short time after the end of the exposure. (Method B). These measurements are performed in a low-level counting laboratory with a low and stable background. One NaI(Tl) detector (125 mm diam., 100 mm height) surrounded by a 20 mm lead cylinder was placed over the subjects head och a second detector placed over the chest. A lead collar was applied around the subjects neck to narrow the sensitive region. See FIG 2.

A few exposures have been performed using method A during the whole exposure time. As the activity of the separate radon daughters deposited can be calculated the measurement with method B is done with known total activity in the whole respiratory tract. Mouth breathing with high aerosol concentration can be assumed to give deposition of radon daughters only in the alveoli region, giving a calibration of the lung counter for this situation.

The results from the few exposures measurements performed is summarized:

Mouth breathing under resting condition with high aerosol concentration (less than 0.5 % unattached fraction) was found to give a total deposition of inhaled radon daughters of 25 %.

Nose breathing under resting condition with low aerosol concentration (~ 78 % unattached fraction) was found to give a total deposition of 85 %. The radon daughters was found to be deposited in the head and bronchial part of the airways, about half of the deposition in the nasal region. There is for the moment not possible to give more exact values due to experimental difficulties to achieve accurate values on the amount of activity deposited in different parts of the respiratory tract. This is in the first place due to the relatively low levels of activity to be measured, but is also influenced by the poor spatial resolution of the lung detector due to relatively high energies of the γ-lines to be measured.

It is also found that exposure to only radon-222 without daughter products contribute to the γ-emission from the body.

Additional exposures and variation of the measuring geometry for the lung counter is planned to be carried out to improve the accuracy of the regional deposition.

The instrumentation to measure the airborne radon daughters and the unattached fraction has been calibrated and an intercomparison has been carried out with the Isotopenlaboratorium in Göttinge. Very good agreement was obtained.

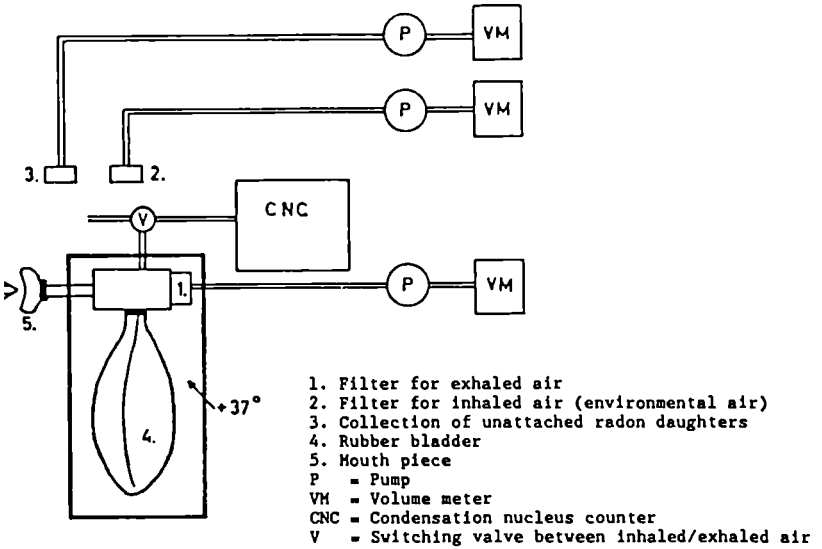


Fig. 1 Experimental set-up for measurement of the total deposition of inhaled radon daughters, Method A.

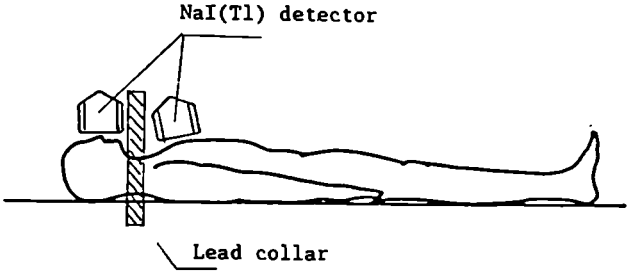


Fig. 2 γ-measurement of the regional deposition of radon daughters, Method B.

Head of Project 7: Dr. Ortega

II Objectives for the reporting period

Training of the spanisch group about metrological methods set up by the Isotopenlaboratorium. Preparation of an experimental facility linked to the german group's techniques.

III Objectives for next period

To do some measurements on radon and thoron decay products concentration in the air, the equilibrium factor and the unattached fraction under realistic natural conditions in the Mediterranean coast.

IV Progress achieved including publications

1 - Development of charcoil dosimeter, "canister", with silicagel layer placed with the aim of controlling the effects of the humidity. The effect of this parameter has been checked.

2 - Determination of gamma thoron daughters emitters by high resolution gamma spectroscopy.

3 - Setting up of several alpha spectroscopy chain detectors for active measurements of short-lived radon decay products concentration in the air and the unattached fraction.

4 - Setting up of a electrostatic chamber, furnished by the Isotopenlaboratorium (IL) from Göttingen for the determination of the radon and thoron in the air.

5 - Training courses at the IL of the University of Göttingen.

TITRE DU PROJET N°1 CONTRAT B16-122-F (D)

Evaluation des irradiations industrielles. Base de données européennes EUROGRID.

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I - Objectifs de la période 1/1/1990 - 31/12/1990

Projet 1.a : **Base de données dans la grille européenne EUROGRID**
Pour permettre son utilisation sur micro-ordinateurs, la base de données EUROGRID a été transférée sur disquettes. Trois logiciels ont été développés pour en faciliter la consultation. Le premier, nommé QUERY, permet de visualiser le maillage de l'Europe, de formuler des requêtes limitant les recherches à un pays, une région, un produit..., de faire apparaître les grandes mailles (10 000 km^2) répondant à la requête et les valeurs des paramètres sélectionnés pour ces mailles. Ce logiciel est disponible gratuitement. Une version purement numérique (EUROGRID) est également disponible. Le troisième (EUROVISU), plus élaboré, nécessite l'acquisition du logiciel MICROSTATION. En plus des propriétés du logiciel Query, il permet d'étendre les recherches aux petites mailles (100 km^2).

Projet 1.b : **Systèmes d'aide à la décision**
En 1990, une maquette du système à été réalisée, permettant une analyse côut-efficacité des contre-mesures concernant les produits alimentaires contaminés à la suite d'un accident. Un système de maintient de la cohérence a été élaboré, dans le but de tenir compte d'éléments qualitatifs appelés "jugements d'experts".

II - Objectifs de la période 1/1/1991 - 31/12/1991

Projet 1.a : **Base de données dans la grille européenne EUROGRID**
Cette période sera consacrée à l'achèvement de la mise au point du logiciel EUROVISU. Par ailleurs, l'actualisation des données sera entreprise, de même que l'extension de la base de données aux pays limitrophes de la Communauté. (Dans un premier temps, cette extension sera limitée aux populations). Une étude de faisabilité sera entreprise pour tenir compte des transferts par l'eau. On tentera d'estimer les transferts de produits agricoles entre mailles, et d'évaluer les paramètres permettant l'estimation des doses recues par inhalation et par irradiation externe.

Projet 1.b : **Systèmes d'aide à la décision**
Il s'agira durant cette période d'identifier les experts, de recueillir l'expertise et de l'organiser sous forme de règles. La participation à des exercices et la rédaction d'un questionnaire seront les moyens utilisés pour parvenir à ces fins. Ces procédures devraient également mettre en évidence les lacunes de la maquette et démontrer l'intérêt d'un système opérationnel.

III - Description des travaux accomplis en 1990

Projet 1.a : **Base de données dans la grille européenne EUROGRID**
Le programme de travail pour l'année 1990 porte sur les points suivants:

1 - Transcription de la base de données EUROGRID sur micro-ordinateurs :
Initialement proposée sur bandes magnétiques, la base de données EUROGRID a été transcrite sur disquettes. Cette nouvelle version à été distribuée aux éventuels utilisateurs (NRPB, KFK, Ecole polytechnique de Madrid), et est disponible sur simple demande.

2 - Généralisation des petites mailles :
Pour rendre compte des conséquences d'un l'accident à courte distance de l'émission, il est

nécessaire de disposer des populations, des surfaces et des productions agricoles sur une maille plus fine. L'acquisition des données dans des mailles de 100 km^2 a donc été entreprise. Deux méthodes différentes sont employées: En ce qui concerne les populations, les recensements permettent d'accéder directement à l'échelle des petites mailles. Pour ce qui est des surfaces et productions agricoles, la notion de secret statistique ne permet pas une définition aussi fine, et un système de pondération à partir de données recueillies à une échelle plus grande est en cours d'élaboration.

3 - Développement de logiciels permettant la consultation de la base de données : L'acquisition du logiciel graphique MICROSTATION a permis de développer un système (EUROVISU) de visualisation des données relatives aux grandes et aux petites mailles (respectivement 10 000 et 100 km^2). Parce qu'il n'existe pas de version run-time de ce logiciel, sa distribution à d'autres utilisateurs est impossible.
Pour compenser cette lacune, nous avons développé nos propres systèmes d'interrogation de la base de données, dont les possibilités sont bien entendu inférieures à celles du produit développé sous MICROSTATION (plus précisément, ils ne permettent que la visualisation des données relatives aux grandes mailles):
Les deux systèmes proposée sont entièrement conversationnels et permettent de formuler des requêtes, et de limiter ainsi les recherches à un pays, un paramètre, une région. Par exemple, une requête peut consister à rechercher quelles sont les mailles recouvrant l'Italie, pour lesquelles la population est supérieure à 500 000 habitants et la production annuelle de blé comprise entre 1 000 et 10 000 tonnes. Les grandes mailles (10 000 km^2) répondant à la requête et les valeurs des paramètres associés apparaissent alors sous forme de tableaux (logiciel EUROGRID) ou de cartes (logiciel QUERY).
La notice d'installation et la description des fonctions de ce système sont en cours d'élaboration, et l'ensemble pourra être distribué en 1991.
Enfin, les quantités de lait collectées dans chaque maille ont été ajoutées à la base de données.

Projet 1.b : Systèmes d'aide à la décision
Les travaux menés au CEA (Système-expert DACFOOD):
Une maquette de système expert a été réalisée: Elle permet une classification, sur la base d'une étude coût-efficacité, des contre-mesures qui peuvent être envisagées vis-à-vis de produits alimentaires contaminés lors d'un accident nucléaire. Pour parvenir à cette classification, l'utilisateur doit définir des lots de produit contaminés (un lot se caractérise par un produit, une quantité et l'activité massique des principaux radionucléides) ou introduire les activités déposées au sol. Le système calcule alors les doses reçues par ingestion, et ajoute éventuellement les doses dues à l'inhalation et à l'irradiation externe (Ces dernières sont introduites en tant que données). L'impact de chacune des contre-mesures (destruction, stockage, transformation,...) est évalué, et le coût de chacune est estimé. Compte tenu du critère adopté par l'utilisateur (dose collective, dose efficace individuelle, dose à un organe,...) une classification est alors proposée.
Un rapport d'avancement est en cours de rédaction. Ce rapport sera essentiellement constitué d'une description des fonctions du système et d'un manuel d'utilisation.
Le recueil d'expertise : dans son état actuel, le logiciel DACFOOD ne prend en compte que les doses et les coûts. Il reste à prendre en compte les jugements d'experts : une méthode a été développée pour maintenir la cohérence des différents jugements, mais la saisie des données d'expertise reste à faire.
Enfin, un contrat est en cours avec TNO (Pays-Bas) sur "l'incorporation des paramètres psycho-sociologiques dans le système expert d'aide à la décision, ainsi qu'une étude de sensibilité des différents paramètres"; il doit aboutir à la rédaction d'un rapport final en Juin 1991.

Publications

Deux résumés ont été soumis au prochain congrès de l'IRPA (Montréal, Mai 1992) :
1 - La base de données européennes EUROGRID : Problèmes méthodologiques et développements récents (S. BONNEFOUS et A. DESPRES);
2 - DACFOOD : Un système d'aide à la décision en cas de contamination de la chaîne alimentaire (A. DESPRES, A. DIAZ, D. SOULATGES).

TITRE DU PROJET N°2 CONTRAT B16-122-F (D)

Evaluation du détriment objectif - Recherches sur l'homme.

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1. OBJECTIVES OF THE PROJECT

Le projet se propose de comparer les méthodes d'évaluation du détriment chimique avec celles utilisées pour le détriment radiologique dans une perspective d'harmonisation des critères utilisés en matière de gestion du risque. En effet, on attend généralement d'une comparaison de l'impact des nuisances notamment sanitaires, qu'elle mette en opposition des données quantitatives cohérentes : c'est une des bases de la gestion des risques. Dans le cadre d'une confrontation du détriment radiologique avec celui des agents chimiques, la cohérence veut qu'on mette en regard des risques cancérogènes sous forme d'incrément par unité d'exposition ce qui, de toute évidence, ne peut être systématiquement envisagé dans le domaine chimique. La comparaison doit se situer en amont et porter sur les choix méthodologiques d'évaluation du potentiel cancérogène : c'est une affaire de rationalité biologique.

2. OBJECTIVES OF THE REPORTING PERIOD : 1990

Dans la suite logique des travaux des années antérieures relatifs à l'examen critique des bases de données exprimentales sur la cancérogénèse chimique, la procédure d'évaluation critique définie pour les composés inorganiques des métaux non ferreux a été appliquée aux radionucléides (plutonium). Il s'agissait dans un premier temps d'établir un relevé des données expérimentales sur la cancérogénèse du plutonium chez l'animal dans des fiches normalisées établies sur la base des recommandations communautaires en matière de bonnes pratiques de laboratoire en utilisant la même grille de lecture que pour les agents chimiques cancérogènes avec : établissement d'autant de fiches que de combinaisons forme physicochimique/forme isotopique du plutonium-voie d'administration-espèce-lignée animale et à l'intérieur de chaque fiche ainsi définie, individualisation d'autant de groupes expérimentaux que de niveaux de dose, de solvants ou de protocoles d'exposition combinée avec d'autres agents cancérogènes. La finalité du relevé est :

1. de rassembler les résultats dans des tableaux synoptiques permettant de confronter de façon homogène, le "plus petit dénominateur commun" (1 forme donnée du composé-1 voie d'administration-1 espèce-1 lignée animale) des expérimentations effectuées sous divers protocoles : ETAPE DESCRIPTIVE

2. de procéder à une analyse de la validité méthodologique des protocoles expérimentaux: ETAPE ANALYTIQUE utilisant l'algorithme d'évaluation mis au point au cours des années précédentes pour les cancérogènes chimiques.

3. de prendre en compte la pertinence des résultats pour évaluer le risque chez l'homme : ETAPE SYNTHETIQUE faisant intervenir la représentativité des voies d'administration vis-à-vis des conditions réelles d'exposition humaine.

La procédure a pour objectif premier L'IDENTIFICATION ET LA SPECIATION DU RISQUE CANCEROGENE en rapport avec les formes physicochimiques d'un élément déterminé : l'approche est qualitative par le classement des résultats en tout ou rien (possibilité de conclure sur la présence ou l'absence d'effet) croisé avec le classement en fonction de l'extrapolabilité du résultat à l'homme (voies relevantes ou non).

L'objectif second est de rassembler les données utiles pour la QUANTIFICATION DU RISQUE CANCEROGENE par la juxtaposition des lots expérimentaux concernant un composé administré sous une voie donnée dans une lignée animale donnée en fonction de la dose (données pouvant provenir d'expérimentations différentes).

Les fiches normalisées des données expérimentales sur les dérivés du plutonium ont été constituées et l'analyse de la validité méthodologique des protocoles expérimentaux a été effectuée. A la différence des cancérigènes chimiques, les expérimentations sur les dérivés du plutonium font l'objet de multiples publications spécialisées (comportement métabolique, effets à court terme, effets à long terme, synthèses sur les relations dose-effet) mais aussi de descriptions partielles (type rapport périodique à des instances subventionnant la recherche) de sorte que le recueil des données relatives à une cohorte donnée d'animaux reste parfois difficile (ce qui impose le repérage des animaux par le code d'identification du laboratoire pour recoupement des données les concernant au travers des publications successives). Au delà de l'exercice d'identification des résultats expérimentaux méthodologiquement acceptables, un complément d'analyse s'avère nécessaire pour intégrer les relations dose-effet (celles ci sont partiellement décrites dans les articles de cancérogénèse et obligent à prendre en compte un système complexe de renvois documentaires sur la dosimétrie). Il s'agit donc de préciser des données quantitatives sur le comportement métabolique du dérivé du plutonium dans la lignée pour laquelle on dispose de résultats sur la cancérogénèse puis d'utiliser un ensemble homogène d'hypothèses en vue de calculer les doses tissulaires.

4. PUBLICATIONS

[1] R. MAXIMILEN, B. DERO. Critical review of animal carcinogenesis by cadmium and its inorganic compounds. Rapport CEA-R- 55165(E), 1990, 226p.
[2] R. MAXIMILIEN. Critical review of animal carcinogenesis by arsenic and its inorganic compounds.Chimie Ecologie Paris, 1990, 139p.
[3] R. MASSE, R.MAXIMILIEN The scientific uncertainties. Related policy perpectives. In: Metals and Human Health Reports and Proceedings. Brussels, Borschette Centre, 17-19 September, 1990

TITLE OF THE PROJECT N°3 CONTRACT B16-122-F (D)

Assessment of the subjective dimension of the radiological detriment, in relation to sociological considerations.

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1. OBJECTIVES OF THE PROJECT

Analysis of public attitudes toward nuclear energy shows the considerable importance of subjective components. As far as risk is concerned, fear and anxiety are not the only explicative factors, and both ideology and culture must be taken into account. Within a comparative approach, are analyzed :

- the subjective dimensions of risk perception for activities, the nuclear one obviously but also others which are common in the everyday life,
- the difference between risk perceptions of specialists and of lay people which can explain the difficulty to communicate.

The final goal is to propose methods or at least recommendations for integrating this subjectivity in risk management and more precisely in risk communication.

2. OBJECTIVES OF THE REPORTING PERIOD : 1990

2.1 Study of risk perception among specialists and of lay people.

The questionnaire addressed the following issues : the opinion on major hazards, the level of risk and its acceptability (as a willingness to do more for risk reduction) for various activities, and elicitation of criterias used by people when they manage the risk. 150 safety experts from various technical domains and a representative sample of lay people in the Bordeaux area (705 individuals) answered. Results of the in-depth analysis are given in a technical report [1].

2.2 Concepts and approaches used in risk perception studies.

The review on risk perception, written in 1990 and presented at the 39th UNSCEAR Session, has been discussed and modified to put a larger focus on radiation [2]. This new text will serve as a basis of discussion for the 40th UNSCEAR Session in Vienna,May 13-17, 1991.

2.3 Social and economical consequences of major accidents.

At the CEC Seminar on Methods and Codes for Assessing the Off-Site Consequences of Nuclear Accidents, held in Athens, on May 7-11, 1990, a paper has been presented which deals with social and economical consequences [3]. More details are given in a report [4].

In the paper, methods for estimating the economic consequences of major technological accidents, and their corresponding computer codes, are briefly presented with emphasis on the basic choices. When applied to hypothetic scenarios, those methods give results that are of interest for risk managers with a decision aiding perspective. Simultaneously the various costs, and the procedures for their estimation are reviewed for some actual accidents (Three Mile Island, Chernobyl,..). These actual costs are used in a perspective of litigation and compensation. From the comparative analysis, some points may be emphasized.

- For large accidents the unit for cost is the billion.
- Costs from scenarios and those observed for actual accidents are of the same order of magnitude, when side effects are not accounted for.
- Side effects cannot be avoided when there are long term consequences.
- Cost estimates obtained from codes are of good value for emergency planning, and

for short term countermeasures associated with rapid recovery.
- On-site costs are not dominating, as it was often supported.
- Environment recovery is a costly action which does not reduce to decontamination.
- Social activities disruption is more or less compensated in actual accidents.

The last two points that always involve costs and compensations in actual situations are neglected in codes; even if the task is difficult, it should be useful to introduce them in the codes in order to achieve better cost assessments.

2.4 Workshop on risk perception of technological hazards.

A restricted workshop on the present situation of risk perception in the Community with respect to technological activities was organized in Paris, November 8-9, 1990. Participants presented their subjects of interest that were, for some people, the social representations of hazards, and for others, the improvement of risk communication. The project of an extended workshop at the end of 1991 was retained.

3. OBJECTIVES FOR THE NEXT REPORTING PERIOD : 1991

3.1 Study of risk perception among specialists and of lay people.

A paper will be prepared for the French review Preventique.

3.2 Radioactive wastes perception.

This topic, studied some years ago, will be re-analysed with French data issued from a national survey which will be performed in June 1990. Two sections in the questionnaire are devoted to beliefs about wastes, and to psychological and social indicators respectively. A study of the relations between the two sections is planned. It is expected to show some of the results at the 3th Conference of the Society for Risk Analysis, Paris, December 1991.

4. PUBLICATIONS

[1] BARNY M.H., BRENOT J., PAGES J.P. Perception des risques majeurs dans la population bordelaise et chez les experts. Note SEGP/LSEES 90/17, Octobre 1990.
[2] BRENOT J. Perception of radiation risks. Note SEGP/LSEES 90/22, December 1990.
[3] BRENOT J. Economic consequences assessment for scenarios and actual accidents. Do the same methods apply ? In: Proceedings of the CEC Seminar on Methods and Codes for Assessing the Consequences of Nuclear Accidents, Athens, 7-11 May 1990.
[4] BRENOT J. et M. MICHOULAND. Accidents Technologiques Majeurs. Evaluation des conséquences socio-économiques. Note LSEES 90/12 Août 1990.

Progress Report

Contract : Bi7-004 Sector : C13

Title : Comparative assessment and management of the health and environmental impact of energy systems and studies related to the expression of the detriment associated with radiation exposure.

1 Lochard	CEPN
2 Wrixon	NRPB
3 Kemp	Univ. East Anglia
4 Friedrich	Univ. Stuttgart
5 Anguenot	CEA-FAR

I Summary of Project and Global Objectives

Over the last decade, there has been a relative stagnation in energy demand whilst protection of the environment has become a matter of growing concern (acid rain, deforestation, global warming, nuclear accidents). Today energy demand is rising again, and programmes must be discussed with a new focus on the link between energy policies and environmental and health effects. In the radiation protection field, re-evaluation of the Hiroshima-Nagasaki data has induced much international discussion about revisions to the system of dose limitation and highlighted inadequacies in the current health detriment indicator, i.e. the number of cancer deaths and serious hereditary effects. In this context it was deemed necessary to develop a methodological framework that would put the radiological and non-radiological risks of energy systems into perspective.

This project has developed in two parts, one dealing with radiological detriment, and the other comparing the coal system in the German region of Baden-Württemberg with the French nuclear cycle in the South-East of France.

A PC-based system for the expression and quantification of radiological risk and detriment is being designed with the possibility of using the new detriment indicators investigated during this contract, i.e. the YOLL (years of life lost), YOLI (years of life impaired), and the QALY (quality adjusted life years) so that measures of both fatal and non-fatal cancers as well as genetic effects can be incorporated. This system will help define the radiological risks of nuclear electricity production, and put them into perspective with the risks of other systems.

The regional studies of the German coal cycle and the French nuclear cycle will form the basis for a methodological framework applicable to all energy systems. The methodology was developed after an overview of past comparative studies so that this work could benefit from previous experience, and outstanding limitations such as the lack of environmental indicators, could be identified. It is important that this work is not just a repetition of that performed in the late 1970's, and so the definition of a framework will ensure that in future results can be updated without redoing all the work, and additional aspects previously ignored or presumed irrelevant, can be treated.

Head of Project 1 : Mr. Lochard

II Objectives for the reporting period :

The CEPN has participated in both the radiological and the non-radiological components of this project, henceforth known as (a) and (b).

(a). - Definition of the general framework for a PC-based system for the expression and quantification of radiological risk and detriment ;
- Review of health effects models;
- Transportation - defaults.

(b). - A synthesis of previous comparative risk studies on energy cycles;
- The definition of a methodology to integrate both health and environmental risk dimensions into a framework for evaluating the risks of energy generation;
- A definition of the French nuclear cycle in conjunction with the CEA-IPSN.

III Objectives for the next period :

(a). - System coding;
- Transportation - additional;
- Sensitivity and uncertainty analyses.

(b). - Completion and improvement of data on the French nuclear cycle;
- Data collection on the French coal cycle, and an application of the French nuclear model to German data;
- A double comparison of the coal and nuclear cycles i.e. between the two countries as well as between the cycles themselves;
- An effort will be made to look at the environmental impacts of energy production in more detail.

IV Progress achieved including publications :

(a). For research purposes, the final package for the expression of radiological risk and detriment will contain all relevant data sets for health effect modelling. Although menu-driven, with an accompanying manual, the user would need to have background knowledge on the relevant principles and calculations, although the package will be suitable for training purposes. In many instances, the user will wish to replicate in part or totally the methodologies employed by various review bodies such as UNSCEAR or BEIR or the more generic or applied studies by the ICRP. These will be put in as default options for the user to follow. Additional defaults other than these have yet to be identified.

For the reference population, the following parameters are required :
- demographic breakdown of the population by age and sex
- death rate from all causes by age and sex
- baseline cancer rates for each organ by age and sex (for a multiplicative risk model).

Transportation - the application of a risk model from one population to prediction of risks for some other population raises concern about the validity of the 'transportation'. In this instance, this concerns the assumption that particular parameter values estimated for one population are also applicable in other populations. This is especially acute if the two populations differ considerably in their baseline rates. The transportability assumption is not directly testable. However, BEIR V considered additive and relative risk models for breast and thyroid cancer across several different populations and concluded that the age and time-specific relative risks are more stable across studies than the corresponding additive risks. This committee subsequently transferred risks from the Japanese to the American population.

The system will be able to calculate risk estimates according to the full range of dose patterns: extended exposure (variable) to the individual; acute exposure to a given population of mixed age and sex; non-uniform exposure; and committed dose. For each situation probability of effect and accompanying years of life lost data will be derived.

A joint paper 'ASQRAD : A tool for the evaluation of radiation detriment' (T.Schneider - CEPN, and J.Robb - NRPB) was presented at the BNES conference on Occupational Radiation Protection in Guernsey 29th April - 3rd May 1991. A document was also written by T.Schneider (CEPN), with P.Hubert (CEA) for the CEC Schloss Elmau workshop in Germany - The Future of Human Radiation Research - March 1991, entitled 'Definition of a standard for comparing life long risk estimates'.

(b). A synthesis of previous comparative risk studies on energy cycles, and the definition of a methodology to integrate both health and environmental risk dimensions into a framework for evaluating the risks of energy generation have been completed. A table giving an overview of some of the most important comparative studies of energy cycles is enclosed (see Table 1), as well as an example of the time-space matrices proposed for the methodological framework (see Table 2).

The joint definition of the nuclear cycle with the CEA-IPSN started by defining the installations to be included in this study. Once these had been agreed upon, the CEPN made the following contributions to the results for nuclear electricity generation :

(1).The risk to workers and the public of constructing and dismantling a 900MWe nuclear power plant. The public risk was assumed to be entirely due to the transportation of materials, and was therefore estimated from the mass of materials needed, the distance travelled, and the type of transport used. The occupational risks of construction were calculated using the investment costs and an input-output model. Data of occupational accidents and diseases exist in the French worker's compensation scheme, and average results from 1984-1988 were used. The radiological risk of power plant dismantling poses the problem of a lack of practical experience in this domain, and as a first approximation an American estimation is quoted.

(2). The collection of data for occupational exposure and accidents at nuclear power plants (from EDF);

(3). A literature review of probabilistic safety analyses on nuclear power plants, and also papers on the possible consequences of a nuclear accident - this work is on-going;

(4). A synthesis of previous CEPN reports on the transportation of hazardous materials, and the disposal of radioactive wastes. Each stage of the fuel cycle was taken into account when considering the transport risks. These risks are for all people, as in the CEPN data base there is no differentiation between the public and workers. For radioactive waste disposal the maximum individual dose to a reference group was calculated using the GEOLE code from the French CEA, for both surface and deep disposal of vitrified and non-vitrified wastes.

Table 1

Overview of some of the most important energy comparison studies

STUDIES	COUNTRY	YEAR	ENERGY SOURCE					CYCLE						INDICATORS			END-USE	SEVERE ACCIDENTS	REGIONAL-ISATION	METHOD-OLOGY
			Coal	Oil	Gas	Nuclear	Renewables (back-up)	Construction	Mines	Transport	Plant Operation	Waste	Dismantling	Health: Public	Health: Occupational	Environment				
WASH-1224	U.S.A	1974	√	√	√	√	x	x	√	√	√	√	x	√ $ / GWa (75% load factor)	√ $ / GWa (75% load factor)	√ qualitative	electricity	x	U.S.A.	Cycle
INHABER	Canada	1979	√	√	√	√	√ (*)	√	√	√	√	nuclear only	x	√ MDL / MWe over life-time	√ MDL / MWe over life-time	x	electricity heat mechanical	√ MDL	Canada	Cycle
HAMILTON	U.S.A	1979	√	√	√	√	√ (x)	√	√	√	√	√	√	√ Deaths / GWa	√ WDL, Deaths No. Cases per GWa	x	electricity heat	nuclear only	U.S.A.	Cycle
UNEP	-	1981	√	√	√	√	√ (x)	x	√	√	√	nuclear only	nuclear only	√ Deaths / GWa	√ Deaths, Injuries per GWa	√ qualitative discussion	electricity heat mechanical + others	nuclear only	x	Cycle
CEPN	France	1981	√	√	x	√ PWR	√ (x) outer	√	√	√	√	x	x	√ Deaths / GWa	√ WDL, Deaths per GWa	√ qualitative	electricity	x	France	Cycle Scenari
FERGUSON	U.K.	1981	√	√	x	√ AGR	x	√	√	√	√	√	x	√ Deaths / GWa	√ Deaths / GWa	x	electricity	√ probabilities	U.K.	Cycle
UNEP	-	1983	√	√	√	√	√	x	√	√	√	√	√	√ Deaths / TWa	√ Deaths, Injuries per TWa	√ qualitative	electricity heat mechanical	nuclear only	x	Cycle
IPSN	France	1987 + 1990	√	√	x	√	x	x	√ except oil	x	√	√	x	√ No. cases & Deaths per year	√ WDL & Deaths per year	√ qualitative	electricity heat	x	EEC/France	Cycle
KALLENBACH	Germany	1988	√	√	√	√ PWR	√ (*)	√	√	√	√	√	x	√ Deaths, Injuries & Diseases per GWa	√ Deaths & WDL per GWa	x	electricity	nuclear only	F.R.G.	Cycle Scenari
FRITZSCHE	Switzerland	1989	√	√	√	√	√ (x)	√	√	√	√	√	x	√ Deaths / GWa (coste & late)	√ Deaths / GWa (coste & late)	x	electricity	√ fatalities / yr & probabilities	Europe	Cycle

Table 2

An example of the time-space matrices proposed for the methodological framework - health effects of the nuclear PWR cycle

NUCLEAR (PWR)	SHORT TERM	MEDIUM TERM	LONG TERM
LOCAL	Occupational injuries : mines, building, power stations Irradiation : transportation, fuel manipulation, major accidents	Pneumoconiosis : mines Cancer : major accidents, irradiation during the fuel cycle	Mutagenic effects : irradiation during the fuel cycle, major accidents
REGIONAL	Irradiation : major accidents	Cancer : emissions and irradiation major accidents, incidents, waste storage	Mutagenic effects : accidents, incidents, waste storage
GLOBAL		Cancer : - major accidents - waste storage	Mutagenic effects : - major accidents - waste storage

Head of Project 2 : Dr. Wrixon

II Objectives for the reporting period

Working together with CEPN, the principal objective for the reporting period was the specification of a computer code, ASQRAD (An Assessment System for Quantification of Radiological Detriment). As part of this, a detailed framework in the form of a planning paper would be prepared prior to coding. This would clarify those areas where the data requirements are relatively simple, and where considerable research effort is still required. In particular, NRPB would be responsible for identifying information on detriment indicators that would be useful outputs from the software.

III Objectives for next period

Continuing development of ASQRAD with final preparation of elements (models and data sets) for input into the code. This will include sensitivity analyses to determine the extent of the necessary information in terms of specific national data. In particular, NRPB will be responsible for finalising the requirements for impairment, somatic and hereditary detriment, and economic data. A second area will be the identification of library and graphics facilities for the code.

IV Progress achieved including publications

One consequence of the latest re-evaluation of the Japanese atomic bomb survivor data has been that various national and international organisations have produced data on the distribution of health effects in populations following exposure to low levels of radiation. However, the approaches used are rarely consistent. Therefore, for both decision-makers and research scientists, a 'user-friendly' system that encompasses all the relevant models, population distributions, and factors important in radiation risk and detriment, was identified as highly desirable.

As a result, the joint CEPN and NRPB code, ASQRAD, is being developed combining the best elements of the two organisations' own systems together with additional information. In general, the models and factors to be included in the code for the calculation of health detriment are not new. The novel approach is to rationalise them into one system as outlined in Fig 1.

To this end, NRPB and CEPN, together with contributions from UEA, have prepared a planning paper identifying the necessary elements, and providing a preliminary assessment of the data needed. This will provide a working basis for the coding of the system, commencing during the summer of 1991, and the final identification of the data requirements. Particular care is being paid to this stage of the work, for too often software programming starts before there is an accurate appreciation of the extent of the work involved. It has also been instructive during this process to consider the requirements of the main users; in research, decision-making and training. As a result, ease-of-use, completeness, graphics facilities and uncertainty analysis are seen as priority features of the code.

One principal area of concern has been the identification of useful measures of detriment as outputs from the code. ICRP publication 60 has established the inadequacy of measures that account solely for the probability of fatal cancer. In general, one would advocate a quantity that includes life-loss, non-fatal effects, and an indication of the relative severity of the effects. Thus, for any given exposure regime the user will be able to calculate probability of effect, years of life lost (YOLL), years of life impaired (YOLI), and quality adjusted life years (QALY) for any combination of fatal and non-fatal cancers and hereditary effects. The QALY is a measure of detriment using subjective psychometric data on the quality of life associated with different conditions to weight severity of impairment. The QALY product encompasses both YOLL and YOLI.

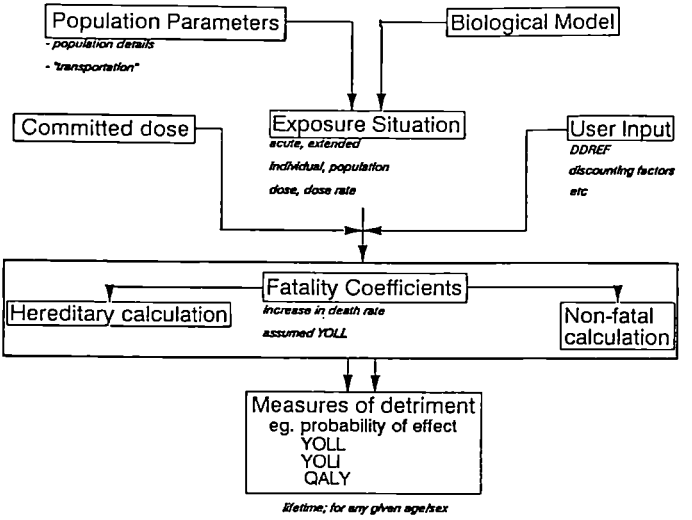

Fig. 1 Outline of components of ASQRAD

There are a number of other attributes that may be important indicators of radiation-induced health detriment. Some of these are noted in ICRP publication 60, and will be considered during the forthcoming year.

A further area of particular concern is that of hereditary detriment. The probability of hereditary effects is a function of the likelihood of conception following exposure. Thus, age-specific fertility rates, that is the probability of individuals of different ages having children, are introduced. These data, for which a range will be available for the purposes of sensitivity analysis, are part of the detriment formulation. Further hereditary detriment variables open to the user are severity weightings according to the category of hereditary effect, and subjective intergenerational weightings.

A joint CEPN/NRPB paper: Schneider T and Robb JD, "ASQRAD: A tool for the evaluation of radiation detriment", discussing many of these issues, was presented at the BNES conference on 'Occupational Radiation Protection' in April 1991.

An joint NRPB/CEPN abstract has been submitted for the IRPA8 conference in Montreal, 1992: Robb JD, Schneider T and Degrange JP, "A tool to aid quantification of radiation detriment".

Head of Project:3 Dr. Ray Kemp
Senior Research Associate: Dr. Michael Thieme
Research Associate: Ms. Dawn Ives

II Objectives for the Reporting Period

The UEA Environmental Risk Assessment Unit's contribution to the radiological part of the project has been related to investigations of the application of the Quality Adjusted Life-Year (QALY) measure for radiological protection. This has involved four related areas of work:

a) an extensive review of the QALY literature in order to advise on the advantages and disadvantages of QALY measures for this field;

b) an investigation of weighting procedures to adjust the QALY value according to (i) the source, and (ii) the consequences of effects of exposure to radiation;

c) calculating the cost of a QALY.

In all of these areas, the UEA team has liaised with the NRPB and other project members throughout, thereby contributing to the direction and progress of the overall research programme.

III Progress Achieved

Excellent progress has been made in each of the above areas. One full person-year's effort has been contributed by Dr. Thieme, supported by approximately 0.25 of a person-year's effort from Ms. Ives, and directed throughout by Dr. Ray Kemp. The results can be summarised as follows:

a) Review of the usefulness of the QALY approach

In general, the concept of the QALY is a good one for radiation protection.

However, QALY methods attempt to be too precise in assigning values to health states and a broader classification system may be beneficial for the radiation protection field than is more generally found in the literature.

Problems arise particularly with respect to discounting. We recommend the employment of a 0% discount rate. Viewing the problem from a social time preference perspective, evidence from our pre-pilot study of indiviudal preferences appears to show that subjects agree with the proposition that all generations should be valued equally. On the other hand, taking a theoretical position that human health and human value are distinct from capital and goods in the market place, we would also argue that discounting procedures should not be employed. While there is some irony in

that this is in essence what the QALY approach attempts to do, i.e. to enable market values to be placed on health care, we recognise that strong ethical objections do exist and so favour a 0% discount rate for the purposes of calculation.

b) Weighting Procedures According to Source and to Effects of Exposure

i) Weighting according to source

There are undeniably strong arguments both for and against differentiating between the sources of radiation in such studies. Nevertheless, we have examined available procedures for determining public weights which might be assigned to different sources of radiation. We recommend that should further work be required, a methodology similar to that employed in a study for the NRPB by the Robens Institute, University of Surrey, would be appropriate. That study examined public weighting of preferences for alternative radioactive waste disposal routes; discussions have been held with one of the authors and with the NRPB, and it does appear to be a viable methodology. Project resources do not allow this work to be taken forward at this stage.

ii) Weighting according to effects

A methodology has been developed and a pre-pilot study of individual preferences has been undertaken at UEA. The methodology is complex and initial results seem to show that it may prove to be impossible to draw meaningful conclusions from a wider study other than with respect to one important variable: namely, that subjects appear to prefer to weight all generations equally. If this is assumed to be the case, then the problem of the appropriate discount rate to be applied to the QALY is resolved (see above), but outstanding difficulties arise with respect to: balancing probabilities of detriment against the Years of Life Lost (YOLL), the Years of Life Impaired (YOLI), and the level (%) of impairment.

c) Cost of a QALY

Two approaches to costing a QALY are available. The first is to take the cost of treatment of radiation induced illnesses. These will vary widely; they will change with technological developments and advances in medical treatment; they are difficult to both quantify and to predict.

The second approach is to take a consensus estimate for the Value of Life (VOL), and then calculate the Value of a Life-Year (VOLY). Then, if we assume that one year spent in perfect health is one QALY, substitute with an estimate for a VOLY in perfect health.

An extensive literature review of relevant VOL estimates has been undertaken by Ms. Ives, and the figures re-calculated from base values to £ 1990.

The median value for VOL is £1.590,498 (£ 1990).

The VOLY is $\frac{£1.59m}{39}$ = £40,782 (£ 1990)

This, assuming a risk factor of 5% per Sv, and an average YOLL value for all cancers of 16 years, results in a revised value for a manSv of £32,626 (£ 1990).

IV Objectives for the Next Period

Project resources under the staff heading have now been more or less expended. Dr. Thieme's contract comes to a close on 31.5.91.

The UEA contribution to the next period of work therefore will be to continue to liaise, advise and to contribute to project discussions. The majority of our effort over the next period of work will be in the form of writing up the study's findings for publication and for dissemination.

Head of the project : Dr. Friedrich

II Objectives for the reporting period

The main objective was the identification and - as far as possible - the quantification of human health effects due to electricity generation from coal in the present German / Baden Württemberg generating system. The main emphasis was put on the development of a consistent methodology to standardize the calculation of health impacts. Indicators for health detriment had to be developed in accordance with the French group to guarantee a coherent and comparable presentation of results.

An introduction to economical valuation methods of health and environmental impacts should be provided.

III Objectives for the next period :

The first task in the next reporting period will be the identification and application of an appropriate dispersion model to quantify ambient air concentrations of several pollutants caused by a coal fired power plant. To estimate the resulting public health effects, health damage models have to be identified and included.

A first quantification of external costs of the coal fuel system will be carried out.

The application of the French nuclear cycle framework to the German conditions has to be prepared by collecting the respective German data.

Some aspects of the health effects of oil heating will be analyzed in a first step.

IV Progress achieved

In the present study, the German / Baden Würtemmberg coal energy system is described and health effects caused by the electricity production from coal are quantified as far as possible.

- CATEGORIES OF HEALTH RISKS ESTIMATES

Before quantifying any health impacts, it has to be clear how the different impacts can be classified. As a first approach, risk estimates are presented according to the risk categories used in former risk assessment studies :

- **occupational** fatalities caused by accidents (direct impacts)
- **occupational** fatalities caused by diseases (medium-term impacts)
- non fatal **occupational** disabilities caused by accidents and diseases which

are summarized in the figure "worker days lost" (WDL) (direct impacts)
- non fatal occupational disabilities which occur after the worker is retired (medium-term impacts)

- public fatalities caused by accidents (direct impacts)
- public fatalities caused by diseases (medium-term impacts)
- non fatal public disabilities caused by accidents and diseases (direct impacts)
- non fatal public disabilities caused by diseases (medium-term impacts).

Because WDLs only indicate the absence from the working place caused by an occupational accident or a disease and do not take into account the state of health of the affected person, this indicator is not appropriate to evaluate permanent disabilities. The indicators "Years Of Lost Life" (YOLL) and "Years with Reduction in Earning Capacity" (YREC) are regarded as helpful for the evaluation of occupational mortality and permanent disability and therefore introduced into the framework as new risk indicators.
An evaluation of public health effects further than just counting the cases of fatalities and diseases might be possible using the "Quality Adjusted Life Years" (QALYs) approach. A reliable use of QALYs still requires scientific research as well as more detailed statistical information concerning public accidents and diseases.

- HEALTH RISKS OF COAL ENERGY TECHNOLOGY

More than producing just a new set of risk figures, emphasize was put on the development of a consistent methodology to calculate human health impacts. This is a first step towards the development of a standardized, computer aided instrument for risk calculation in the future.

To quantify the health risks of coal energy technology the complete fuel system including the different process steps mining, cleaning, transport, combustion and waste disposition is analyzed. Additional risks from the power plant construction and dismantling are also regarded. For each of the process steps a methodology to calculate the respective health impacts is developed. The required data sets are defined and the processing of the data is fixed. The calculation procedure can be displayed graphically as a risk tree with the electrical energy production as the starting point.

To calculate public risks from air pollution dispersion models and health damage functions have to be used. The modelling of secondary pollutants like ozone and acid rain requires rather complex models. Several dispersion models are evaluated to include an appropriate one into the framework.

Underground coal mining is the main source of the occupational impacts, these values are calculated with a certain reliability.

Public health risks are much more uncertain, so a wide range of possible results is estimated.

The implementation of the described methodology on a PC was started in the reporting period. Objective of this part of the work is to facilitate the use of a computer aided

framework in Germany as well as in France with its respective site specific or country specific data sets.

- ECONOMIC VALUATION

The fundamental principles of economic valuation of externalities from electricity production are described and several valuating methods are presented.

Head of Project 5: Dr. Anguenot

II Objectives for the reporting period

Inventory of French nuclear units. Description and releases data of the PWR system (mining and milling, transformation of uranium concentrates, uranium enrichment, production of nuclear fuel and assemblies, electricity production and reprocessing of spent fuel). Study of the risks for the public by assessment of atmospheric and food chain transfers.

III Objectives for next period

- Complementation of the data bases ;
- Information on the French coal energy system to make comparison with the coal cycle in Germany ;
- Calculation with German data and French model of the public exposure resulting from German nuclear energy system ;
- Comparison of French and German systems ;
- Thinking about the best way to carry on this study.

IV Progress achieved including publications

FRENCH URANIUM FUEL CYCLE
Assessment of public risks during normal operation

1. Nuclear energy in France

The uranium PWR cycle from mining up until reprocessing and wastes storage includes several steps which are presented separately. The PWR system assuming 95 % of nuclear production UNGG and FRB have been neglected.

The evaluation of nuisances and risk is done for normal operation.

2. Mining and milling

The French uranium mining industry (reserves > 100 000 t) is divided between "COGEMA" and "TOTAL Compagnie Minière" (Vendée, Massif Central, Hérault).

The uranium concentration is performed close by the mines. Uranium concentration is generally between 0.5 and 1.3 ‰. The main exposure for public results mainly from radon emission which is estimated at about 0.5 t Bq/Twh(e).

3. Transformation of uranium concentrates

This step is handled by COMURHEX in Malvesi (Aude). The "yellow cake" is transformed into UF4.

The amount of U discharge is about 0.8 kg/Twh(e).

4. Conversion of U concentrates

Transformation of UF4 into UF6 is carried out by COMURHEX in Pierrelatte (Drome). The discharge at the chimney after filtering is about 90 kBq/Twh(e) for uranium (α activity).

5. Uranium enrichment

The plant EURODIF is situated in Tricastin close to Pierrelatte. The U discharge is about 8 g/TWh(e) while fluoride and chloride ions are respectively estimated at 0.8 and 2 g/TWh(e).

6. Production of nuclear fuel and fuel assemblies for PWR

FBFC carries out fuel fabrication in two plants : ROMANS (Isère) and PIERRELATTE (Drome). A part of the enriched UO_2 powder is sent to DESSEL (Belgium). The total activity discharged is of the order of 1.5 kBq/TWh(e) (Uranium + daughter products).

6. Production of energy (PWR)

Four nuclear plants are taken into account to find the following emissions :

- 1.2 to 2.8 TBq/TWh(e) for noble gases ;
- 0.008 to 0.04 GBq/TWh(e) for iodines ;
- 0.74 TBq/TWh(e) for tritium ;
- 22.8 GBq/TWh(e) for carbon-14.

7. Reprocessing of spent fuel

Information is mainly obtained from LA HAGUE plant (Manche) :

- 3.4 MBq/TWh(e) ß total ;
- 1 GBq/TWh(e) tritium ;
- 2.3 PBq/TWh(e) krypton-85 ;
- 0.1 MBq/TWh(e) cesium-137 ;
- 34.9 MBq/TWh(e) iodine-131 ;
- 0.9 GBq/TWh(e) iodine-129 ;
- 5.5 kBq/TWh(e) plutonium-239.

8. Risks for the public

Exposure of the public is calculated, as a mean annual value, from concentrations of the various pollutants in the environment, with the help of various transfer models.

The exposure of the most exposed group of population is an hypothetic situation where all kinds of pollutants are at their maximum concentration value in the same sector of maximum transfer.

The exposures calculated for each step of the fuel cycle are reported in the table below :

TABLE

Exposure of the most exposed group

	MAXIMAL VALUE [Sv/y)/(TWh/y)]
Mining and milling	$8.32\ 10^{-6}$
Transformation	$7.03\ 10^{-7}$
Conversion	$4.10\ 10^{-10}$
Enrichment	$6.64\ 10^{-9}$
Fuel fabrication	$3.27\ 10^{-10}$
Energy production	$1.22\ 10^{-7}$
Reprocessing	$3.24\ 10^{-7}$
URANIUM CYCLE	$9.48\ 10^{-6}$

The collective exposure to which general public are subjected have been assessed for about 10 and 50 miles from emission point.

Progress Report

Contract Bi6-126

Sector:C14

Title : Statistical methods for the analysis of geographical correlations, application to the analysis of the correlation between population radiation exposure and cancer mortality

1 Hémon INSERM

I - Summary of Project and Global objectives

The research project presented here has a double purpose : first to investigate statistical methods suited to the analysis of models of association between spatially defined variables, then to apply these methods to the study of the joint variations of risks factors such as low dose radiation or industrial pollution together with some health indicators such as mortality for cancer of specific sites.

Head of Project 1 : Prof. D. Hémon

II - Objectives for the reporting period

• study the robustness of the modified tests of association previously developped to departure from normality assumptions

• define non parametric tests of association between spatially distributed variables

• analyse the geographical association in France between the mortality rates for some radio-sensitive sites (lung, breast, thyroïd, leukemia) and background radiations, taking into account appropriate geographical confounders.

III - Objectives for next period

not applicable as this is the final report

IV - Progress achieved including publications

We first summarize the progress achieved in statistical methodology.
The development of parametric tests of association between spatially distributed variables was complemented by a study of non parametric methods.

1. Non parametric tests of association

As a preliminary step, the robustness of the modified tests of the correlation coefficient to some patterns of departure from normality was investigated.

1.1 Performance of the modified $t_{\hat{M}-2}$ test for non gaussian variables

The development of modified $t_{\hat{M}-2}$ tests for simple or partial correlation between spatially distributed variables has been previously reported (1,2). The construction of these tests as well as the study of their performance was done under Gaussian hypotheses. The performance of the modified $t_{\hat{M}-2}$ test was subsequently investigated when the normality of the underlying variables was perturbed. Three types of perturbations were considered : (a) truncated Gaussian variables, (b) lognormal variables, (c) mixture of Gaussian variables. The $t_{\hat{M}-2}$ test was shown to be quite robust for these patterns of departure from normality in terms of its significance level, with a tendency to be over-conservative in cases of high autocorrelations (3). Alternative non parametric tests would therefore be of interest.

1.2 Modification of Spearman's rank correlation test for large samples

A classical non-parametric measure of association between two variables is Spearman's rank correlation r_s which evaluates a correlation coefficient between the ranks of the two variables. When N is reasonably large ($N \geq 30$), r_s is tested using the transformed variables, $(N-2)^{1/2}\, r_s/(1-r_s^2)^{1/2}$, which follows approximately t-distribution with N-2 d.f. When X and Y are measured at N sites of a spatial domain and are positively spatially autocorrelated, this procedure is no longer correct as it leads to inflated significance levels. Similarly to the case of the empirical correlation coefficient, a modified rank correlation test can be defined, based on an effective sample size $\hat{M}$ (instead of N) which takes into account the spatial autocorrelation of each variables. $\hat{M}$ is estimated as for the modified $t_{\hat{M}-2}$ statistic defined in (1). Table 1 presents results from a simulation study which shows one hand that the type I errors of the classical r_s test are inflated in the case of high positive autocorrelation and on the other hand that the observed type I errors of the modified r_s test are very close to their nominal level. The simulation model was the same as used in the previous reports.

Table 1

Type I errors (per cent) of the classical and the modified Spearman's test between X and Y.
500 simulations were carried out for several levels of autocorrelation in $\rho(1)$ in X and Y (disc model) and a nominal 5% significance level

autocorrelation $\rho(1)$ for X and Y	0.0	0.2	0.4	0.6	0.8
type I errors (%) for the classical r_S test [95 % CI]	4.4 [8.6;6.2]	5.2 [3.3;7.1]	10 [7.4;12.6]	15.6 [12.4;18.8]	37 [32.8;41.2]
type I error (%) for the modified r_S test* (95 % CI)	4.6 [2.8;6.4]	5.0 [3.1;6.9]	6.6 [4.4;8.8]	5.6 [3.6;7.6]	4.7 [2.8;6.5]

* test of $\sqrt{\hat{M}-2}\ r_S/(1-r_S^2)^{1/2}$ as a t-distribution with $\hat{M}-2$ d.f.

1.3 Tests of association based on permutations

Monte Carlo tests can be defined which rank an observed statistic among a set of statistics simulated under the null hypothesis, defining in this way a Monte Carlo significance level for the t-statistic. In the case of spatially autocorrelated variables X and Y, using all the permutations of the values of X or Y without restrictions to simulate the set of statistics, lead to over significant Monte Carlo tests, as could be expected since the spatial structure of X and Y is destroyed (Table 2).

To overcome this problem, only those permutations leading to realisations of X or Y which preserve in some way the internal autocorrelation structure should be retained. In practise, a distance measure between the original autocorrelations of X, $\{\rho_X(k)\}$, where k represents increasing distance lag for the autocorrelation classes, and the autocorrelations of the permuted X, $\rho_{\tilde{X}}(k)$ has to be defined. Results concerning an irregular network of 21 points and the statistic defined by the correlation coefficient are presented in Table 2. Two criteria suited to the size of the network were studied :

i) $D_1 : |\rho_X(1) - \rho_{\tilde{X}}(1)| < d$, ii) $D_2 : \frac{1}{2}\{|\rho_X(1) - \rho_{\tilde{X}}(1)| + |\rho_X(2) - \rho_{\tilde{X}}(2)|\} < d$

for several choices of d.

Several remarks can be made : for moderate autocorrelation ($\rho(1) = 0.4$), values of d can be found for both D_1 and D_2 which give correct significance levels. Note that these values are much smaller for D_1 than for D_2. With the same values of d, D_1 and to a lesser extend D_2 give somewhat inflated significance levels in the more strongly autocorrelated cases. An attempt to improve the results with criterion D_1 by reducing the values of d in the highly autocorrelated cases was unsucessfull as realisations of permuted X satisfying D_1 with $d = 0.005$ for instance were too rare. On the other hand, it was feasible to find realisations of permuted X which satisfied D_2 with d=0.1 and this gave better results than with d=0.05. The resulting Type I errors are indeed reasonably close to their nominal level.

In summary this first study shows that permutation tests for spatial association can be defined for irregular spatial networks. This method needs to be studied further with other spatial networks and autocorrelation structures.

Table 2
Type I errors (percent and 95% confidence intervals) for permutation tests of r_{XY}+ in the case of spatially autocorrelated variables X and Y.

Autocorrelation ρ(1) for X and Y	0.4	0.6	0.8
Permutations without restriction	17.2 [14.9;19.5]	21.4 [18.9;23.9]	28.4 [25.6;31.2]
Permutations following :			
Criterion D_1*			
d = 0.01	4.3 [2.4;6.2]	13.6 [9.6;17.6]	9.9 [7.1;12.7]
d=0.05	7.2 [4.3;10.1]	13.2 [9.9;16.5]	13.0 [9.5;16.5]
Criterion D_2**			
d = 0.05	6.9 [4.0;9.8]	7.8 [4.7;10.9]	11.0 [7.9;14.1]
d=0.1	5.4 [3.3;7.5]	7.9 [5.2;10.6]	8.7 [6.6;10.8]

+ r_{XY} is the empirical correlation coefficient between Xi and Yi defined in a irregular network of 21 points

* $D_1 : |\rho_X(1) - \rho_{\tilde{X}}(1)| < d$ ** $D_2 : \frac{1}{2}\{|\rho_X(1) - \rho_{\tilde{X}}(1)| + |\rho_X(2) - \rho_{\tilde{X}}(2)|\} < d$

2. Geographical analysis of mortality rates for radio-sensitive sites

2.1 Data file

• The cancer sites chosen were those known to be radio-sensitive. They include lung, thyroid, breast cancer and leukemia. Age standardised mortality rates were computed for 3 periods : 1968-1969, 1974-75-76 and 1984-85-86, which are close to census dates.

• Mean gamma radiation was measured indoors and outdoors in 50 French départements (Tirmarche et al, Radiation Protection Dosimetry, 24 : 479-482, 1988). For a smaller group of départements (42) mean radon level, an exposure which could be linked to lung cancer is also available.

• In view of important known risk factors for some cancer sites, adjustment variables will be taken into account. Specifically male lung cancer mortality rates will be adjusted on cigarettes sales per inhabitant and percentage of blue collar workers, female lung cancer mortality rates will only be adjusted on cigarette sales per inhabitant and finally breast cancer rates will be adjusted on the proportion of urban communes per départements as an indicator of socio-economic level of the département.

2.2 Method

Modified $t_{\hat{M}-2}$ test of simple or partial correlation between cancer mortality rates and radiation exposure were calculated for the 3 periods. In view of the moderate number of areas only four strata (<150, 150-300, 300-450, >450 kms) were chosen for calculation of the effective sample size $\hat{M}$. It was checked that this particular choice of strata had little influence on the results.

2.3 Results

• There is weak evidence of a link between lung cancer mortality and gamma radiation exposure for the period 1968-1969 (Table 3) with similar correlations coefficients for men and women, indoor or outdoor exposure. For the other 2 time periods, there is no evidence of any pattern and the correlations are non significant contrary to what would be expected if the 1968 correlations reflected a direct link. No geographical association was found between lung cancer rates and radon exposure.

• No positive association was found between gamma radiation exposure and either breast cancer or leukemia for any of the 3 periods analysed.

• There is some evidence of geographical association between female thyroïd cancer rates and gamma radiation (Table 4). Simple correlation coefficients are broadly similar for the 3 periods even though the modified $t_{\hat{M}\text{-}2}$ statistic is only statistically significant for the first and third period. Some evidence of positive association is maintained after adjustment on a gradient of distance to sea, a covariate which is significantly linked to female thyroïd cancer for the periods 1968 and in 1984. No geographical association was found for male thyroïd cancer.

2.4 Discussion

The only positive result to have emerged from our geographical analysis is that of a possible link of gamma radiation with thyroïd cancer in females. For this site, mortality represents only a small fraction of the incident cases and hence the analysis would have been considerably more powerful if geographical incidence data were available. Thyroïd cancer mortality is approximately twice as high in women than in men which could explain the negative finding for men. Further analyses at a more detailed geographical level would be interesting as the averaging of the exposure over a whole département produces certainly some ecological bias (4) which could explain the negative findings for the other mortality sites. For rare cancer sites such as thyroïd cancer or leukemia, an analysis which takes into account the fluctuations of the observed mortality counts around their mean is also warranted.

Table 3

Modified tests of correlations between lung cancer mortality and mean gamma radiation exposure

lung cancer (male)+	Indoor				Outdoor			
	r	$t_{\hat{M}\text{-}2}$	$\hat{M}$-2	p	r	$t_{\hat{M}\text{-}2}$	$\hat{M}$-2	p
1968-69	0.29	2.37	62	0.02	0.22	1.75	60	0.09
1974-75-76	0.02	0.17	49	NS	-0.06	-0.46	49	NS
1984-85-86	-0.16	-1.14	48	NS	-0.19	-1.33	48	NS

+ after adjustment on cigarette sales per inhabitant and proportion of blue collar workers.

lung cancer (female)++	Indoor				Outdoor			
	r	$t_{\hat{M}\text{-}2}$	$\hat{M}$-2	p	r	$t_{\hat{M}\text{-}2}$	$\hat{M}$-2	p
1968-69	0.24	1.56	39	0.13	0.25	1.60	40	0.12
1974-75-76	0.07	0.51	59	NS	0.11	0.87	58	NS
1984-85-86	0.00	0.00	47	NS	0.02	0.14	47	NS

++ after adjustment on cigarette sales per inhabitant

Table 4
Modified tests of correlation between thyroïd cancer mortality (female) and mean gamma radiation exposure

thyroïd cancer female		indoor				Outdoor			
		r	$t_{\hat{M}-2}$	$\hat{M}-2$	p	r	$t_{\hat{M}-2}$	$\hat{M}-2$	p
1968-69	(a)	0.26	1.74	42	0.09	0.32	2.20	42	0.03
	(b)	0.22	1.53	45	0.13	0.28	1.94	44	0.06
1974-75-76	(a)	0.22	1.59	48	0.12	0.22	1.53	47	0.13
	(b)	0.21	1.48	49	0.15	0.20	1.41	49	0.16
1984-85-86	(a)	0.18	1.73	85	0.09	0.20	1.93	85	0.06
	(b)	0.14	1.55	111	0.12	0.16	1.66	107	0.1

(a) simple correlation coefficient
(b) partial correlation coefficient after adjustment on distance to sea

PUBLICATIONS

(1) P. CLIFFORD, S. RICHARDSON, D. HEMON. Assessing the significance of the correlation between two spatial processes. Biometrics, 1989 45 (1) 123-134.

(2) S. RICHARDSON. A method for testing the signifiance of geographical correlations with application to industrial lung cancer in France. Statistics in Medicine, 9, 515-528, 1990.

(3) S. RICHARDSON. Some remarks on the testing of association between spatial processes. Syracuse Symposium on Spatial Statistics, April 1989. Monographies de l'Institute of Mathematical Geography "Spatial Statistics : Past, Present, and Future". pp. 277-312, Août 1990.

(4) S. RICHARDSON. Statistical methods for geographical correlation studies to be published (chapter 16) in : "Geographical and Environmental Epidemiology : Methods for Small Area Studies", Eds P. Elliott, J. Cuzick, D. English, R. Stern, Oxford University Press.

Contract: Bi6-221 **Sector: C14**
Title: Epidemiological studies of radiation carcinogenesis and its biophysical basis.

1 Gössner GSF Neuherberg

I. Summary of Project and Global Objectives

Project 1: Late effects in ^{224}Radium treated ankylosing spondylitis patients
Prof. Gössner, GSF
Project 2: Late effects in ^{224}Radium treated juvenile and adult patients
Prof. Spiess, Universität München
Project 3: Epidemiology of radiation carcinogenesis
Prof. Kellerer, GSF (früher Universität Würzburg)

The three projects of the research programme are aimed at the epidemiological study of long-term radiation effects in patients injected with ^{224}Radium. The data from these patients are a unique source of information for risk analysis of incorporated bone seeking radionuclides in man.

Project 1 is concerned with more than 1500 ankylosing spondylitis patients treated between 1948 and 1975 with repeated intravenous injections of ^{224}Radium. The α-doses to the skeleton, on average 0.67 Gy, are considerably lower than the doses in the earlier patients that are being studied in Project 2. The causes of death, and occurrence of other lesions possibly related to the ^{224}Radium treatment, are analysed and compared with results in a control group of ankylosing spondylitis patients not treated with radioactive drugs or X-rays.

Project 2 continues the study of patients that were treated in a German hospital shortly after World War II as juveniles for bone tuberculosis and as adults for ankylosing spondylitis. The data, particularly for bone sarcomas, are largely complete, but additional recent observations on various other radiation effects are still being collected and special attention is given to the determination of these stochastic and non-stochatic radiation effects and their dose, time, and age distribution.

Project 3 continues and extends the mathematical and statistical studies for the evaluation of the data from Project 1 and Project 2 and for the comparison of these data with the broader experience from other epidemiological studies and other experimental work on radiation carcinogenesis. It is also concerned with the biophysical basis of the effectiveness of different types of ionising radiation, and generally the risk evaluation at low doses. Continued attention has been given to the development of algorithms and to the definition of data sets for general use in radiation carcinogenesis studies.

Head of Project 1: Prof. Dr. W. Gössner

II Objectives for the reporting period

Contact and follow-up of patients of the exposure group and the control group. Registration of causes of death. Comparison of results from the exposure and control groups with respect to the risk of bone tumours, leukaemias, and other diseases known or supposed from Project 2 to be related to the ^{224}Radium treatment.

III Objectives for next period

The follow-up of patients in the exposure and control groups will be continued and the results evaluated with special regard to the late effects in bone, haematopoietic tissue, kidney, liver, and other organs known or supposed from Project 2 to be affected by injected ^{224}Radium. The remaining patients of the exposure group will be addressed with a new questionnaire compiled together in close cooperation with Project 2. Continued effort will be undertaken to include as many ankylosing spondylitis patients as possible who have been treated with ^{224}Radium in hospitals of the former GDR.

IV Progress achieved including publications

In this study we have been following the health of ankylosing spondylitis patients collected from all orthopaedic hospitals in the old countries of the F. R. Germany which were known to have treated notable number of patients with ^{224}Ra. The majority of these patients have been treated in the years 1948 - 75 and most of them received one series of 10 weekly injections of about 1 MBq of ^{224}Ra each. This dosage leads to a cumulative α-dose of 0.56 Gy to the marrow-free skeleton of a 70 kg man. As of December 1990 the study consists of 1472 patients in the exposure group and of 1342 patients in the control group (ankylosing spondylitis patients not treated with radioactive drugs or X-rays). Patients found during current follow-up or from hospital records to be treated previously with X-rays have been deleted from the original study groups.
Personal and treatment data for the patients of the exposure and the control groups have been drawn from the hospital records. Information on current status is gained from re-examinations at different times after treatment and from questionnaires sent periodically to the patients. Causes of death were determined preferably from hospital records, reports from family physicians, or death certificates. For several patients there were autopsy protocols available additionally. The underlying causes of death were classified and registered according to the 7th revision of the International Classification of Diseases. Up till end of 1990, 532 patients in the exposure group and 703 patients in the control group have died (Table 1). Causes of death have been ascertained in 514 patients in the exposure group and in 622 patients in the control group. The remaining cases are still being investigated.
Table 2 shows the skeletal and soft tissue diseases observed so far. In this table we restricted our interest to those diseases which are known or implied from Project 2, the higher dose study, to be associated with a former administration of ^{224}Ra. There is, however, in contrast to the findings in the high dose group, no significant difference between observed and expected cases for cancers of stomach, liver, urinary system, or the female breast neither for the exposure nor for the control group.

We furthermore observed three malignant primary bone tumours (according to the histological typing of bone tumours of the WHO) in the exposure group of our study: one fibrosarcoma of bone, one reticulum cell sarcoma (malignant lymphoma) of bone, and one medullary plasmocytoma (multiple myeloma). The types of the bone tumours in our exposure group, however, are different from those observed in Project 2 where mostly osteosarcomas have been observed.
For total leukaemias the increase is highly significant for the exposure group and striking also for the controls compared to a standard population (exposure group: 9 cases observed vs. 2.2 expected, $p < 0.001$; control group: 6 cases observed vs. 3.0 expected, $p = 0.084$) but not yet for the exposure group compared to the controls. This indicates an effect of the mostly considerable intake of painkilling or other drugs related to the basic disease which is observed not only in the control group but also in the exposure group prior to irradiation with ^{224}Ra. It is well known that phenylbutazone e. g., a drug which in former times was widely used in the treatment of ankylosing spondylitis, can cause bone marrow depression. Acute leukaemias occurring in association with phenylbutazone treatment have repeatedly been reported in the literature.
Subclassification of the leukaemias shows a certain preference for the chronic myeloid leukaemia in the exposure group (3 cases observed vs. 0.7 expected, $p = 0.034$) whereas in the control group (1 case observed vs. 1.1 expected) the observed cases are in the range of expectancy. This apparent difference seems somewhat parallel to the fact that in this study bone marrow tumours are more dominant than in the higher dose study of Project 2.
Certain disorders of the haematopoietic system following treatment with ^{224}Ra were observed earlier by other authors even at the same low dose level. An increased rate of leukaemias would also correspond with results from animal experiments with bone seeking α-emitters given at very low dose rates, lower than those found to cause bone tumours. Also for ^{239}Pu, a bone surface seeker like ^{224}Ra, the induction of myeloid leukaemia has been demonstrated in mice down to dose rates of a few mGy/day or even less.

Table 1: Follow-up status of ankylosing spondylitis patients in the exposure and control groups (Dec. 1990)

	Exposure Group	Control Group
Total number of patients	1578	1469
- Treated with X-rays additionally	106	127
Remaining patients	1472	1342
Deceased patients	532	703
- Cause of death certified	514	622
- Cause auf death not yet known, still in work	18	81

Table 2: Skeletal and soft tissue diseases (Dec. 1990)

	Exposure Group		Control Group	
Observed patients	1472		1342	
- Deceased, cause of death certified	514		622	
Total cancers	109	(20L*)	138	(13L)
- Malignant primary bone tumours	3		1	
- Fibrosarcoma	1		0	
- Malignant lymphoma	1		0	
- Medullary plasmocytoma	1		1	
- Myeloproliferative diseases	6	(1L)	3	
- Myeloid leukaemia	5		3	
- Chronic myeloid leukaemia	3		1	
- Acute myeloid leukaemia	2		2	
- Osteomyelosclerosis	1	(1L)	0	
- Lymphatic leukaemia	3		2	
- Leukaemia of unknown type	1		1	
- Non-Hodgkin-Lymphoma	2	(1 L)	1	
- M. Hodgkin	1		0	
- Extramedullary plasmocytoma	0		1	
Bone marrow failure	12	(8 L)	9	(3L)
Cataracts	36	(33L)	20	(19L)
Exostosis	1	(1L)	1	

* living

Publications:

Late effects after Ra-224 treatment of ankylosing spondylitis patients
EULEP Task Group Meeting, Chilton (UK), November 6 - 7, 1990
EULEP Newsletter (in press)

Head of Project 2: Prof. Dr. H. Spiess

II Objectives for the reporting period

Standard questionnaires have been sent out to 243 adult patients. Nearly 80% (192) of the adult patients answered. 23 of the adult patients died during the reporting period. In addition to the contact via questionnaires some of the patients who live in Northern Germany were visited by a colleague of Prof. Spiess. One member of the project staff attended two epidemiology courses at the University of Michigan in summer 1990, and followed this by a stay at NIH, Radiation Epidemiology Branch, Bethesda, to discuss scientific planning for the project. A cooperation with Dr. C. Land (National Cancer Institute/NIH, Radiation Epidemiology Branch, Bethesda/MD, USA) has been initiated. To determine whether the elevated number of breast cancers in the collective is radiation-induced, we have considered the possibility to build up a control group of female patients treated for bone tuberculosis in the same time period as our patients.

III Objectives for next period

- Standard questionnaires will be sent out to the juvenile patients
- Patient visits are planned for patients living in Northern Germany
- Invite juvenile patients to University of Munich to complete their record history and to perform detailed examinations
- Cooperation with Prof. F. Stefani to examine patients eyes with Scheimpflug-camera (Eye Hospital of the University of Munich)
- Cooperation with Dr. C. Land (National Cancer Institute/NIH, Radiation Epidemiology Branch, Bethesda/MD) in the cataract and kidney study

IV Progress achieved including publications

At 3 year intervals we are following the health of 900 patients (509 men, 173 women, 111 boys, and 107 girls) who received repeated injections of ^{224}Ra after World War II, for treatment of ankylosing spondylitis or bone tuberculosis, but also for other non-cancerous diseases. At the time of last contact 553 of 900 patients were deceased. 54 patients developed bone sarcomas, 2 patients developed a second bone sarcoma. The last bone tumour appeared one year ago, but the risk appears nearly extinct by now. There were 107 observed soft tissue malignancies, and this number is close to the expectation based on the distribution in age, the length of the follow-up of the cohort, and the age specific population rates for the different malignancies. The age specific rates were taken from the Saarland and the former GDR tumour registry. For a few cancer types, however, the observed numbers are in excess of the expectation values.
The breast cancer excess came as a surprise, with 16 cases observed versus 4.1 - 6.1 cases expected. All but one case occurred more than 10 years after irradiation and all but one occurred after the age of 35. In the patients given ^{224}Ra as adults the 8 cases observed are similar statistically to the 3.5 - 5.2 expected cases. However, for those given ^{224}Ra as juveniles, a ninefold increase occurred (8 cases observed vs. 0.6 - 0.9 expected, $p < 0.000005$).
Liver cancers have occurred in seven ^{224}Ra patients, i.e. in a significantly larger number than the 1.1 - 1.2 cases expected ($p < 0.001$). One "probable" liver cancer may possibly have been a metastasis, but the clinical and pathology reports clearly indicate that the other five were primary liver cancers, and did not originate from the gallbladder or external bile duct. Even for the 5 confirmed cases versus 1.1 - 1.2 expected, the difference is still significant ($p < 0.008$).
Kidney cancers have occurred in 6 patients versus 2.4 - 2.6 cases expected ($p < 0.05$). All of these cancers were hypernephromas, the most common form of

kidney cancer. The tumour appearance times ranged from 12 to 35 years after the start of ^{224}Ra injections. Leukaemias occurred in 6 patients compared to 2 cases expected based on German Cancer Statistics. The elevated leukaemia rates observed in the control group of Project 1 would correspond to 5 leukaemia cases among the ^{224}Ra spondylitis patients, whereas from German population rates 2.2 cases would be expected. Of the 6 leukaemias among the ^{224}Ra patients in our study, 4 occurred among the 396 spondylitis patients; only two occurred among the other 504 patients who mostly had tuberculosis for which few drugs were available at that time in Germany. Only one of the juvenile patients has developed a leukaemia; she, a tuberculosis patient, had chronic lymphatic leukaemia, a type not assumed to be associated with radiation exposure.
Statistical and radiobiological evidence suggests that ^{224}Ra and its decay products can induce bone sarcomas and may induce breast, liver, and kidney cancers in humans. In the continued follow-up, the possibility of increased rates of additional types of cancers will need to be monitored.

Table: Summary of diseases of the ^{224}Radium patients (Dec. 1990)

	age at first injection		
	0-19 yr	adult	total
Skeletal diseases			
Bone sarcoma	38	18	56
Exostosis	29	0	29
Growth retardation	28	0	28
Tooth breakage	40	20	60
Soft tissue diseases			
Cataract	38	47	85
Liver (non-cancer)	4	35	39
Kidney (non-cancer)	11	69	80
Diabetes	3	28	31
Cancers of soft tissue	22	85	107
Lung	2	15	17
Breast	8	8	16
Urogenital tract	2	21	23
Bladder	0	6	6
Prostate	0	7	7
Uterus	1	5	6
Fallopian Tube	0	1	1
Ovar	1	2	3
Gastro-intestinal tract	3	20	23
Stomach	1	11	12
Pancreas	0	2	2
Colon	1	5	6
Rectum	1	2	3
Skin	2	3	5
Liver	2	5	7
Kidney	1	5	6
Others (f. e. brain)	2	8	10
Leukaemia	1	5	6

Publications:
Chmelevsky, D., Spiess, H., Mays, C. W., Kellerer, A. M.: The reverse protraction factor in the induction of bone sarcomas in Ra-224 patients.
Rad. Res. 124, 69-79 (1990)

Head of Project 3 : Prof. Dr. A. M. Kellerer

II Objectives for the reporting period

- Analysis of the incidence of soft tissue tumours in the bone tuberculosis and Morbus Bechterew patients treated with ^{224}Radium.
- Computation of expected incidences on the basis of the age distribution and the time at risk of the patients.
- Derivation and application of suitable statistical methods to determine excess frequencies and possible correlation with injected activity.

III Objectives for next period

- Examination of the feasibility of the formation of the control group for a case control study of the breast cancer patient.
- Reassessment of the dosimetry of ^{224}Radium for different organs.

IV Progress achieved including publications

In the collective of patients followed by Prof. Spiess (Project 2) a variety of soft tissue neoplasms has been observed during recent years. In their entirety the tumour frequencies are in line with the increasing incidences expected in the aging collective; the quantitative comparison is included in the discussion of Project 2.
For a few tumour types, breast tumours, liver tumours and kidney tumours the expected numbers are substantially smaller than the observed frequencies. The difference is particularly striking for the breast cancers, and there is specific interest in this apparent excess in view of the recent reports on elevated breast cancer risks in the women who have been irradiated against *tinea capitis* as children.
However, the age specific breast cancer rates for Germany are somewhat uncertain, since data are available only from the registries of Saarland and of the former GDR. The numbers are, again, included in the report and the tables for Project 2.
Beyond the mere observation of an excess, it is important to examine the correlation of the incident cases with injected activity. We have for this purpose utilised suitable rank-order procedures - which were also topic of our methodological studies within the project - and have concluded, that, at this point in the follow-up there is no significant trend with injected activity. This underlines the need for a special control group that may still be formed among bone tuberculosis patients. In this planned investigation particular attention will have to be given to X-ray diagnostics that may have been frequent among the patients.

Publications:

Breckow, J., Kellerer, A. M.: Wirkungen kleiner Strahlendosen
Phys. uns. Zeit 21, 63-69 (1990)

Kellerer, A. M., Rossi, H. H.: A generalized definition of dosimetric quantities
Int. J. Radiat. Biol. 57, 859-864 (1990)

Progress Report

Contract: Bi6-298 **Sector:** C14

Title: Thorotrast-investigations to evalute the long term effects caused by artificial radiation in man (thorotrast patients follow-up study)

1 Van Kaick Deutsch.Krebsforsch.Zent.Heidelberg

I. Summary of Project and Global Objectives

The aim of the German Thorotrast Study is to uncover the late effects of incorporated colloidal thoriumdioxide by epidemiological observations and clinical and biophysical examination of the patients; to compare the results of those of a corresponding control group and to assess the relationship between late effects and radiation dose. Furthermore we try to offer appropriate diagnostic and if possible therapeutic facilities and to give advice to the family physicians of the patients.

The German Thorotrast Study comprises 2326 Thorotrast patients and 1890 contemporary matched patients in the control group to be evaluated. 899 Thorotrast patients and 662 controls had clinical and biophysical follow-up examinations every two years since 1969. The recent most important results of the study are: a high excess rate of primary liver cancer (411/2) was observed beginning after the 15th year of exposure. 31% of the tumors are combined with cirrhosis and 6% with other neoplastic diseases. A clear (mean) dose rate effect relationship exists. The tumor frequency depends on the time of exposure or the cumulative dose to the liver respectively and not primarily on the age at injection. The lowest cumulative dose at 10 years before diagnosis of liver cancer were about 2 Gy. Risk estimates for liver cancer after 40 years of exposure are 500 malignant tumors per 10^4 person-Gy for man and 300 for women.

A high excess rate exists also for nonlymphocytic leukaemia starting already 5 years after Thorotrast injection (39/4). The lowest cumulative dosis to the red bone marrow at time of death were about 0.5 Gy. According to the present results an excess rate can be expected for carcinomas of the extrahepatic bile ducts, pancreas, esophagus, larynx, as well as non-Hodgkin's lymphoma, bone sarcomas, plasmacytomas and mesotheliomas.

Head of Project 1: Prof.Dr. van Kaick

II Objectives for the reporting period

- Clinical, biochemical and radiological examinations of the Thorotrast patients and the control group
- Biophysical examinations to calculate the tissue dose due to the thoriumdioxide deposits and their radioactive daughter products
- Identification of the causes of death of Thorotrast patients and members of the control group
- Statistical evaluation of the epidemiological, clinical and biophysical data

III Objectives for next period

- The working program will be continued according to the recommendations of the coordinating committee
- Regular correspondence with about 500 patients of the Thorotrast and control group and the family physicians
- Out-patient examinations of Thorotrast patients and patients of the control group at two year intervals
- Supplementation and controlling of the stored data and preparation of final statistical evaluation

IV. Progress achieved including publications

Clinical examinations

In the reporting period we examined 183 patients (98 Thorotrast patients and 85 control patients). We detected 13 primary liver tumors and 3 recurrences. Furthermore we diagnosed cancer of the following organs (Thorotrast/control): kidney (1/1); pancreas (1/0); prostate (2/0).
The evaluation of the causes of death of 35 patients of both groups resulted in: primary liver cancer (8/0); nonlymphocytic leukaemia (2/0); chronic lymphocytic leukaemia (0/1); lung cancer (1/1); cancer of the GI-tract (1/1).

Epidemiological studies

2171 (of n=2326) Thorotrast patients and 1504 patients of the control group (of n=1890) have died. Table 1 summarizes neoplastic diseases with a high or probable excess rate.

Liver cancer ist the most common Thorotrast induced tumor. The shortest latency period was 16 years; the longest up to now 51 years. 336 (82%) of the liver tumors are confirmed by biopsy or autopsy; 55(14%) by clinical follow up, and only 19 (4%) by death certificates. Pathohistological findings describe cholangiocarcinoma (126/31%), haemangiosarcoma (95/23%) and hepatocellular carcinoma (71/17%). Others are histologically diagnosed as carcinomas of the liver without classification (44/10%).
The remainders were clinically proven cancers of the liver but without histological confirmation (74/18%). The male to female ratio of liver cancer in total was 1.7/1.

Table 1.
German Thorotrast Study - Diseases with High or Probable Excess Rate

Status '91 Cause of Death	Thorotrast n=2,326		Control n=1,890	
Liver cancer*	411 [+5]	(17,88%)	2	(0.11%)
Liver cirrhosis	187 [+171]	(15,39%)	49 [+2]	(2.70%)
Nonlymphocytic leukaemia	36 [+3]	(1.68%)	5	(0.26%)
Bone marrow failure+	29	(1.25%)	5 [+1]	(0.32%)
Ca ext. bile ducts	28 [+3]	(1.33%)	6	(0.32%)
Ca. pancreas	18	(0.77%)	5	(0.26%)
Ca. esophagus	7 [+1]	(0.34%)	1	(0.05%)
Ca. larynx	6 [+1]	(0.30%)	1 [+1]	(0.11%)
Non-Hodgkin's lymphoma	14 [+2]	(0.68%)	3	(0.16%)
Bone sarcoma	4 [+1]	(0.21%)	1	(0.05%)
Plasmacytoma	7 [+2]	(0.38%)	1	(0.05%)
Mal. Mesothelioma				
Pleural	3	(0.17%)	0	
Peritoneal	2	(0.09%)	0	

[] Additional cases with another disease leading to death
* 5 patients with combined carcinoma and sarcoma
() Inclusive additional cases related to n
\+ Aplastic anaemia, agranulocytosis, and thrombocytopenia

The risk for nonlymphocytic leukaemia (NLL) is tenfold higher in the Thorotrast group compared to the controls. The earliest latency period was 5 years; the longest 47 years (Figure 1). The acute NLL were classified as: acute myelocytic leukaemia (11); acute promyelocytic leukaemia (15); acute monocytic leukaemia (5); erythroleukaemia (3); acute plasmacell leukaemia (1). Chronic myeloid leukaemia (CML) were observed in 4 patients having long exposure times of 16, 28, 39 and 42 years.
In 3 cases NLL were combined with liver cancer. All cases except 4 patients were clinically and haematologically confirmed; 15 deceased patients were autopsied.

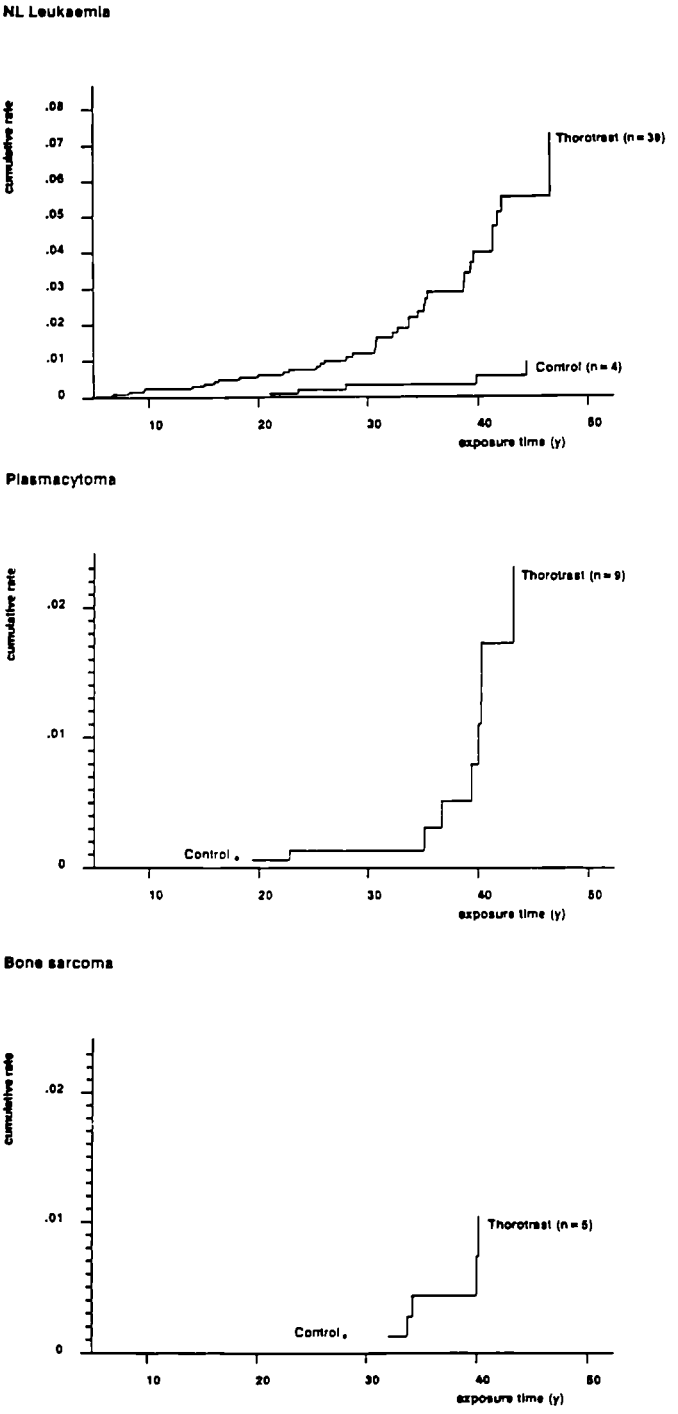

Figure 1.
Cumulative rate of nonlymphocytic leukaemia, plasmacytoma and bone sarcoma of all Thorotrast and control patients.

The last years have shown an increase of neoplastic diseases in different organs (Table 1); an excess rate is very probable. However we have to keep in mind that twice of control patients are still alive so that this figures may change in the following years. Excess of carcinomas of the gall bladder and extrahepatic bile ducts, of plasmacytoma and of bone sarcoma were observed also in other Thorotrast series (Mori et al., 1989; Olssen et al., 1989)(Figure 1).

PUBLICATIONS

van Kaick, G., Wesch, H., Lührs, H., Liebermann, D., Kaul, A. (1991):Neoplastic diseases induced by chronic alpha-radiation; epidemiological, biophysical and clinical results of the German Thorotrast Study. J Rad Research (in press)

Görich, J., Liebermann, D., Lührs, H., van Kaick, G. (1991) Regional lymphnodes of liver and spleen - topographic examinations in Thorotrast patients. Br J Radiol (in press)

Progress Report

Contract: Bi6-333 Sector: C14
Title: Late effects of thorotrast among Danish patients

1 Jensen Danish Cancer Society

I. Summary of Project and Global Objectives

The aim of this study is to describe and analyze by epidemiological methods the potential long-term health effects of intra-arterial exposure to Thorotrast in Danish patients injected for cerebral angiography during 1935-1947.
Thorotrast, a 20-25 % colloidal solution of thorium-232 dioxide, is known to be retained lifelong principally in the reticuloendothelial system (RES) (liver, spleen, bone marrow, lymph nodes adjacent to liver and spleen) subsequent to systemic administration. Distribution outside the RES is not well characterized but probably of minor importance. Thorium-232 is a long lived mainly alpha-emitting nuclide (physical half-life > 10^{10} years). A minor redistribution to bones of the retained Thorotrast is believed to find place during the decay and one daughter product of the decay is thoron (Rn-220), a gas which is partially dissolved in the blood and exhaled by the lungs.
Thus, Thorotrast-injected persons are subject to a continuous, long lasting exposure to internal alpha radiation of the RES and perhaps of the epithelium of the airways.
Only few other populations known to have been exposed to alpha emitters have been studied (e.g. luminous dial painters and spondylitics exposed to radium) and among these increased risks of bone cancer have been observed. This is in contrast to external radiation which numerous populations have been exposed to and subsequently followed up (e.g. survivors after nuclear bomb explosions and patients medically irradiated for different purposes). Leukaemia and solid tumours in all organs occur with increased frequency in such populations.
Alpha-emitting nuclides such as plutonium and americium are important in nuclear industry. It is anticipated that these nuclides may be widely dispersed in the environment in case of nuclear power plant break downs. The potential health effects on human populations exposed to such nuclides by ingestion or inhalation are unknown, but it is believed that the results from studies of persons exposed to thorium may be applicable to these situations as well.
In Denmark, a study aimed of identifying and following up Thorotrast-injected persons was set up as early as 1949. It was elaborated in the years following, but it has not been active during the latest years and some criticism as to the methodology employed has been raised.
The present study will reidentify all persons, reverify Thorotrast exposure, comprehensively follow up all persons with regard to vital status, cancer incidence, cause of death, and incidence of non-malignant diseases and relate the incidences to that of the general population and to Thorotrast dose and demographic variables. A study of cancer incidence and mortality among offspring of Thorotrast injected will also be included.

Head of Project 1: Dr. Jensen

II Objectives for the reporting period

During the reporting period the existing historical data on Thorotrast-exposed persons were computerized. Verification of identity, of Thorotrast exposure, of vital status, of cause of death, of cancer incidence, and of hospital discharge diagnoses was obtained by computerized record-linkages to the Central Population Registry, to the National Registry of Causes of Death, to the Danish Cancer Registry and to the National Registry of Patients, and, in cases of doubt, by manual queries to different bodies. Thorotrast exposure was verified by searching and copying original hospital records. A preliminary analysis of cancer incidence has been conducted.

III Objectives for next period

The next period of the study will be aimed of further analyzes of cancer incidence related to a number of variables. The non-cancer mortality will be assessed and related to standard rates of the population. The relative mortality of the cohort will be assessed controlling for Thorotrast related causes of death. The pattern of hospital discharge diagnoses will be analyzed in a case-control design. In depth studies including histo-pathologic review of relevant diseases will be carried out if possible. The offspring of the Thorotrast injected will be searched for by manual queries to local population registries. The cancer incidence and mortality of the offspring will be analyzed by record-linkage to relevant registries.

IV Progress achieved including publications

By computerized record-linkage with the Central Population Registry and by two preliminary record-linkage procedures with the National Registry of Causes of Death confirmation of identity and verification of vital status was obtained for most of the patients. However, in approximately 250 cases it has been necessary, usually due to incorrect data in the original material, to trace persons through manual queries to local population registers and to church officials. Further, approximately 200 hospital records have been traced in order to verify causes of death and to collect pathology reports etc. Verification of Thorotrast exposure has been obtained by tracing and copying approximately 800 hospital records from 1930s and 1940s describing the Thorotrast injection.

A record-linkage with the Danish Cancer Registry has been conducted. Persons having had a cancer diagnosed and alive after 1st April 1968 have been identified computerized by means of the unique person identifying number given to all Danish residents at that time while persons with a diagnosis of cancer but dead before 1st April 1968 have been searched for manually in the Cancer Registry. The incidence of cancer in the whole cohort has been analyzed and by life table methods the incidence has been related to the cancer incidence of the Danish population specified for sex, age, calendar period and cancer site.

For persons who have died the cause of death has been identified in the National Registry of Causes of Death by record-linkage. This registry also holds rates specified for cause of death, age, sex and calendar period (with the necessary regulations due to changing classifications during time) for the whole population. Data are currently being analyzed in a similar manner to data regarding cancer morbidity.

For persons alive 1977 or later (249 persons) data regarding all hospital admissions in the period 1977-1989 have been obtained through linkage to the National Register of Discharge Diagnoses. As no rates regarding discharge diagnoses exist for the whole population, these data will be analyzed in a case-control design where matched controls will be drawn randomly from the background population.

Results:

From table 1 can be seen that information regarding 1095 persons was found in the original material. 96 persons have been excluded mostly because Thorotrast exposure could not be verified and was considered to be less possible (56 persons). Thus, the study population consists of 999 historical prospectively registered Danish residents with Thorotrast exposure for cerebral angiography with fully identified vital status and information of date of Thorotrast injection and volume of Thorotrast injected.

Details regarding the material are given in tables 2-4.

The overall sex and site specific cancer incidence with relative risks has been listed in table 5. In general the cohort experiences an elevated cancer risk compared to the Danish population.
Remarkable is the high incidence of cancer of the central nervous system. This is due to an extremely high incidence in the years immediately following the Thorotrast exposure and is a reflection of brain tumours often having caused symptoms due to which the cerebral angiography was performed.

Notable is also the extremely high incidence of liver cancer. This has been described earlier in other trials. In contrast to the German trial there is no tendency to a higher incidence of liver cancer among men than in women. Likewise, in contrast to the German study, there is a significantly increased risk of lung cancer among males in the Danish study and a similar, however, not statistically significantly, increased risk in females.

Other sites which present a higher than expected incidence is breast (RR 1.80), ovary (RR 2.44), other skin among females (RR 2.41), eye among females (RR 15.2), metastases and other unspecified sites among both females and males, multiple melanoma among females (RR 11.0) and leukaemia in men and women (RR 10.6). Finally three cases of cancer of peritoneum (2 mesotheliomas) appeared among men (RR 18.9). Data are being further analyzed and related to age at injection, injected volume etc.

Table 1 MATERIAL

Total registered		1095
	Number of persons	
Duplicate registration	3	
Foreign resident	8	
No cerebral angiography	14	
Registered in study at the time of a cancer diagnosis	7	
Not identified or traced	8	
Thorotrast not verified	56	
	96	96
Study population		999 persons

Table 2 Age at Thorotrast injection

	Number of persons		
age (years)	males	females	total
0 - 4	0	2	2
5 - 9	9	8	17
10-14	27	16	43
15-19	44	31	75
20-29	99	104	203
30-44	188	132	320
45-59	155	112	267
60+	42	30	72
Total	564	435	999

Table 3 Injected Thorotrast volume

Thorotrast Dose (ml)	number of persons males	females	total
1 - 10	236	182	418
11 - 20	210	175	385
21 - 30	65	45	110
31 - 40	34	22	56
41 - 50	14	4	18
51+	5	7	12
total	564	435	999

Table 4 Time at risk after Thorotrast injection (46 persons still alive)

Time after Thorotrast injection (years)	number of persons males	females	total
0 - 1	115	73	188
1 - 2	18	17	35
2 - 3	23	12	35
4 - 9	49	40	89
10 - 19	98	47	145
20 - 29	103	86	189
30 - 39	89	92	181
40 - 49	68	66	134
50+	1	2	3
total	564	435	999

Table 5

Observed (O) and expected (E) numbers of cancer among 999 Thorotrast exposed persons 1935 - 1988. Modified ICD7. *: p< 0.05

	MALES				FEMALES			
Cancer site ICD7	O	E	O/E	95% CI	O	E	O/E	95% CI
All sites 140-205	193	47.6	4.06*	(3.5-4.7)	177	46.1	3.84*	(3.3-4.4)
Sarcoma 140-205	18	0.69	26.0*	(15.5-41.2)	10	0.73	13.8*	(6.6-25.2)
Buccal cavity and Pharynx 140-148	3	1.73	1.73		0	0.54	0	
Esophagus 150	1	0.64	1.54		0	0.26	0	
Stomach 151	5	4.60	1.09		2	2.41	0.83	
Small intestine 152	1	0.15	6.60		1	0.12	8.61	
Colon 153	5	3.22	1.55		4	3.58	1.12	
Rectum 154	3	3.37	0.89		1	2.17	0.46	
Liver 155.0	42	0.40	105*		38	0.23	163*	
Gallbladder 155.1	5	0.37	13.4*		10	0.67	14.8*	
Liver, not specified as primary 156	8	0.24	33.3*		6	0.22	26.9*	
Pancreas 157	2	1.55	1.29		3	1.14	2.64	
Peritoneum 158-159	3	0.15	18.9*		0	0.19	0	
Nasal cavity 160	0	0.14	0		2	0.06	31.0*	
Larynx 161	1	0.72	1.38		0	0.10	0	
Lung primary 162	19	8.29	2.29*		4	1.81	2.20	
Pleura 162.2	1	0.17	5.94		0	0.06	0	
Breast 170	0	0.08	0		18	10.0	1.80*	
Cervix uteri 171					6	5.06	1.18	
Corpus uteri 172					1	2.69	0.37	
Ovary 175					7	2.87	2.44*	
Other gync. 176					1	0.46	2.18	
Prostate 177	6	3.95	1.52					
Testis 178	2	0.62	3.22					
Other male gen. 179	1	0.23	4.35					
Kidney 180	2	1.46	1.37		3	0.98	3.06	
Bladder 181	4	3.32	1.21		2	0.95	2.10	
Melanoma of skin 190	1	0.54	1.85		1	0.71	1.41	
Other skin 191	2	4.97	0.40		8	3.32	2.41*	
Eye 192	0	0.18	0		2	0.13	15.2*	
Brain,CNS 193	47	1.36	34.5*		24	1.14	21.0*	
Thyroid 194	0	0.14	0		1	0.26	3.84	
Endocrine 195	1	0.07	14.4		0	0.05	0	
Bone 196	0	0.13	0		1	0.07	11.5	
Connect. tissue 197	0	0.23	0		1	0.18	5.64	
Metastases 198	8	0.73	11.0*		8	0.64	12.5	
Other, unspec. 199	5	0.34	14.6*		5	0.56	8.92*	
Non-Hodgkin Lymphoma 200,202	3	0.86	3.50		0	0.61	0	
Mb Hodgkin 201	0	0.42	0		1	0.24	4.09	
Mult. myeloma 203	0	0.51	0		4	0.37	11.0*	
Leukaemia 204	12	1.38	8.72*		12	0.88	13.7*	

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Istituto Superiore di Sanità
Viale Regina Elena 299
I-00161 Roma

Contract no.: BI6-F-313-I

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: (06) 4990

Title of the research contract:

Epidemiologic study on respiratory cancer among miners with low dose radiation exposures.

List of projects:

1. Epidemiologic study on respiratory cancer among miners with low dose radiation exposures.

Title of the project no.:

Epidemiologic study on respiratory cancer among miners with low dose radiation exposures

Head(s) of project:

Dr. P. Comba
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Scientific staff:

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I. Objectives of the project:

The aim of the present project was the study of cause specific mortality among Italian miners, with special reference to respiratory malignancies. The contrast between observed and expected mortality rates for lung cancer in various cohorts of miners exposed to different levels of radiation can infact provide figures for estimating the number of extra cases of lung cancer per Working level Month and person years of exposure.

II. Objectives for the reporting period:

III. Progress achieved:

METHODOLOGY

The Gorno mine is located in the Central Alps , north of Milan . Zinc , lead and silver were mined in this site by the Etruscans , by the Romans and , after several centuries , by the Republic of Venice and subsequently by the Italian Government. Exploitation of the ore was discontinued in 1981 . A survey by ENEA detected radon concentrations ranging between 27 and 130 pCi/l , corresponding to 0.20 - 1.03 working levels, the median being 0.60 WL. A concurrent survey by CEA estimated 0.10 - 0.90 WL, with a median value of 0.36 .

A mortality cohort study design was adopted. All male subjects who were at work in 1950, or were subsequently employed, till 1980, were included in the cohort. Company files provided information about name , date of birth , dates of hiring and dismissal (if any), residence and job title. This last was categorized in underground and surface worker; subjects were allocated to the surface worker category only if they had never worked underground.

Altogether 1392 subjects were enrolled in the cohort ; 25 females and 10 subjects with missing data were excluded from the study.

Vital status of cohort members was ascertained through search in the Registry Office of the competent municipalities , and by use of the data base on mortality operating at ENEA (Altavista et al 1984).

Expected deaths and standardized mortality ratios (SMR) with their 95 % confidence intervals were computed using the program Life Table Analysis System , realized by Nosh and installed at I.N.S. Cause - sex - age - calendar time specific mortality rates of the Italian population were used as reference.

RESULTS

The study included 1357 subject , 60 of whom were lost to follow-up . The total number of deceased subjects was 527; for 30 of them it was not possible to ascertain the cause of death.

Table 1 shows cause specific mortality among underground miners . Overall mortality and mortality for all neoplasms were significantly increased with respect to expected figures. Oesophageal, gastric and lung cancer occurred at significantly increased rates.
Mortality for tuberculosis and for non malignant respiratory diseases showed a significant excess : 52 out 68 cases of death from respiratory disease were attributed to silicosis, which was also mentioned on 10 out of 12 causes of death from respiratory tuberculosis. Mortality for violent causes was significantly increased, while diseases of the circulatory and digestive systems did not show significant departures from expected figures. Finally, a significant decrease in mortality for diabetes should be mentioned.

As far as surface workers are concerned, mortality for all causes and for all neoplasms did not exceed significantly expected values ; a significant excess of liver cancer was detected. Silicosis accounted for 10 out of 21 deaths for respiratory diseases and was mentioned in 2 out of 4 death certificates referred to tuberculosis. Circulatory diseases showed a significant decrease and no case of diabetes was observed. Liver cirrhosis was increased, but not significantly.

The main finding of the study appears to be the elevated mortality for respiratory diseases observed in underground workers ; among surface workers no excess of lung cancer was detected , while the occurrence of silicosis caused an increase in mortality for non malignant respiratory diseases.

If lung cancer mortality is considered taking into account duration of exposure and latency time, it can be seen that the extra cases among underground workers tend to occur after about 20 years from beginning of exposure.

The number of extra cases of lung cancer among underground miners was 16.4 ; since average duration of exposure was 17.93 years, and median level exposure to radon was 0.6 WL according to ENEA and 0.30 WL according to CEA , it was estimated that between 9.78 and 16.31 extra cases per 10^6 person years and WLM had occurred.

DISCUSSION

The mortality study concerning zinc - lead miners in Gorno resulted in an estimate of 9.78 - 16.31 extra cases of lung cancer per 10^6 person years and WLM. This estimate is coherent with the findings of a previous study by Battista et al. (1988) concerning iron ore miners in Tuscany : in that case the estimate was 13 per 10^6 person year and WLM.

REFERENCES

Altavista P., Belvisi M., Mastrantonio M., Miniero O.,: Base: una base di dati per analisi e studi epidemiologici . Atti del II Convegno Nazionale sugli Studi di Mortalita' , Firenze 10-12 Ottobre 1984: 195-197.

Battista G., Belli S., Carboncini F., Comba P., Levante G., Sartorelli P., Strambi I., Valentini F., Axelsen O.: Mortality among pyrite miners with low - level exposure to radon daughters. Scand J Work Environ Health 1988; 14: 280-285.

Table 1. Mortality of underground workers

Cause of death	Observed	Expected	SMR	95% I.C.
All causes	348	217,11	160	143 - 170
All cancers	95	58,68	161	130 - 190
Mouth and pharynx	1	2,03	49	1 - 270
Digestive tract	37	20,46	180	127 - 240
Oesophagus	7	1,55	451	180 - 920
Stomach	18	8,93	201	119 - 310
Intestine	4	3,40	117	32 - 300
Rectum	1	1,85	53	1 - 290
Liver	3	2,45	122	25 - 350
Pancreas	3	1,88	159	32 - 460
Peritoneum	1	0,30	326	8 -1810
Respiratory tract	36	19,67	182	128 - 250
Larynx	3	2,36	126	26 - 370
Trachea,bronchi and lungs	33	16,60	198	136 - 270
Prostate	3	2,60	115	23 - 330
Genitourinary tract	5	3,33	149	48 - 350
Kidney	2	0,95	210	25 - 750
Bladder	3	2,38	125	25 - 360
Other sites	9	6,56	137	62 - 260
Lyaphatic and hemopoietic system	3	3,93	76	15 - 220
Tuberculosis	12	3,95	303	156 - 530
Respiratory tuberculosis	12	3,71	323	166 - 560
Diabetes	0	3,75		
Diseases of blood	0	0,42		
Mental disorder	2	0,71	280	34 -1010
Diseases of the nervous system	3	2,52	118	24 - 340
Diseases of circulatory system	82	85,62	95	76 - 110
Respiratory diseases	68	16,95	401	311 - 500
Acute infections	0	0,21		
Influenza	0	0,48		
Pneumonia	10	3,80	262	125 - 480
Bronchitis	3	9,78	30	6 - 80
Other	55	2,66	2060	1552-2680
Diseases of the digestive system	21	19,43	108	66 - 160
Hepatic cirrhosis	14	12,47	112	61 - 180
Diseases of the genito-urinary tract	1	3,76	26	0 - 140
Diseases of the skin	0	0,12		
Diseases of bones	0	0,25		
Ill-defined causes	15	2,59	577	322 - 950
Accidents	23	13,55	169	107 - 250
Suicide	1	2,37	42	1 - 230
Other and unknown	25	2,22	1121	725 -1650

Table 2. Mortality of surface workers

Cause of death	Observed	Expected	SMR	95% I.C.
All causes	179	165,51	108	93 - 125
All cancers	46	40,63	113	82 - 151
Mouth and pharynx	0	1,42		
Digestive tract	21	14,47	145	89 - 221
Oesophagus	1	1,08	91	2 - 509
Stomach	8	6,32	126	54 - 249
Intestine	4	2,46	162	44 - 414
Rectum	0	1,34		
Liver	6	1,69	355	129 - 772
Pancreas	1	1,30	76	1 - 426
Peritoneum	1	0,20	484	12 -2692
Respiratory tract	14	12,92	108	59 - 181
Larynx	4	1,57	254	69 - 651
Trachea,bronchi and lungs	10	10,88	91	43 - 168
Prostate	1	2,14	46	1 - 258
Genitourinary tract	3	2,32	129	26 - 377
Kidney	0	0,63		
Bladder	3	1,68	178	36 - 520
Other sites	5	4,56	109	35 - 255
Lymphatic and hemopoietic syste	2	2,70	73	8 - 266
Tuberculosis	5	2,55	195	63 - 456
Respiratory tuberculosis	4	2,39	166	45 - 426
Diabetes	0	2,77		
Diseases of blood	1	0,31	322	8 -1790
Mental disorder	1	0,49	204	5 -1133
Diseases of the nervous system	2	1,83	109	13 - 394
Diseases of circulatory system	51	69,72	74	55 - 97
Respiratory diseases	21	13,63	153	95 - 235
Acute infections	0	0,21		
Influenza	0	0,44		
Pneumonia	6	3,51	170	62 - 371
Bronchitis	3	7,58	39	8 - 115
Other	12	1,89	634	327 -1108
Diseases of the digestive system	18	13,46	133	79 - 211
Hepatic cirrhosis	15	8,39	178	99 - 294
Diseases of the genito-urinary tract	1	3,18	31	0 - 174
Diseases of the skin	0	0,09		
Diseases of bones	0	0,18		
Ill-defined causes	12	3,34	359	185 - 627
Accidents	6	9,98	60	21 - 130
Suicide	1	1,64	60	1 - 336
Other and unknown	14	1,54	907	459 -1522

Table 3. Mortality for lung cancer as a function of duraton of exposure and latency time

Latency	Exposure							
	Underground				Surface			
	=< 20 ys		> 20 ys		=< 20 ys		> 20 ys	
	O	E	O	E	O	E	O	E
=< 20 ys	3	2,75	-	-	1	2,34	-	-
> 20 ys	12	5,64	18	8,22	2	3,56	7	4,99

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. M. Di Paola, FAFA, Roma
Prof. G. Battista, University of Siena
Dr. P.L. Cocco, University of Cagliari
Dr. M. Rocchio, Local Health Unit, Clusone
Dr. R. Pagnoni, Local Health Unit, Albino

V. Publications:

IN PROGRESS

Dr D.M. Parkin, IARC, Lyon
ob/520

Contract no.: Bi6-319-C14

EUROPEAN CHILDHOOD LEUKAEMIA/LYMPHOMA INCIDENCE STUDY

Progress Report to 31.10.1990

Data collection from all participants should have been received for the entire period 1980-1987. By the end of October, this target had been largely achieved (See attached Table).

A summary of the scientific background to the study (updating that contained in the study protocol, and including estimates of the expected increases in observed numbers of cases) was presented at the 9th Conference of the American Statistical Association on Radiation and Health in Colorado, USA (8-12 July, 1990). An extended abstract of this presentation will be published in Radiation Research in December, 1990 (Copy attached as Annex A).

Incidence rates of leukaemia by cell type for the period 1980-85 have been calculated for national populations. These are shown as histograms in Figures 1 and 2, attached. Incidence rates for subsequent years 1986 and 1987 have also been calculated for all national populations. A selection of results, in graphic form, is shown in Figures 3-7.

The next steps in the analysis of the data received to date are:

(1) The calculation of baseline incidence rates for leukaemia (by subtype), for the subnational areas for which UNSCEAR provides dose estimates (Figure 8). Definitions of these areas, in terms of the geographic subdivisions of the participating countries, have been obtained.

(2) As 1988 data become available for the different centres, comparisons of observed cases, by age-group, with numbers expected from 1980-85 rates, for

(a) each year 1986, 1987, 1988
(b) period 1987-1988

These calculations will be performed for national areas, and for subnational units. Subnational units will then be reaggregated into areas with similar first-year estimated effective dose equivalents (e.g. ≥1000; 500-999; < 500 μSv).

During 1989/1990 agreement was obtained to extend the ECLIS study to two other important areas. Bulgaria had relatively high estimated exposure (760 μSv average first year effective dose equivalent). Agreement was reached that data for Bulgaria would be supplied for the study, however it is probable that this will have to undergo critical evaluation before it can be accepted. As for Austria, this will probably require the comparison of data available from several sources. Agreement was also reached with the Petrov Research Institute, Leningrad, to supply data from Byelorussia and parts of the Russia Republic. Again, however, it seems likely that a special ad hoc exercise will be necessary to validate the routine statistical data from the USSR. This has been the subject of separate negotiations concerning the extension of the present contract to permit the inclusion of data from the Soviet Union (See Annex B, attached).

Continuation of the Study

A full analysis of results for the baseline period (1980-85), and three years post-accident, will be completed during 1991. During the year, the third meeting of investigators should be held. Field work required during 1980 includes visits to Bulgaria, to investigate problems of identification and registration of leukaemia cases, and to USSR. Work within the USSR will be included within an extension to the present contract.

Dr J. Kaldor has now resigned from IARC. Statistical aspects of the analysis of the time trend data, and assistance in the field of radiation epidemiology, will continue to be provided through the Biostatistics Programme.

Attachments: Update Table
Annex A: Copper Mountain Abstract
Annex B: Report of meeting on Chernobyl 7-9 November 1989
Figures 1-7

23 November 1990

EUROPEAN CHILDHOOD LEUKAEMIAS/LYMPHOMAS INCIDENCE STUDY

22/10/90

Data received

Austria	1980-87	Leukaemia/Lymphoma
Czechoslovakia, Bohemia	1980-87	Leukaemia/Lymphoma
Czechoslovakia, Moravia	1980-87	Leukaemia/Lymphoma
Czechoslovakia, Slovakia	1980-87	Leukaemia/Lymphoma
Denmark	1980-87	Leukaemia/N.H.L.
Finland	1980-87	Leukaemia/Lymphoma
France, Bas-Rhin	1980-84	Leukaemia/Lymphoma
France, Dijon	1980-87	Leukaemia/Lymphoma
France, Doubs	1980-87	Leukaemia
France, Isere	1980-87	Leukaemia/Lymphoma
France, Lorraine	1983-87	Leukaemia/Lymphoma
France, PACA & Corsica	1984-88	Leukaemia/Lymphoma
German Dem. Rep.	1980-87	Leukaemia/N.H.L.
Germany, Fed. Rep.	1980-87	Leukaemia/Lymphoma
Hungary	1980-88	Leukaemia
Italy, Piedmont	1980-86	Leukaemia
Netherlands	1980-87	Leukaemia
Norway	1980-87	Leukaemia/Lymphoma
Poland	1980-87	Leukaemia
Sweden	1980-85	Leukaemia
Switzerland, Basel	1980-85	Leukaemia
Switzerland, Geneva	1980-87	Leukaemia
Switzerland, Vaud	1980-87	Leukaemia
UK, England & Wales	1980-87	Leukaemia/Lymphoma
UK, Scotland	1980-87	Leukaemia/Lymphoma
USSR, Estonia	1980-87	Leukaemia/Lymphoma
USSR, Lithuania	1980-87	Leukaemia/Lymphoma
Yugoslavia, Slovenia	1980-87	Leukaemia/Lymphoma

ANNEX A

FOR PUBLICATION IN RADIATION RESEARCH, DECEMBER 1990

THE EUROPEAN CHILDHOOD LEUKAEMIA/LYMPHOMA INCIDENCE STUDY

D.M. Parkin, IARC, Lyon, France
(on behalf of the ECLIS Study Group)[1]

Following the accident at the Chernobyl nuclear power plant on 26 April, 1986, radioactive materials were deposited over large areas of Europe. There were three successive 'plumes' of material affecting (1) the Eastern USSR, Poland and Sweden (2), Central Europe - especially Austria, Bavaria, North Italy and part of Switzerland, and finally (3) Romania and Bulgaria. Most exposure was to ^{131}I, ^{134}Cs and ^{137}Cs, and was generally of rather low magnitude. Exposure to humans was both external (mainly from ground deposition) and internal (from ingestion of contaminated food). Estimated average exposures require rather complex models, and differ according to the methods used. UNSCEAR[1] has produced estimates for all national populations in Europe (and by sub-region within some countries). Nationally, the highest average exposures outside the USSR were in Bulgaria (760 μSv) and the lowest in Portugal (2 μSv). These figures can be compared with the average (worldwide) exposure from natural sources of 2400μSv per person - although there is considerable geographic variation .

Outside the immediate vicinity of the accident, the predicted health effects due to radiation are rather small. Nevertheless, these health effects should be monitored for several reasons. Firstly, it is a matter of great public concern, and already there are reports of clusters of leukaemia[2], excess infant mortality[3] and excess premature births among malformed children[4] in sub-national areas with higher than average exposures. Investigation of apparent clusters is greatly facilitated by a large scale systematic study. Secondly, it is possible that either dose estimates or the models used in predicting cancer risk are in error, and the excess will be higher than expected.

Childhood leukaemia is the most logical choice of adverse health effect for monitoring[5]. Radiation-induced leukaemias appear early (2-10 years) after exposure, and provide the largest excess incidence of any cancer. Background incidence of leukaemia is relatively constant in Europe[6]. Finally, a fairly comprehensive monitoring scheme is already in place in the form of registers of cancer and childhood cancer, so that a study of geographical and temporal trends in incidence requires no special data collection systems in most countries. It should be noted that the relatively good prognosis of childhood leukaemia in many countries, and likely improvements in therapy and survival in others, means that mortality data are virtually useless for monitoring risk.

[1] ECLIS Study Group (July 1990), Dr D.M. Parkin, Dr J.M. Kaldor (Dr D.R. English) Mr E. Masuyer (IARC), Dr H. Hansluwka (Austria), Dr J. Augustin (Czechoslovakia-Bohemia/Moravia), Dr I. Plesko (Czechoslovakia-Slovakia), Dr H. Storm (Denmark), Dr S. Karjalainen (Finland), Dr J-M. Lutz (France), Dr W. Staneczek (GDR), Dr J. Michaelis (FRG), Dr M. Vargha (Hungary), Dr B. Terracini (Italy), Dr J.W. Coebergh (Netherlands), Dr F. Langmark (Norway), Dr W. Zatonski (Poland), Dr L. Barlow (Sweden), Dr L. Raymond (Switzerland), Mr C.A. Stiller (UK., England & Wales), Dr R. Black (U.K., Scotland), Dr M. Rahu (USSR, Estonia), Dr R. Kriauciunas (USSR, Lithuania), Dr V. Merabishvili (USSR Statistics), Dr V. Pompe-Kirn (Yugoslavia).

The European Childhood Leukaemia/Lymphoma Incidence Study (ECLIS) was set up in 1987. It involves cancer registries in 17 European countries (Table I). Some 60 million children live in the areas covered, and approximately 2400 cases of leukaemia are expected every year. BEIR[7] provides a formula for calculating relative risk. Assuming (a) a latent period of 2 years, and (b) that risk at 2–4 years is the same as that at 5–10 (to which the BEIR data apply). The approximation RR = 1 + (0.243d) x exp (4.885) can be obtained, where d = dose in Sv. For an average exposure of 500 μSv, the relative risk is thus 1.016. Table I shows the estimated excess cases in each country using this formula. The average is an 0.8% increase, with a maximum in Byelorussia (around 6%).

The study involves the collection of data on all recorded cases of childhood leukaemia and lymphoma occurring in the populations covered by the registries. Collation of data from the different centres and its analysis is coordinated by IARC. Registries send an updated file of every case registered, with details of age, date of birth, date of diagnosis, place of residence, and histological diagnosis, at annual intervals. Data collection to the end of 1987 is now almost complete.

The period 1980–85 will serve as a baseline to investigate differences in the incidence between regions, and to provide information on underlying trends in incidence in the 6 years preceding the accident. Changes in 1986 and subsequent years will be studied to see if they bear any relationship to the estimated exposure levels in different regions. Since the place of residence of all cancer cases is recorded, it is possible to study almost any geographic units. The basic analyses will, however, comprise either national populations or broad subregions within certain countries, for which exposure estimates have been prepared by UNSCEAR.

Preliminary results confirm the rather constant background incidence of leukaemia, around 40 per million children per year (Table I). There is a slight male preponderance in almost all countries. Time trends in 1980–85 are rather variable in different countries, but several show small increases in incidence, against which future changes (post accident) will have to be evaluated.

A first complete analysis of background incidence, and age-specific incidence rates in 1986–87 will be completed during 1990. However, it is too early to relate any of the incidence data to the exposure estimates. Future analyses will also examine birth-cohort specific incidence, with particular interest focussed on children born in May 1986 – January 1987 (prenatal exposure).

ACKNOWLEDGMENTS

We wish to acknowledge the financial support of the Radiation Protection Programme, Directorate-General for Science, Research and Development, Commission of the European Communities (Contract B16-D-319-F).

We also acknowledge the assistance of Dr Burton Bennett and the United Nations Scientific Committee on the Effects of Atomic Radiation.

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[2] Gibson BES, Eden OB, Barrett AA, Stiller CA & Draper GJ. Leukaemia in young people in Scotland. (Letter) Lancet, ii, 630 (1988)

[3] Lüning G, Schmidt M, Scheer J & Ziggel H. Infant mortality after Chernobyl. Lancet, 335, 362 (1990)

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[6] Parkin DM, Stiller CA, Draper GJ, Bieber CA, Terracini B & Young JL. International Incidence of Childhood Cancer (IARC Scientific Publications No. 87), Lyon, International Agency for Research on Cancer (1988)

[7] Committee on Biological Effects of Ionizing Radiations. Health Effects of Exposures to Low Levels of Ionizing Radiation. National Academy Press, Washington D.C. (1990)

ECLIS STUDY (Children 0-14)

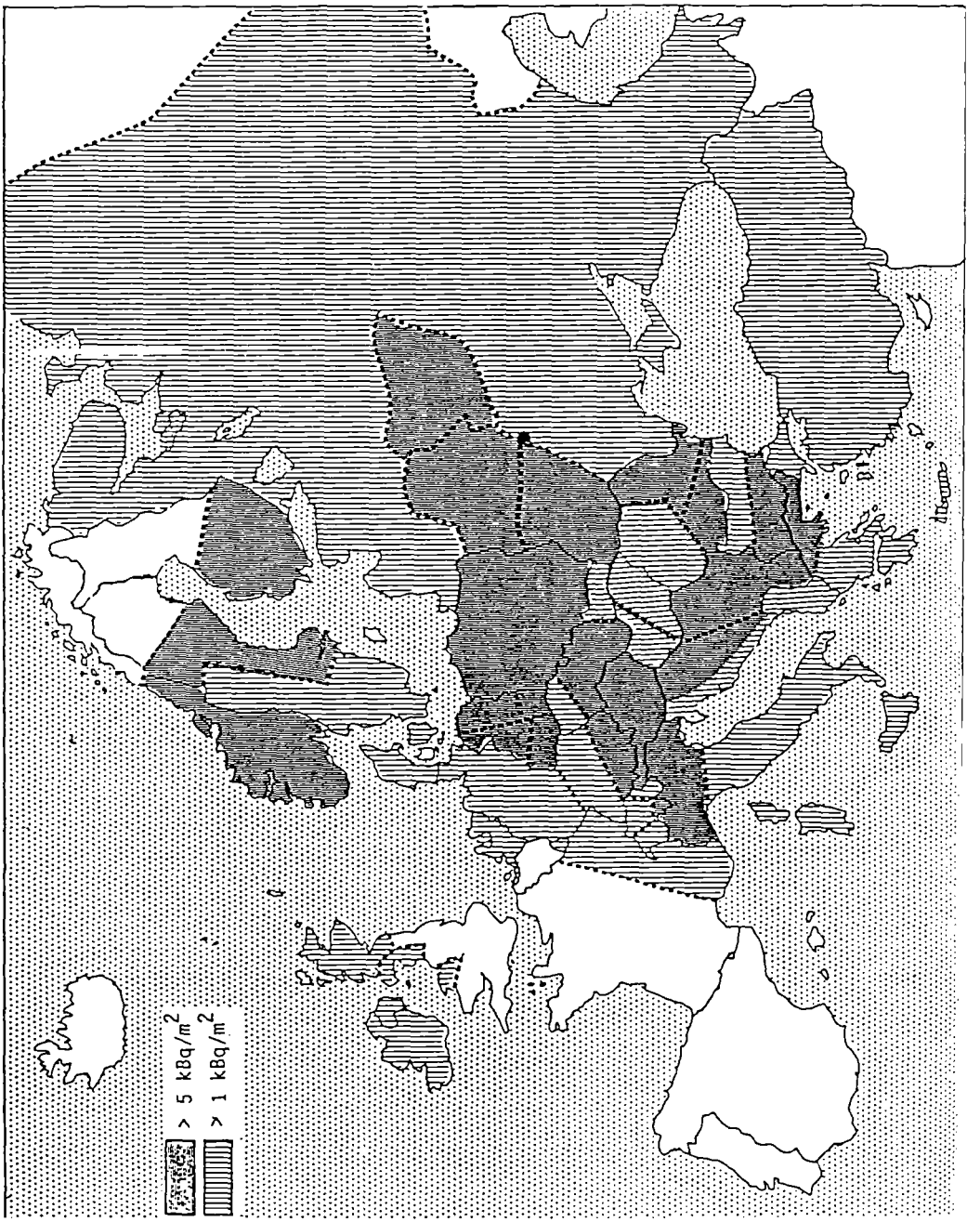
Average first year effective dose equivalent of radiation, observed incidence of leukaemia in 1980-85 and estimated cases of leukaemia post-accident.

	Dose (uSv)	Pop (mill)	Annual incidence rate 1980-85 (per mill)	Annual cases 2 years post-accident	
				Expected (baseline rate)	Estimated excess (radiogenic)
Austria	670	1.3	45	59	1.3
Czechoslovakia	350	3.8	40	152	1.7
Denmark	30	1.0	47	47	0.0
Finland	460	0.9	47	42	0.6
France (part)	155	1.6	(37)	59	0.3
German Dem. Republic	210	3.1	37	115	0.8
Fed. Rep. of Germany	130	9.6	42	403	1.7
Hungary	230	2.4	36	86	0.6
Italy (part)	370	1.3	(47)	61	0.7
Netherlands	58	2.5	41	103	0.2
Norway	230	0.8	43	34	0.3
Poland	270	9.4	29	273	2.4
Sweden	150	1.5	41	62	0.3
Switzerland (part)	270	0.5	(40)	20	0.2
UK:					
England and Wales	27	11.1	39	433	0.4
Scotland	27	1.3	38	49	0.0
USSR:					
Byelorussia	1950	2.5	[37]	93	5.8
RFSFR(3)	440	1.6	[37]	59	0.8
RFSFR(4)	135	2.3	[37]	85	0.4
Estonia	135	0.2	37	7	0.0
Lithuania	135	0.8	37	30	0.1
Yugoslavia (Slovenia)	625	0.5	42	21	0.4
TOTAL		60		2293	19.0

() Rates from IARC Scientific Publication Nº87
[] Rates for Estonia/Lithuania

RFSFR (3): Brjanskaya, Smolenskaya, Orlovskaya, Tulskaya and Kaluzhskaya oblasts
RFSFR (4): Leningrad, Karelenian A.R. and Leningradskaya, Pskovskaya and Novgorodskaya oblasts

Figure 8



ANNEX B

DMP/EC/ob
19.11.1990

REPORT OF DISCUSSIONS ON THE STUDIES WITHIN THE USSR OF NEOPLASMS FOLLOWING THE CHERNOBYL ACCIDENT
7-9 NOVEMBER 1990

Present: Dr E. Cardis
Dr J. Estève
Dr D.M. Parkin
Mr K. Saita
Dr L. Tomatis
Academician L.A. Ilyin
Dr E. Ivanov
Dr E.I. Komarov

I. CURRENT RESEARCH IN THE USSR

1. Organization of follow-up

The Chernobyl follow-up programme is the responsibility of the Ministry of Public Health of USSR and is being coordinated by a committee which was set up within the USSR Academy of Medical Sciences. The main institution involved in the follow-up is the Institute of Radiation Medicine (in Kiev), although each of the three primarily affected republics (Byelorussia, Russia and Ukraine) is carrying out work and has set up central state registries of exposed persons - through its own radiological institute (Minsk, Leningrad and Moscow, Kiev). A centralized register, covering approximately 530 000 persons exposed as a result of the Chernobyl accident, has been established in Obninsk. Data are collected in the registries from Byelorussia, Russia and Ukraine, as well as from other republics where persons exposed as a result of the accident clean-up are now living, and then sent to Obninsk.

Following a memorandum of understanding between the Ministry of Health of the USSR and WHO, an International Programme on the Health Effects of the Chernobyl Accident (IPHECA) has been planned in collaboration with WHO. The establishment of a WHO Collaborative Centre - International Centre on Radiation Health Issues (ICRHI) - in Obninsk has also been proposed as part of this programme. This Centre would coordinate all research programmes within the USSR. A meeting of a Scientific Advisory Committee was held in Hiroshima on 23-26 October 1990 to discuss these issues. It was recommended that the first priority of the IPHECA should be epidemiology, and that epidemiological

studies be developed in collaboration with IARC and RERF. The aim of Academician Ilyin and Drs Ivanov and Komarov's visit to IARC was to discuss the basis of this collaboration.

2. Study populations and dosimetry

In summary, four main groups of persons with reliable individual dosimetry, exposed as a result of the Chernobyl accident, were identified:

- children with thyroid exposure (70 000);
- emergency accident workers (40 000);
- residents still living in "strict control" areas (40 000)
- residents evacuated after accident (45 000);

One of the problems of concern for epidemiological studies is the lack of complete individual exposure information. Detailed average dose estimates have been made for the populations of 780 settlements in the strict control area - covering 270 000 persons - in five regions of the three republics: Mogilev and Gomel in Byelorussia; Briansk in Russia; Zhitomir and Kiev in Ukraine. These are based on TLD and whole body count measurements and on modelling of environmental transport and consumption of milk and contaminated food. The distribution of these doses in settlements has also been characterized. The question of how these dose estimates could be used in epidemiological studies was discussed.

The contribution of external radiation and internal contamination to individual doses varied with different subgroups of the population. Overall, approximately 30% of the dose is due to short lived radioisotopes in the first year following the accident.

The problem of radiation limits for relocation of populations and for destruction of contaminated milk and food was briefly discussed.

Previous radiation accidents, recently made public, were also described. The results of a health study of residents of Kishtim have now been published. Further work is planned in collaboration with scientists at RERF in Hiroshima.

3. Registration of Cancer

Population-based cancer registration in the Soviet Union relies primarily on the central notification of cases treated in oncology centers. It is known to be incomplete, and variably so in different regions. In Byelorussia, figures on leukaemia 'incidence' have been reported for the different districts, but these may be based on hospital attendances, uncorrected for place of residence of the cases, thereby inflating incidence. Increased interest in leukaemias and lymphomas may also have led to improved recording of cases in recent years. For these reasons, the study of neoplasms in relation to exposures from Chernobyl requires special mechanisms, either for population-level studies of incidence by geographical area and time, or for identifying incident cancers in cohorts of individuals in the high-exposure groups.

In Byelorussia, a special register of 'hemoblastoses' (haematological malignancies, lymphoma, myeloma) has been established. It works by collecting data from all possible sources - haematological departments in all districts, oncology centres, autopsies, and death certificates. Data on all cases are entered onto 'epidemiological cards', which are then carefully sorted to eliminate duplicates. The register was established in 1988, and includes neoplasms back to 1979/80. Analyses have concentrated on incidence rates in the residents of the two most highly exposed districts: Gomel and Mogilev. The annual incidence of acute leukaemia in children is very similar to that found elsewhere in Europe, and fluctuates between 15 and 48 per million. Incidence of acute leukaemia (all ages) in 1983-85 was 36 per million in Mogilev and 37 per million in Gomel. There have be no significant changes in acute leukaemia incidence in 1986-1988. The only apparent change is an increased incidence of chronic lymphatic leukaemia in 1987-1988, the significance of which is unclear.

II. RESEARCH AT IARC

1. European Childhood Leukaemia/Lymphoma Incidence Study (ECLIS)

This was started in 1987, and now involves cancer registries in 18 countries, covering a population (aged 0-14) of over 60 million. Financial support has been provided by the Radiation Protection Programme, Commission of the European Communities.

The study involves collection of data on all recorded cases of childhood leukaemia and lymphoma occurring in the populations covered by the registries. Collation of data from the different centres and its analysis is coordinated by IARC.

Preliminary results confirm the rather constant background incidence of leukaemia, around 40 per million children per year. There is a slight male preponderance in almost all countries.

Results for 1986-87 have been completed for most national populations, but it is too early to relate any of the incidence data to the exposure estimates. The latter are available for national populations, or broad sub-regions within countries, from the report of UNSCEAR. Future analyses will also examine birth-cohort specific incidence, with particular interest focused on children born in May 1986- January 1987 (prenatal exposure).

2. Studies in radiation risk assessment

It is desirable to obtain additional direct human data on the effects of low doses, protracted exposures, internal contamination with various radionuclides (in particular iodine), and on transgenerational and age at exposure effects in order to improve the reliability of existing risk estimates.

IARC has initiated a very large epidemiological study to directly assess the effects of chronic exposure resulting in very low doses from low LET radiation. This study is based on follow-up of workers in the nuclear industry for whom detailed individual yearly dosimetry is available. The protocol of this study was presented; it involves three phases: a retrospective dosimetry study to ensure comparability and quantify the uncertainty of individual dose estimates; combined analyses of data from existing cohort studies (from Canada, the UK and the US); a collaborative international study of workers whose cancer mortality has not yet been studied (12 countries have currently accepted to participate and the feasibility is being evaluated).

III. PROPOSED FUTURE COLLABORATION

Studies may include:

1. Studies of Leukaemia: Population level/ecological studies

a. Participation of USSR centres in the ECLIS study

The Byelorussia centre is at present ready to join the study group. Although data has been promised (via Petrov Institute, Leningrad) for Ukraine and parts of the Russian Republic, it is unclear as to how complete these are at present, and whether a system of multi-source registration has been established for the relevant areas.

Technical assistance in the form of provision of micro-computers and software suitable for recording cancer cases could be made available as part of the agreement to extend the ECLIS study to Byelorussia, Ukraine and Russian Republic. The Radiation Protection Programme of EEC is anxious to support such an extension of the European study.

b. Extension of the ECLIS protocol in the USSR

To include adults - viz all ages, or, perhaps better, to age 65. This is already undertaken by the Byelorussian Centre.

The opportunity to record detailed residential history (since January 1985) and any information on exposure, for all registered leukaemia cases, should be taken.

Accurate subtyping of all leukaemia and lymphoma cases is at present difficult in the USSR, and appropriate chemical and immunological reagents to allow this could be funded from part of the extended study within USSR. Extension to the adult age range (15-64) would mean approximately three times as many acute leukaemias recorded. This would permit study within the USSR for small geographical areas. Dosimetry by detailed geographical area is available for five districts (Mogilev, Gomel, Briansk, Kiev, Zhitomir). In certain areas of these districts the estimated average dose is such that, in theory, an excess of leukaemia cases could be detected. Interest lies in investigating

whether this is so, and the relationship, at the population level, between excess incidence and estimated average dose.

The question as to whether this type of extension to adults is feasible elsewhere in Europe will be discussed at the next meeting of the ECLIS group.

2. Cohorts for follow-up and dosimetry

For epidemiological purposes, average doses at a regional level will be very useful for geographical correlation studies and for population registry based studies (as described in 1a and 1b, above). For studies aimed at risk estimation at fairly low levels of exposure, however, reliable individual dose estimates are necessary.

As discussed during Dr V. Ivanov's (Obninsk Institute) visit to IARC in September 1990, follow-up of specific subcohorts included in the Obninsk registry and for whom reliable dose information is available, would be of interest, if feasible. Particular subcohorts include: 1) the Emergency Accident Workers, in particular those who started work several months after the accident, who received fairly low doses of predominantly external radiation in a relatively short time period; 2) children and adults living in contaminated areas, whose exposure is protracted and whose doses result both from external exposures and from ingestion and inhalation of radionuclides.

In addition, the study of offspring of persons exposed either pre-conception or perinatally would be of particular interest .

By restricting extensive follow-up to several subcohorts, some of the data collection problem could be alleviated. Risk estimation for individual cancer sites could then be carried out in specific case-control studies within the framework of those pre-defined cohorts, and additional information on potentially confounding factors (chemical exposures, smoking, ...) could be obtained, at relatively low cost.

3. Nuclear industry workers

Interest was expressed in a Soviet participation in the study. Academician Ilyin will be in contact with IARC to discuss a possible Soviet collaboration. Such a collaboration, because of the size of the nuclear workforce in the USSR, would be of great value to the international study.

IV. CONCLUSIONS

The discussed on-going and projected activities of IARC could be associated to the WHO collaboration programme on the Health Effects of the Chernobyl Accident (IPHECA) and be carried out in close interaction with the planned WHO Centre on Radiation Health Issues (ICHRI). IARC is expected to be a member of the scientific council of the Obninsk centre.

During Dr Cardis' visit in Kiev in December 1990, she will hold further discussions on the present state of cancer registration in Kiev, and the feasibility of cohort follow-up, and about the possible role IARC could play in advising the IPHECA in the design of epidemiological studies.

In 1991 (March/April), a formal visit by an IARC delegation should be made to the USSR, specifically Minsk (including haematology, oncology and radiation medicine institutes, and possibly Gomel region), Obninsk, Moscow, Leningrad and Kiev. The purpose is to plan the details of the joint projects described above, and to estimate realistic budgets for the different components.

It was suggested that a workshop be held in Minsk in 1991 (September/October), in collaboration with the Radiation Protection Programme of the European Community. The purpose would be to evaluate the present situation regarding availability of information within the USSR, and to review ongoing studies in USSR and IARC.

ANNEX

LIST OF POTENTIAL PARTICIPANTS

1. Minsk - Institute of Haematology (Dr E. Ivanov)
2. Kiev - Institute of Radiation Medicine (Dr O. Tsvetkova)
3. Obninsk - Institute of Medical Radiology (Dr V. Ivanov, Dr A.F. Tsyb)
4. Moscow - Institute of Biophysics (Academician L.A. Ilyin)
   Oncology Center (Professor N.N. Trapeznikov)
   Center for Children - Immunohaematology (Dr Rumyantsev)
5. Leningrad - Petrov Research Institute (Dr V.M. Merabishvili)
   Institute of Radiology (Dr E.I. Komarov)

COORDINATORS

For internal activities

Academician L.A. Ilyin (Moscow)
Dr E. Ivanov (Minsk)
Dr E.I. Komarov (Leningrad)
Dr V.M. Merabishvili (Leningrad)
Dr O. Tsvetkova (Kiev)
Dr V.G. Bebechko (Kiev)
Dr V. Ivanov (Obninsk)

Figure 1

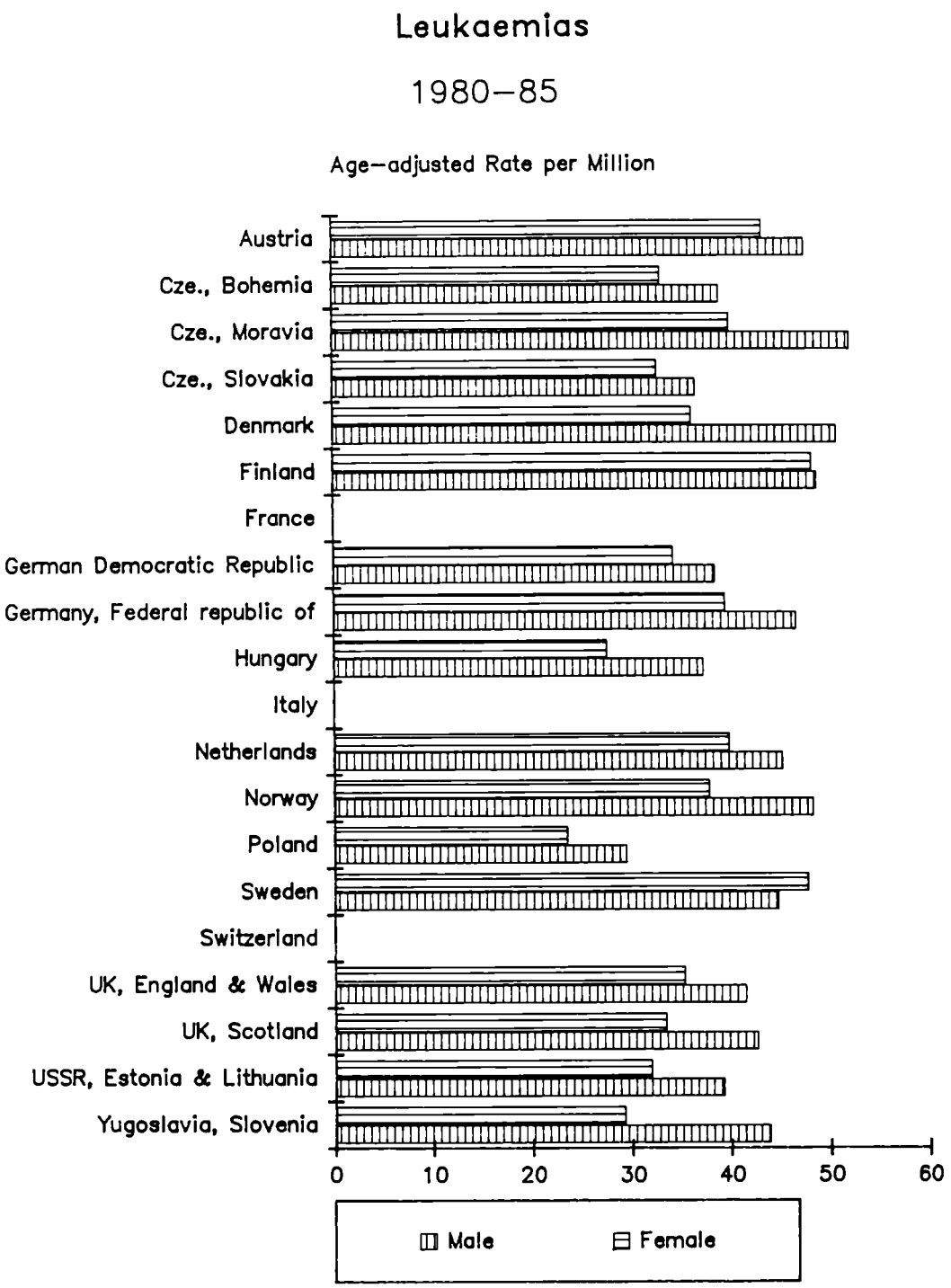

Leukaemias
1980–85
Age–adjusted Rate per Million
Austria
Cze., Bohemia
Cze., Moravia
Cze., Slovakia
Denmark
Finland
France
German Democratic Republic
Germany, Federal republic of
Hungary
Italy
Netherlands
Norway
Poland
Sweden
Switzerland
UK, England & Wales
UK, Scotland
USSR, Estonia & Lithuania
Yugoslavia, Slovenia
0
10
20
30
40
50
60
Male
Female

Figure 2

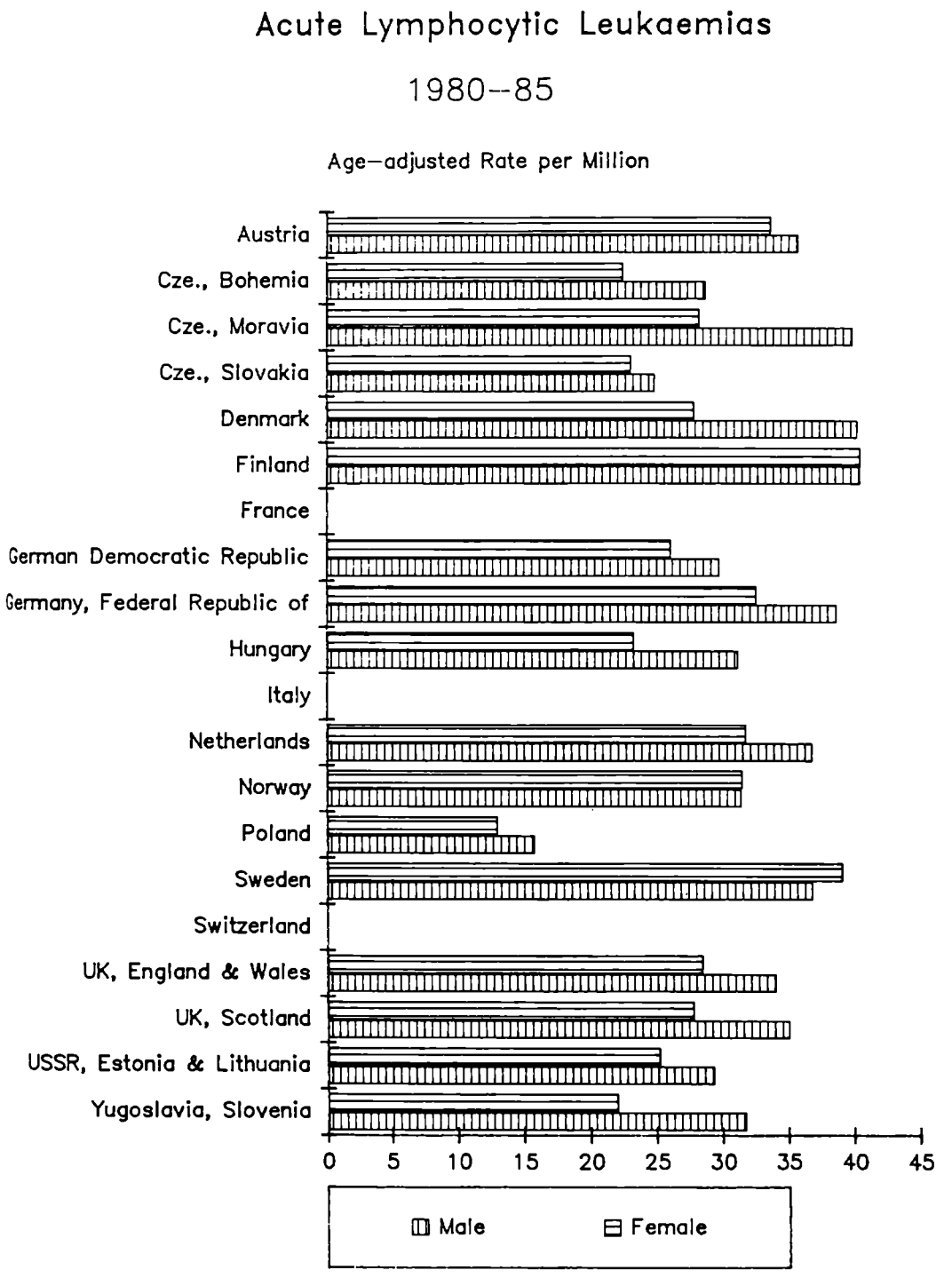

Acute Lymphocytic Leukaemias
1980--85
Age-adjusted Rate per Million
Austria
Cze., Bohemia
Cze., Moravia
Cze., Slovakia
Denmark
Finland
France
German Democratic Republic
Germany, Federal Republic of
Hungary
Italy
Netherlands
Norway
Poland
Sweden
Switzerland
UK, England & Wales
UK, Scotland
USSR, Estonia & Lithuania
Yugoslavia, Slovenia
0
5
10
15
20
25
30
35
40
45
Male
Female

Figure 3

AUSTRIA

LEUKAEMIAS, BOTH SEXES, 1980-87

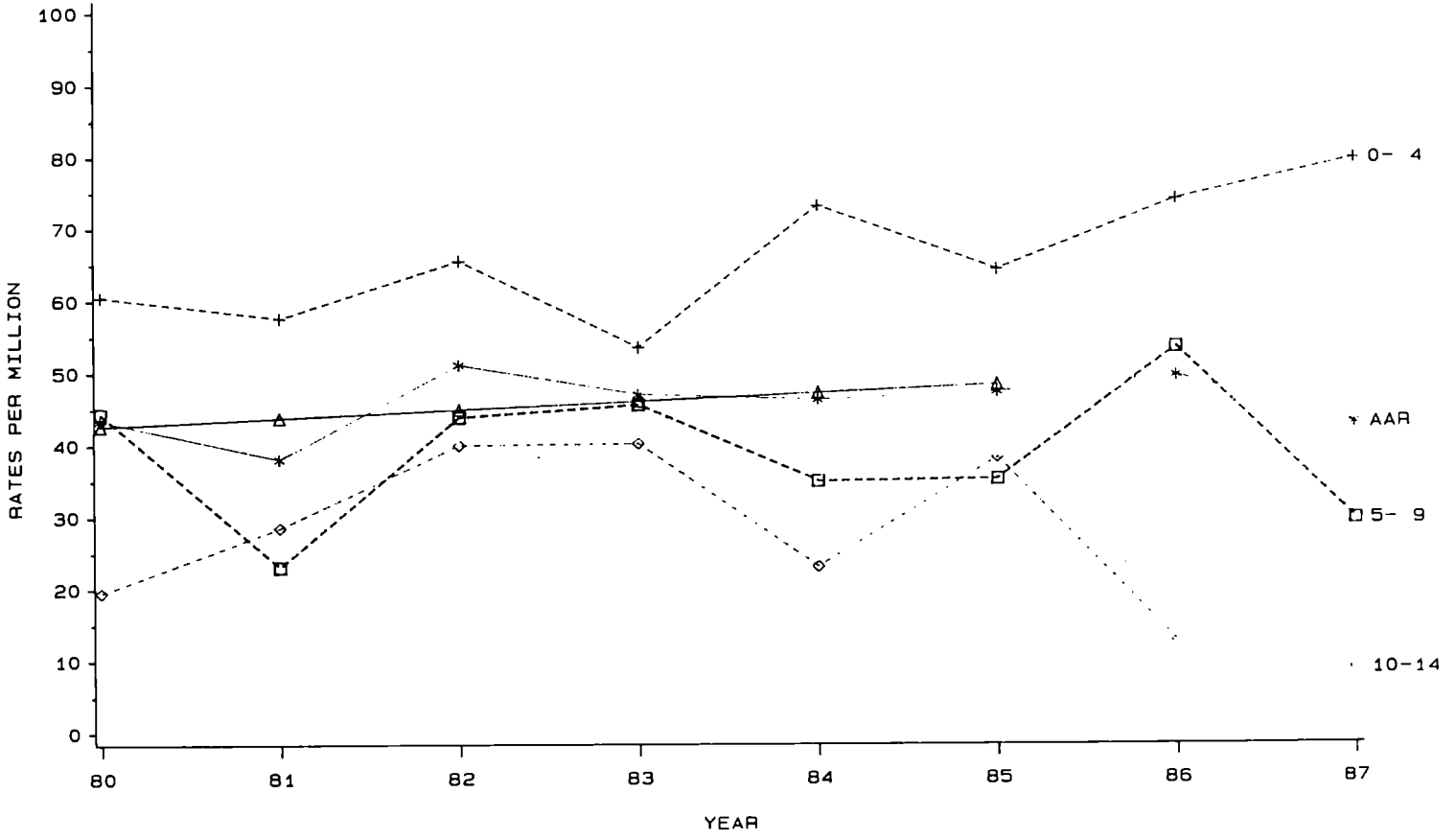


Figure 4

FINLAND

LEUKAEMIAS, BOTH SEXES, 1980-87

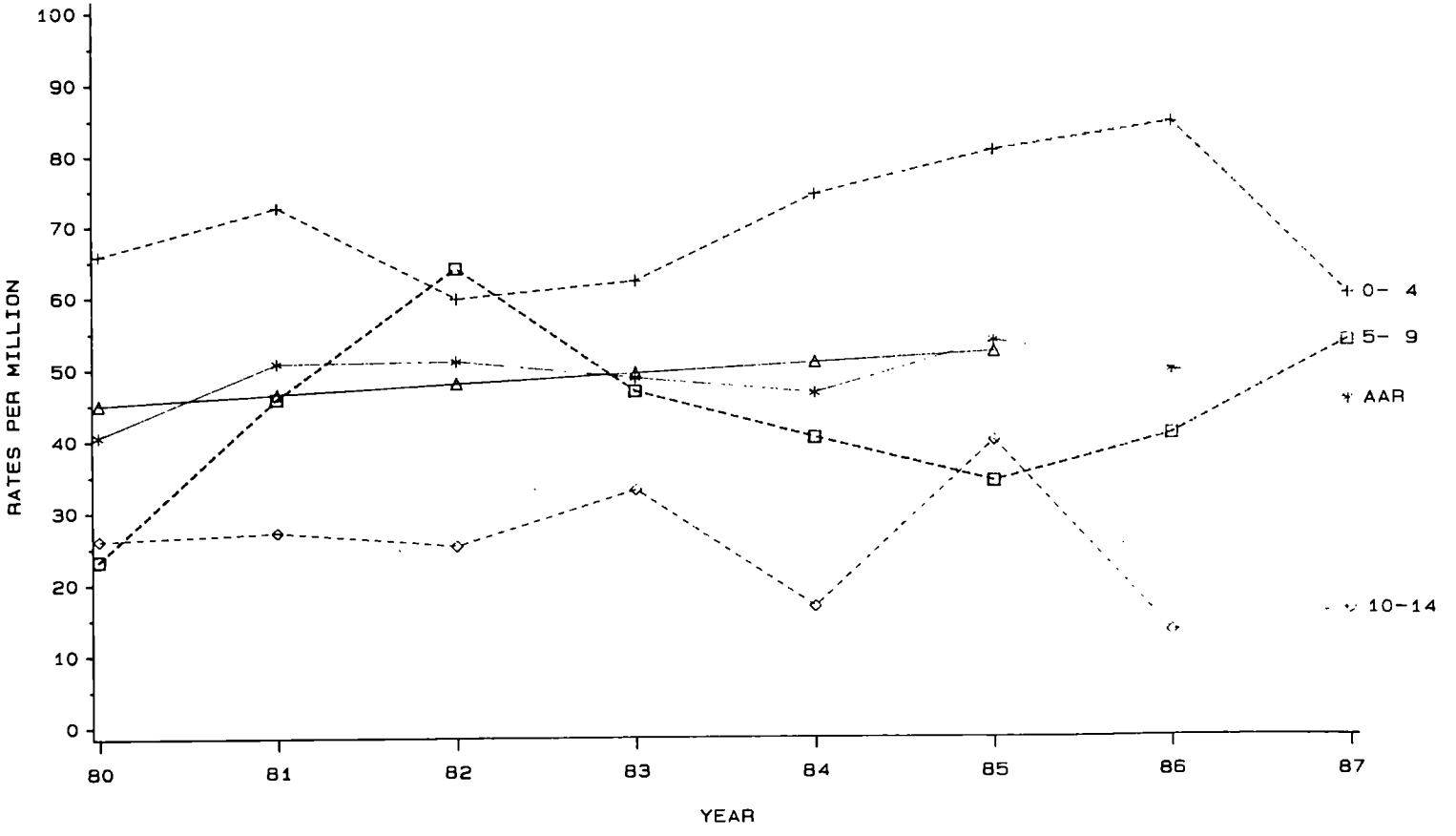
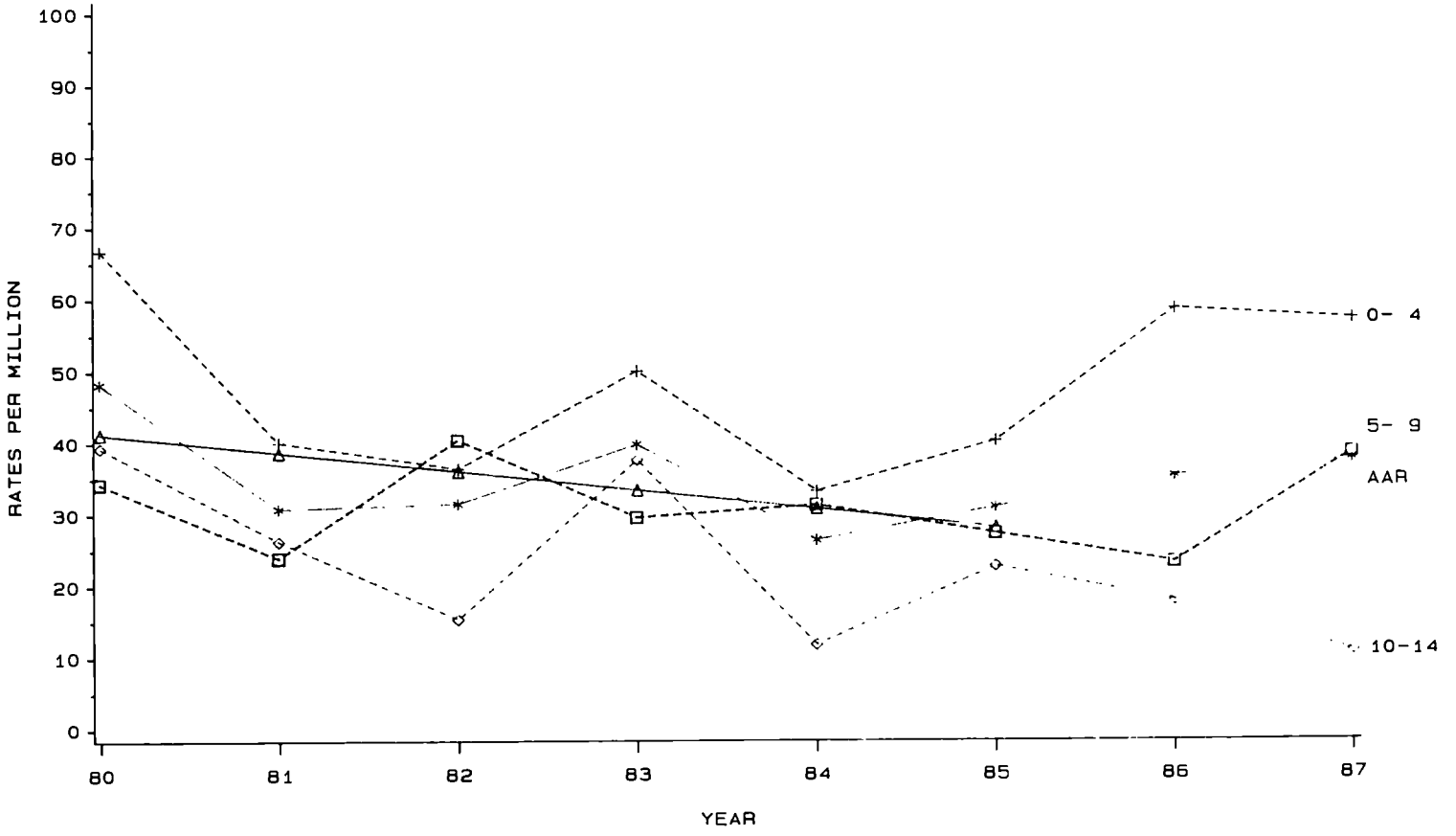


Figure 5

CZECHOSLOVAKIA, SLOVAKIA

LEUKAEMIAS, BOTH SEXES, 1980-87



POLAND

LEUKAEMIAS, BOTH SEXES, 1980-87

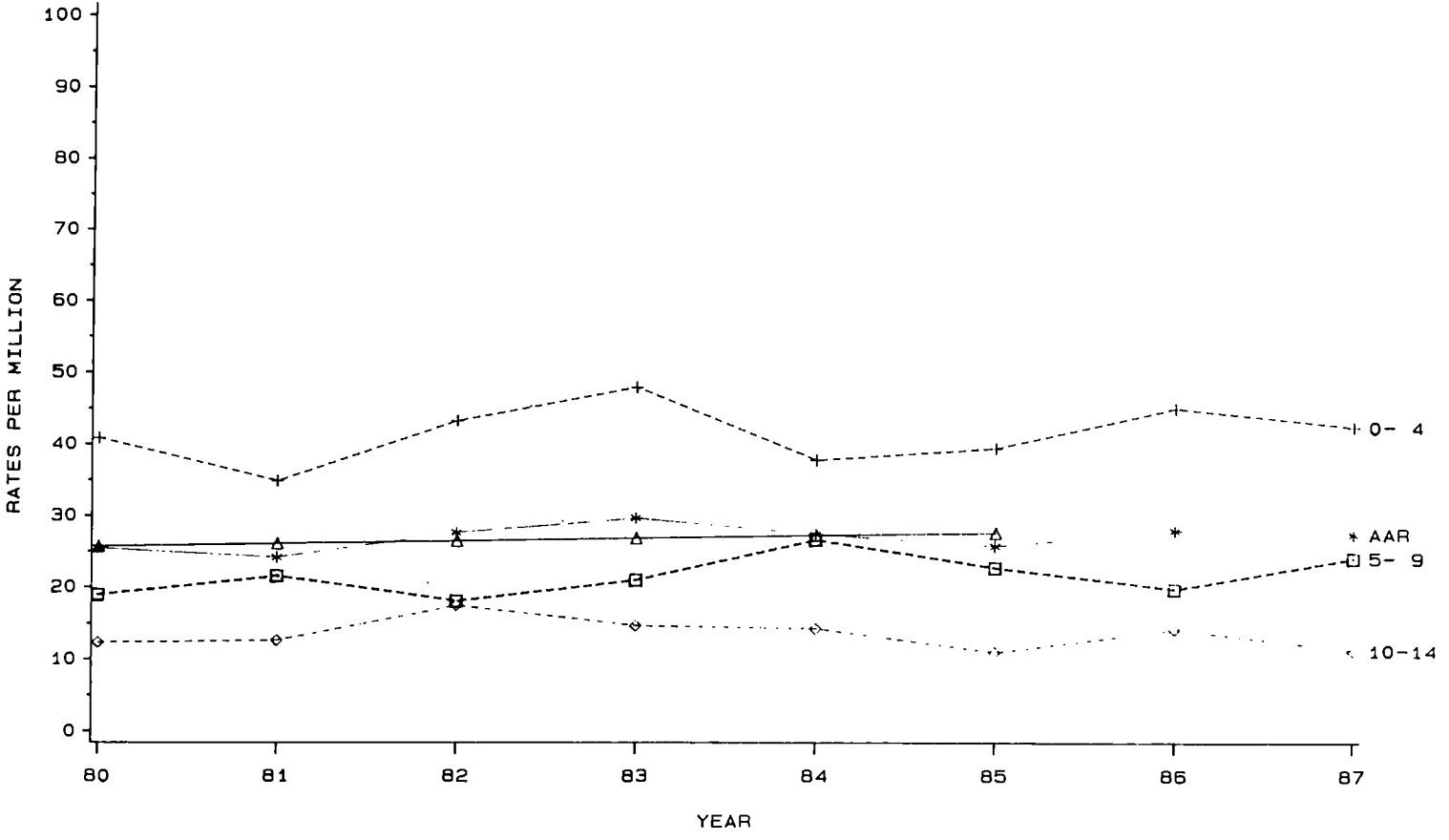
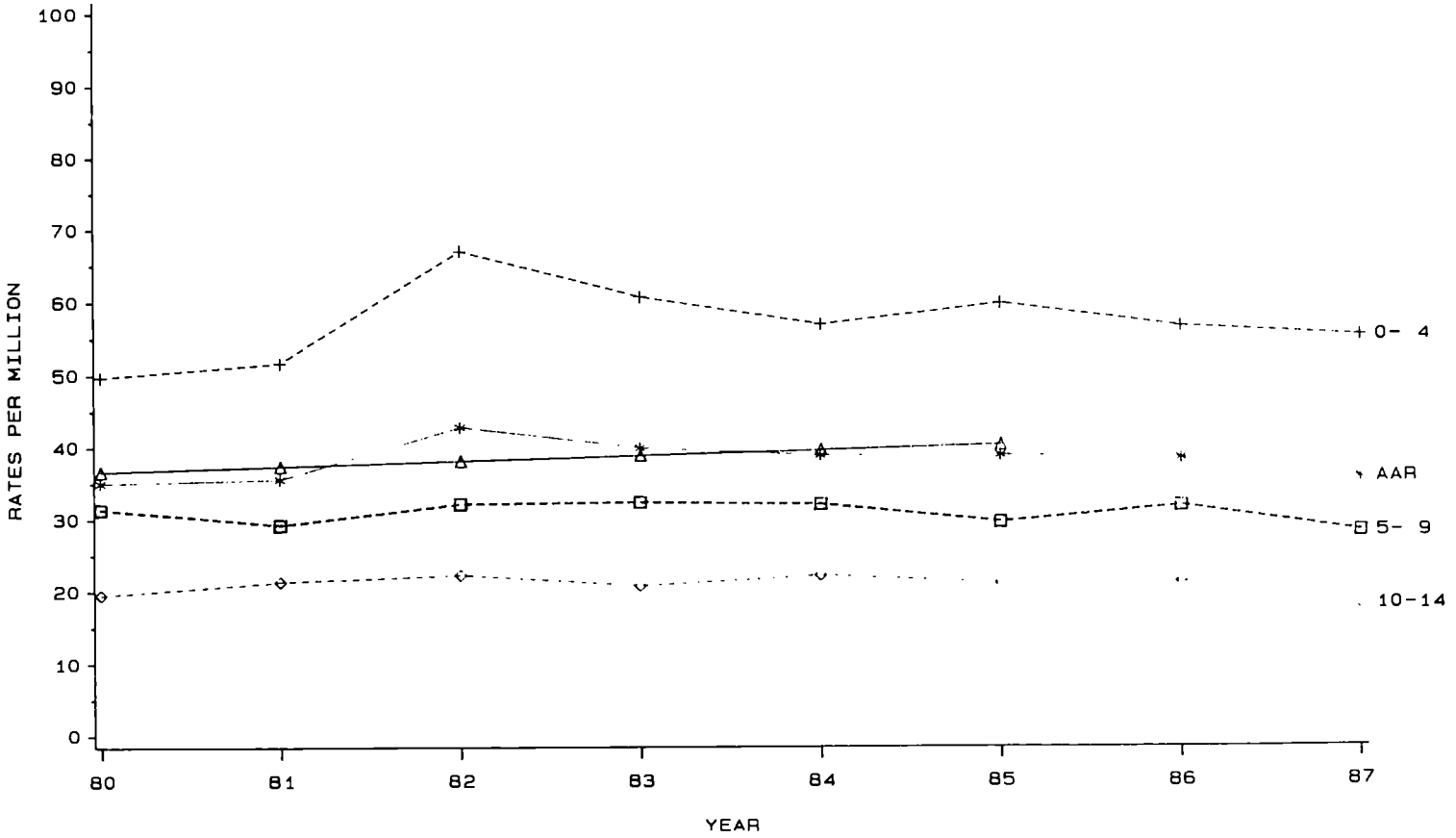


Figure 6

Figure 7

UK, ENGLAND AND WALES

LEUKAEMIAS, BOTH SEXES, 1980-87



Progress Report

Contract: Bi6-347h Sector: C14

Title: Epidemiological studies of radiation risks (NRPB Association)

1	Muirhead	NRPB
2	Kellerer	GSF Neuherberg
3	Chmelevsky	GSF Neuherberg
4	Oberhausen	Univ. Saarlandes
5	Holm	Karolinska Hospital
6	Becciolini	Università degli Studi di Firenze

I. Summary of Project and Global Objectives

Objectives of Project

(i) To analyse data on populations exposed to high doses of radiation, such as the Japanese atomic bomb survivors and groups of uranium miners.

(ii) To examine data on populations exposed at low doses and methods for analysing such data.

(iii) To perform preparatory work for the compilation of 'probability of causation' tables that are specific to EC countries and that also cover radon daughter exposures.

(iv) To study the incidence and mortality from thyroid cancer in a cohort with medical exposures to I-131.

(v) To study cancer incidence and mortality among Swedish patients given radiotherapy for skin haemangioma in childhood.

(vi) To examine the incidence of second tumours among Italian patients given radiotherapy for cancer of the head, neck, breast, endometrium, uterine cervix or thyroid.

Progress during 1990 on certain areas of this project has been affected by some delays in commencing work, in particular owing to the movement of one of the project heads between institutions and because two of the participating organisations (Karolinska Institute and University of Florence) entered into the Association Agreement only in the later part of the year. However, all of the organisations are now participating fully.

Concerning the epidemiological studies being performed under this project, a first examination of the observed number of thyroid cancer cases among the cohort of patients given I-131 in Homburg (Saarland) suggests an excess relative to regional rates, although based on fairly small numbers. Information being obtained from questionnaires may provide more accurate estimates of the person-years-at-risk. A second database of I-131 patients has also been created within the Saarland, which should serve to increase the statistical power. In Italy, the examination of clinical records for cancer patients in Florence has started, and this process will also be carried out at other centres. It will be important, from an epidemiological viewpoint, to obtain information on the length of follow-up of these patients so that incidence rates for second cancers can be calculated. In Sweden, the further follow-up of the skin haemangioma patients and calculation of individual doses is just commencing.

Concerning the calculation of estimates of radiation-induced cancer risks, an examination has been made of risk projection models and their implications. In particular, the BEIR V models have been applied to the UK population, whilst a problem with the BEIR V model for leukaemia has been highlighted. The detailed investigation of data on groups such as the Japanese atomic bomb survivors and US and Czech uranium miners will commence in 1991; results from these analyses will be used in developing European 'probability of causation' tables, for which work on collecting baseline cancer incidence and mortality data in various countries and regions has started.

At low doses, the results of an analysis of the geographical distribution of childhood leukaemia and non-Hodgkin's lymphoma throughout Britain in relation to natural radiation will be published shortly. The properties of a statistical method for detecting whether a disease has a 'natural' tendency to cluster have been studied, and this method will be applied to the above childhood leukaemia database.

Head of Project 1: Dr Muirhead

II. Objectives for the reporting period

(i) To examine the implications and sensitivity of models for projecting radiation-induced cancer risks across time and populations.

(ii) To enhance software written for the analysis of data from epidemiological studies of radiation workers. To examine statistical methods for detecting whether a disease has a 'natural' tendency to cluster. To analyse data on the geographical distribution of childhood leukaemia throughout Britain in relation to natural radiation.

III. Objectives for next period

(i) Analyses will be undertaken (in conjunction with the University of Munich) of detailed data sets on populations exposed to high doses of radiation. In particular, models will be fitted to describe how the radiation-induced cancer risks among the Japanese atomic bomb survivors vary with dose and time since exposure, while diagrams will be constructed to illustrate the integrated cancer mortality rates for the two cities, both sexes, different age groups and different tumour sites. Analyses will also be performed of data on lung cancer among uranium miners on the Colorado Plateau (USA) to address issues such as the joint effect of radon exposure and smoking on the lung cancer risk.

(ii) Further analyses will be conducted, in collaboration with the Childhood Cancer Research Group (CCRG) (University of Oxford) of the CCRG's database on the geographical distribution of childhood leukaemia and Hodgkin's lymphoma (NHL) in Britain. The statistical method developed to test whether a disease has a 'natural' tendency to cluster will be applied to this database. A paper will be prepared on the risk of childhood leukaemia and NHL in relation to both natural radiation and socio-economic factors.

The documentation for the programs (ARFAR, etc.) written for the analysis of cohort studies of radiation workers will be updated to reflect recent modifications.

IV. Progress achieved including publications

(i) Modelling Radiation Risk in Populations Exposed to High Doses

A review has been undertaken of methods for projecting radiation-induced cancer risks across time and populations (Muirhead, 1990(a), 1990(b)). As part of this, an examination was made of models developed by the US BEIR V Committee, whose report was published recently. These models predict constancy of relative risks across populations but, for certain cancers such as respiratory and breast cancers, the relative risk is assumed to decrease at long times after exposure. These models have been used to calculate estimates of radiation-induced cancer risks that are applicable to a UK population, based on the same baseline cancer rates as utilised in the report by Stather *et al* (1988; NRPB-R226). The total

cancer risk following high dose rate exposure was estimated to be 11% Sv^{-1} based on the BEIR V models; this lies towards the upper end of the range of 4-13% Sv^{-1} calculated by Stather *et al* under the assumption that the raised relative risk either ceases after 40 years following exposure (the current period of follow-up for the Japanese atomic bomb survivors) or remains constant until the end of life. These calculations also indicated that the BEIR V Committee may have over-estimated the leukaemia risk by assuming the relative risk for chronic lymphatic leukaemia (CLL) to be the same as that for other types of leukaemia, whereas it seems that CLL is not radiation-inducible.

(ii) Assessment of Data from Populations Exposed to Low Radiation Doses

Further modifications have been made to software for analysing data from cohort studies of radiation workers. NRPB has been analysing data from the UK's National Registry for Radiation Workers (NRRW) under CEC contract BI6-F-213-UK. The statistical software used in this analysis includes the program ARFAR (At Risk For Any Reason) and relate programs.

These software allow an analysis to be made of any trends in mortality rates with radiation dose, after adjusting for factors such as age and calendar period. The modifications to these programs allow results to be obtained simultaneously both for a number of different causes of death and for various strata defined by, for example, sex, industrial classification, longest employer, etc. The effect of these modifications is to produce considerable savings in the computer time required for the NRRW analysis.

Study has been made of a particular statistical method which may be used to test whether a disease has a 'natural' tendency to cluster. This topic is important in attempting to assess results around nuclear installations. The method, which was derived originally by Potthoff and Whittinghill (Biometrika, 1966), does not seem to have been used previously in this type of application. The method has been modified to allow any clustering effects that occur within small geographical areas to be distinguished from those occurring over larger areas. NRPB was invited to apply this method to 50 simulated data sets of the geographical distribution of childhood leukaemia in Northern England as part of the International Agency for Research on Cancer (IARC)'s Comparative Study of Statistical Methodologies for Investigating Localised Clustering of Disease. NRPB's contribution to this study will be published as part of the IARC report on this subject during 1991.

There have recently been claims by Henshaw *et al* (Lancet, i, 1009-13 (1990)) that the incidence of leukaemia (as well as certain other cancers) and mean indoor radon concentrations in different countries are correlated to a statistically significant extent. However, the quality of both the cancer and radon data varies widely between countries, and makes the interpretation of such international correlation studies very difficult. A re-analysis was carried out (Butland *et al*, 1990) which excluded countries for which the quality of the data was known to be poor; this tended to reduce the magnitude of the correlations. More recently, an analysis has been performed by NRPB and the Childhood Cancer Research Group (CCRG) (University of Oxford) of the CCRG's database on the

geographical distribution of childhood leukaemia and non-Hodgkin's lymphoma throughout Britain in relation to the NRPB's data on the geographical distribution of indoor radon concentrations and gamma dose rates. In contrast to studies looking for differences between countries, this study uses the best available data within a single country (Britain) and is based on small areas (county districts). The results of this analysis will shortly be submitted as a letter to the Lancet.

Publications covering work of reporting period

Butland, B K, Muirhead, C R and Draper, G J, Letter to the Editor: Radon and leukaemia. Lancet, i, 1338-9 (1990).

Muirhead, C R, Projection of radiation-induced cancer risks across time and populations, Radiation Protection Dosimetry (to appear).

Muirhead, C R, Projecting radiation-induced cancer risks across time and populations. Sozial-und Präventivmedizin (to appear).

Head of Project 2: Prof Kellerer

II. Objectives for the reporting period

To undertake preparatory work for the analysis of data in populations exposed to high doses.

III. Objectives for next period

Analyses will be undertaken in conjunction with NRPB of detailed sets on populations exposed to high doses of radiation. In particular, diagrams will be constructed to illustrate the integrated cancer mortality rates for the two cities, both sexes, different age groups and different tumour sites. Explanatory diagrams on lung cancer among Czech uranium miners will also be constructed. This is a subject that has taken added interest because similar data are now becoming available from the vast uranium mining operations that took place in the former German Democratic Republic.

In co-operation with the Institute of Radiation Protection at GSF, work will be performed towards the establishment of radioepidemiological tables for west European countries and specifically for the Federal Republic of Germany. The division of work will be such that the incidence and mortality data will be prepared at GSF, while the formulation of the relevant algorithms will be responsibility of the Institute of Radiation Biology at the University of Munich.

IV. Progress achieved including publications

Owing to the move of Professor Kellerer from the University of Wurzburg to the University of Munich and GSF, only the initial steps in his Group;s research programme have been performed to date. In the past, the algorithms for the proportional hazards model and its modifications that were used by Professor Kellerer's group had been written at the University of Wurzburg. For better comparison with the work performed at the Radiation Effects Research Foundation (RERF) in Japan and at other scientific institutions, the latest versions of the programs AMFIT and EPICURE developed by Dr D L Preston (RERF) have now been implemented. These have been tested in applications to the data from RERF on cancer mortality data among the Japanese atomic bomb survivors up to the end of 1985 based on the new DS86 dosimetry system.

Head of Project 3: Dr Chmelevsky

II. Objectives for the reporting period

To start to collect national and regional data on cancer incidence and mortality as part of the preparatory work for the construction of European 'probability of causation' tables.

III. Objectives for next period

The collection and comparison of national and regional data on cancer incidence and mortality will continue, with the aim of calculating approximate incidences for Germany. In addition, the computation of preliminary probability of causation tables for Germany will commence, based on the present estimates of baseline cancer incidence and new estimates of radiation-induced cancer risks. The formulation of the relevant algorithms will be developed by the University of Munich.

IV. Progress achieved including publications

Radioepidemiological Tables of the Probability of Causation for Use in the Countries of the EC and their Extension to Radon Daughter Exposures

A central problem for the generation of radioepidemiological tables for some European countries such as Germany is the lack of a national cancer registry. However, national mortality data are generally available. Therefore, in order to estimate national incidences of different cancer types, one possibility is to use data from regional registries within a country or from foreign registries (such as the SEER program in the USA), and to make adjustments based on the comparison of national mortality data with the corresponding regional or foreign mortality data.

Within Germany there is a regional cancer registry in the Saarland. This covers a population of approximately one million and has been operating since the early 1970s. This registry provides reliable data, but the population that it covers may not be representative of the national population.

Another German cancer registry, namely that covering the former DDR (German Democratic Republic), may be more representative of the general population. However, data from this registry seems to be far less reliable than those from the Saarland registry.

In addition to the above, there was a cancer registry in the city of Hamburg which operated for 20 years until the early 1980s. Another recent registry, initiated in 1980, is the childhood registry based in Mainz which provides national cancer incidences for those aged less than 15 years.

During the last few months GSF has started to collect the available data on German cancer incidence, as well as the corresponding mortality data. Steps have also been made to obtain incidence and mortality data for several European countries, as well as for the USA.

Head of Project 4: Prof Oberhausen

II. Objectives for the reporting period

To contact members of the cohort of patients given I-131 at Homburg (Saarland). To compare the observed number of cases of thyroid gland carcinoma in this cohort with that expected on the basis of regional rates. To create a second database, consisting of patients given I-131 at a hospital in Saarbrücken.

III. Objectives for next period

It is intended, with the help of registration offices, to obtain names and addresses for the 30% of the patients given I-131 at Homburg (Saarland) who have not yet replied to the questionnaire. For those who have died, dates of death will be obtained. A second comparison of the observed number within this cohort of thyroid gland carcinoma cases entered in the Cancer Registry of the Saarland and the expected number based on regional rates will then be performed. A similar comparison will be carried out for the second cohort of patients, who attended a hospital in Saarbrücken.

IV. Progress achieved including publications

Epidemiological Research with the Aim of Determining the Morbidity and Mortality Risks of Thyroid Gland Carcinoma

During the past year a questionnaire has been sent to all 11,776 patients who had been given I-131, either for reasons of diagnosis or treatment, at the Department of Nuclear Medicine at the University of Homburg between 1962 and 1977. This questionnaire concerns the health of these patients since that time. Up until now about 70% of these patients have replied. Reasons for non-response are likely to be change in address, in some cases change in the name of the patient, and death.

A first comparison has been carried out between the observed number of thyroid gland carcinoma cases in the cohort of patients and the expected number of cases, based on incidence rates for the population of the Saarland. Because the dates of death are not yet known for many of those (1,132) patients who have died, these members of the cohort were excluded from the calculation of the person-years-at-risk. During 1972-1987, 17 cases of thyroid cancer among members of the cohort were registered in the Saarland cancer registry, compared with an expected number of 8. Preliminary calculations suggest that the observed excess risk is less than that predicted using the US radioepidemiological ('probability of causation') tables by about a factor of 7.

A second database of patients in the Saarland who received I-131 has now been created. This database consists of about 17,000 patients who attended a large hospital in Saarbrücken.

Publications covering work of reporting period

Incidence of thyroid gland carcinoma after I-131 treatment: first results of an epidemiological study with the patients of the Department of Nuclear Medicine in Hamburg/Saar. Technical Progress Report, University of Saarland (1990).

Head of Project 5: Dr Holm

II. Objectives for the reporting period

To start work on extending the follow-up period for the Swedish skin haemangioma patients given radiotherapy, and on calculating organ doses for individuals treated with radium-226.

III. Objectives for next period

New and updated record linkages will be performed between the cohort of 18,460 patients treated for skin haemangioma and (i) the Swedish Cancer Register for the period 1958-86, and (ii) the Swedish Cause-of-Death Register for the period 1951-88. Estimates will be made of doses to thyroid, breast, lung, stomach, colon, gonads and bone marrow for all 14,647 patients who were younger than 18 months at the time of treatment and who were treated with radium-226. Based on the above, dose-response analysis for both cancer incidence and cancer mortality will be carried out.

IV. Progress achieved including publications

Cancer Incidence and Mortality following Radiotherapy for Skin Haemangioma in Childhood

The Karolinska Institute in Sweden, where this work is being performed, signed the Memorandum of Agreement late in 1990. Consequently there is little work to report at present.

Head of Project 6: Dr Becciolini

II. Objectives for the reporting period

To examine the clinical records of patients treated for tumours of the breast, uterus, ovaries, head and neck in Florence during 1973-85. To obtain information on second cancers among these patients.

III. Objectives for next period

The analysis of clinical records of selected groups of patients will continue. In particular, records of cases of breast cancer, carcinoma of uterine cervix, and head, neck and thyroid carcinomas will be examined in order to find out the incidence of second tumours during follow-up or of their occurrence after diagnosis of previous malignancies. The analysis will be performed on all the cases referred to Florence during 1977-87. The incidence of second tumours in groups of patients submitted to different types of therapy will be studied.

Evaluation of the results obtained at the Universities of Pisa, Siena and Modena will commence.

IV. Progress achieved including publications

Epidemiology of Second Tumours in Radiotherapy Patients

Among patients treated for cancer of the uterus at the University and Hospital Departments of Radiation Therapy in Florence between 1973 and 1985, the cancer was diagnosed as arising in the uterine body for 429 of the cases. Of these, 247 were primitive endometrial adenocarcinomas, while 32 were local recurrences after surgery among patients who had not been submitted to post-operative radiation therapy. Fifty-one patients were not evaluated owing to reasons such as a histology other than adenocarcinoma or an age of 80 years or older; another 100 patients were not considered because of unknown or uncertain site of origin of the tumour, lack of histological definition of the tumour, etc.

The overall 10-year survival for those with endometrial adenocarcinoma was 71%. Post-operative radiation therapy was performed on 212 patients, all of whom received external beam therapy with doses ranging between 50 and 56 Gy and conventional fractionation. Thirteen cases were submitted to radiation therapy only (12 with external beam and 1 with intracavity treatment only). No treatment was performed in the remaining 22 cases after surgery.

The 225 patients submitted to radiation therapy were associated with a total of 19 other neoplasms. For five of these cases the other tumour was detected at the same time as the endometrial carcinoma, whilst in four of these cases it had arisen beforehand. Ten new malignancies were diagnosed following radiotherapy for endometrial carcinoma. Of these, 4 were of the breast, 3 of the large bowel, 2 were adenomas and 1 was a papilloma of the intestine, and 1 was an *in situ* carcinoma of the cervix. The intestinal tumours and the *in situ* carcinoma of the

cervix were diagnosed with 2-7 years of the completion of the radiotherapy course, and all of the sites were included in the treatment volume.

Among the 22 patients not submitted to radiotherapy after surgery, 4 other tumours were diagnosed. Three of these were found before the endometrial carcinoma, while the remaining one (a breast tumour) arose three years after treatment.

PROGRESS REPORT

Contract : Bi 7-007
Sector : C 14

Title : Radon and Lung Cancer in the Ardennes and Eifel Region

1. Poffijn	Univ. Gent
2. Tirmarche	CEA - FAR
3. Wichmann	Univ. Wuppertal
4. Kayser	Direction de la Santé
5. Darby	Imperial Cancer Research Fund
6. Jacobi	GSF Neuherberg
7. Tirmarche	CEA - FAR

I. Coordinator of the project : A. Poffijn

II. General goals

Epidemiological study on the role of radon in the etiology of lung cancer in the Ardennes-Eifel region and in Brittany.
In each of the participating countries, Belgium, France, Germany and Luxemburg cases and controls will be collected in a series of hospitals, situated in the respective regions of interest.
The radon exposure for the last 35 years is reconstructed through 6 months measurements in the living and bedroom of the different dwellings occupied by the study objects during the considered time interval.
Through a common "core"questionnaire items such as occupationnal exposure, smoking habits and environment tobacco smokes (for non smokers and occasional smokers only) are estimated. The coding scheme for these questions will be identical for all participants.
The study is set up as a 5 year project in order to arrive at completely analysed data for 1.200 cases and 3.600 controls.
Through contacts with epi-research teams in U.K., Sweden and the U.S., the possibility of pooling the data from different studies on a larger scale is considered.

III. Objectives for next period

During the next period the collecting of the data will be completed in all the hospital centers.
The conclusion will also be drawn out of the result of the field intercomparison (3 and 6 month exposure) between the radon detectors used by the different participants.
The draft coding schedule for data collection will be finalized and generally procedures for the transfer of data will be set up.

IV. General progress

During the reporting period (May 90 - May 91) major progress was achieved in the following domains :

* Development of a common cove protocol (see appendix I)
* Draft doding scheme for data collection
* Quality assurance exercise (lab + high radon house) 2 days exposure
* Contact with clinics, training of interviewers, start of field work
* Publications :
  The general concept of the study and its current state will be presented at the DOE/CEC meeting in Washington (22 and 23 July 1991) and at the 5th International Symposium on the Natural Radiation Environment (Salzburg 22-28 September 1991).

CASE-CONTROL STUDY OF RADON AND LUNG CANCER IN THE ARDENNES-EIFEL REGION
COMMON PROTOCOL.

Residential Criteria

1. All subjects, whether cases or controls, should be currently living within the defined study area which corresponds roughly to the geological Ardennes and Eifel region.

2. In Belgium the study area comprises the four provinces of Hainault, Liège, Luxembourg and Namur. In Germany the study area comprises the three administrative districts of Trier, Koblenz, and Köln. In Luxembourg the study area comprises Ösling (i.e. the northern part of the country). In France the study area comprises the northern half of the département of the Ardennes.

3. Only subjects who have lived in the study area for at least 25 out of the last 35 years.will be included.

4. In Belgium, France and Luxembourg there will be no exclusion criteria based on ethnic origin or nationality. In Germany only persons with a German passport who have lived in the Federal Republic of Germany since 1965 will be included.

5. There will be no exclusion criteria based on occupation.

Cases

1. Within each country a starting date and a finishing date for the study will be defined, and attempts will be made to identify all the incident (i.e. newly diagnosed) cases of lung cancer satisfying the residential criteria occurring in between these two dates. In Belgium and Luxembourg case enrolment began on 1 September 1990. In France and Germany case enrolment began on 1 October 1990.

2. Cases aged 75 years or over on their date of diagnosis with lung cancer will be excluded.

3. In each centre patients with lung cancer will be interviewed as early as practicable in their clinical history, to minimise the risk that the patient will die before the measurement in his home is complete. In Germany, patients who are not interviewed within three months of diagnosis are excluded.

4. The aim is to include all cases with primary lung cancer, with reliability of diagnosis grade 1 or 2 on the Oxford classification (see Appendix A).

5. If, in some centres, patients are interviewed before a definite diagnosis is made those who subsequently turn out not to have lung cancer will, if their disease is eligible, eventually be transferred to the hospital control group.

6. In Belgium the clinical notes of each patient will be reviewed by the study physician to obtain the final diagnosis. In Germany and Luxembourg the final diagnosis of lung cancer will be based chiefly on the histology or cytology report. In France the final diagnosis will be based on the histology report and clinical opinion.

7. In each country one local pathologist will review all the pathological material for that centre.

Hospital Controls

1. All participating countries will include for each case of lung cancer entered into the study 2 hospital controls (3 in Belgium and Luxembourg). Each hospital control will be a person who is a hospital in-patient who has not had lung cancer, whose current hospital admission is for a disease that is not strongly related to tobacco and who is not suffering from a disease that is likely to render them incapable of participating in the study. The detailed disease criteria are given in Appendix B. In each centre attempts will be made to include patients with a wide variety of diseases in the hospital control group.

2. The matching criteria for hospital controls are age (in 5 year groups: ... 35-39, 40-44, 45-49, ... 65-69, 70-74), sex and broad area of residence. In Belgium the broad areas of residence will be provinces, and also a "local" area will be defined around each hospital where cases are recruited. In Germany it will be a telephone area. In Luxembourg and France it will not be necessary to match by area of residence. In Germany, Belgium and Luxembourg frequency matching based on the margins will be carried out. In France individual matching will be carried out.

3. It is likely that for many hospital controls the final diagnosis will not be available at the time of interview. In Belgium the case notes of each hospital control will be reviewed by the study physician to obtain the final diagnosis. In France and Luxembourg the local physician will confirm the diagnosis. In Germany the final diagnosis will be taken from the hospital records as soon as it is available.

Community Controls

In Germany and Luxembourg community controls will also be included.

In Germany community controls will also be included with a sampling modification of the random digit dialling or using information of the town registries.

For random digit dialling in all telephone books of the study area a simple random sample of private numbers (=households) is taken as primary units. Within these units controls persons are selected as secondary units.

For selecting controls from the town registries a method of double phase sampling is used. In the first phase an overall sample of persons in all towns is taken; in the second phase the sample of control persons is chosen.

To control the matching criteria sex, age and region both samples are designed as a stratified sampling scheme with a quota plan to fit the frequencies of the matching variables.

In Luxembourg the selection will take place on a population list of the local authorities, on an individual matching criteria using 5 years age groups and same sex. The controls will be identified randomly on this list.

Questionnaire

1. There will be a "core" questionnaire including items such as residential history since birth, occupational history, smoking history, and exposure to passive smoke (for non-smokers and occasional smokers only). As far as possible this "core" section of the questionnaire will be identical in all four countries, and the code generated from these core questions will be identical.

2. In addition each country will include some questions on educational attainment and current occupation of the subject and his/her current partner (or last partner in the case of widows/widowers) to allow classification of social status into high (i.e. professional), middle (i.e. intermediate), low (i.e. manual). If the subject and his/her partner would be classified differently then the subject will always be assigned to the higher of the two.

3. Each country is also free to include additional items in its own questionnaire.

Radon Measurements

1. For each subject (case or control) included in the study an attempt will be made to measure the radon concentration in each home in the study area where the subject has lived for more than 1 year during the last 35 years. (In Belgium if 1 extra measurement will give a lifetime exposure, an attempt will be made to get it).

2. Detectors will be of the Karlsruhe or Makrofol type. In each house that is measured two detectors will be placed, one in the living room and one in the major bedroom.

3. The period of measurement will be six months in all centres except Germany where it will be 2 consecutive six month periods. Where the period of measurement is only six months, seasonal adjustments will be estimated from the study data e.g. by assuming that the true radon concentration in houses measured at different times of the year is the same.

4. The comparability of the detectors and associated procedures used by the different centres will be investigated by intercomparisons carried out both in the laboratory and in the field.

5. For periods of residence outside the study area but within the last 35 years an attempt will be made to measure the likely exposure to radon. In cases where measurement is not feasible, the possibility of estimation will be reviewed later.

Additional Data

In all centres some basic data will be recorded on potential cases who live in the study area, but who do not meet the criterion of having lived there for at least 25 years in the last 35 years or who die before they can be interviewed, and also on potential cases and controls who refuse to take part in the study, or who are too ill to be interviewed or who are not interviewed for any other reason. The data will include the reason that no interview was obtained, the date that an attempt was made to carry out the interview, and the patient's sex, age, current address, and final diagnosis. These items are necessary to calculate the refusal rate in the study, and also to demonstrate good coverage of the numbers of lung cancers occurring in the study area during the time period of the study e.g. by comparison with official mortality or cancer registration data. In addition for each such person the total number of years lived in the study area will be collected wherever possible.

Pooling of data

Each centre will submit data periodically to the co-ordinator in Gent for validation and consolidation into a single database before transfer to Wuppertal for analysis. It is intended that submissions should occur approximately every six months, approximately 1 month before each meeting of the collaborators, so that the co-ordinator can present some tabulations of the accumulating data to the group for discussion.

Head of Project 1: Dr. Poffijn

II Objectives for the reporting period (May 1990-May 1991)

* Development of a common protocol for the Ardennes-Eifel study, based upon the experience gained in a previous pilot project conducted in one hospital in southern Belgium.
* Organization of a radon intercomparison exercise for the different participants to this study, under controlled laboratory conditions and at a similar exposure level in a high radon test house in the Belgian Ardennes.
* Development of a draft coding schedule.

III Objectives for next period

* Extension of the study to the subregion of Liège (hospital La Citadelle).
* Organization of an intercomparison exercise between the interviewers involved at the different centers.
* Review of all medical slides by reference pathologist.
* Evaluation of the on-going 3 and 6 month radon quality control exercise between the different participants to this common study project.
* Testing and finalization of the draft coding schedule.
* Collection of all data (every 6 months) for each participant and transfer in appropriate form for statistical analysis to Wuppertal.

IV Progress achieved including publications

* The questionnaire as used in the pilot phase at the Mont Godinne hospital center (Namur) was adapted according to the common protocol worked out with the other participants during a series of work sessions.
* The field work in this hospital started in September 1990.
* After analysis of the entry rate of lung cancer patients in different hospitals in the province of Hainaut, the hospitals of Warquignies and Jolimont were selected as reference centers for this province.
  Interviewing started here in February '91. At the same time radon detectors were also distributed.
* During the reporting period a total of 170 interviews have been performed and 392 radon detectors were installed at current and previous addresses of the patients.
* Publications :
  The role of radon in the etiology of lung cancer, proceedings Health Risks of Radon and Tobacco Smoke, Gent 1990, Annals Belgian Radiation Protection Association, Vol.15, n°9, p.441-448.

Project: CASE CONTROL STUDY IN THE FRENCH ARDENNES REGION, EVALUATING THE RISK OF LUNG CANCER LINKED TO DOMESTIC RADON EXPOSURE

Head of Project 2: Dr. TIRMARCHE

II Objectives for the reporting period (1990 - may 1991)

1) Collaboration to an international European protocol defining the epidemiological study in the Ardennes-Eifel region.

2) Contact with the physicians and pneumologists to define the hospitals recruiting most of the lung cancers of this region.

3) Test of feasibility of this study.

III Objectives for next period (may 1991 - may 1992)

- The collaboration between CEA and the Society "Ardennes-Epidemiologie" having been defined by common agreement, and the training of the interviewer being realized with satisfaction, we consider that "at the hospital basis", the Ardennes study is evoluating in a feasable way. The introduction of dosimeters in the houses is actually linked to a high percentage of refusal and has to be discussed with the persons directly involved, in order to get a higher percentage of success.

IV Progress achieved including publications

By questioning a sample of physicians and pneumologists of the region concerned in this study, we have got the conclusion that most of the lung cancer patients passed by the hospitals of CHARLEVILLE-MEZIERES, and Dr. M. COHEN of the "Société ardennaise de Cancérologie" accepted to coordinate this study on a local basis (the hospitals and clinics concerned are in CHARLEVILLE-MEZIERES and or SEDAN).

Patients are all interviewed by the same interviewer and about 20 interviews have actually been conducted.

The dosemeters are presented and explained to the patients during the hospitalization. They are of the same type as those measuring Radon in the Belgian study.

An intercomparison of the different dosemeters involved in the European study has been conducted in laboratory conditions and in standard dwelling conditions for 3 and 6 months of exposure.

Publications : We intend to present the actual state of the study at the future DOE and CEC meeting in Alexandria (22-23 July, 1991) and at the 5th Int. Symposium on Natural Radiation Environment in SALZBURG, Austria (September 22-28, 19 ' ').

Head of Project 3 : Prof. Dr. H.E. Wichmann

II Objectives for the reporting period (May 1990 - May 1991)

- Recruitment of lung cancer hospitals in the study region :
- Universitäts- und Polikliniken Homburg
- Hufelandklinik Bad Ems
- Mutterhaus der Boromäärinnen Trier
- Klinik Köln-Merheim, and of hospitals for control persons :
- Universitäts- und Polikliniken Homburg
- Dryanderklinik Bad Ems
- Development of case and control recruitment within the hospitals
- Training of the interviewers
- 48 interviews with lung cancer patients and population controls (including rough data check) - participation on 2 radon-intercomparisons.

III Objectives for next period

- Carry on field work of case and control interview
- Development of data analysis approaches

IV Progress achieved including publications

see general progress

Head of Project 4 : Dr. Kayser

II Objectives for the reporting period (May 1990 - May 1991)

- Participation to the development of a common core protocol
- Training of the interviewer
- Recrutement of lung cancer hospitals
- Participation to the quality assurance exercise

III Objectives for next period

- Continuation of field work
- Improvement of data transfer from clinics
- Testing of the draft coding schedule

IV Progress achieved including publications

- 44 patients interviews and all detectors installed in their current and past homes
- Intercomparison of involved dosimeter in lab conditions and in high radon house
- Publications : see general progress

Head of Project 5: Dr. Darby

II Objectives for the reporting period

Development of a protocol for the Ardennes-Eifel study, and development of a draft coding schedule for data collection.

III Objectives for next period

Finalisation of coding schedule for data collection, and finalisation and implementation of procedures for collecting data centrally from the various areas.

IV Progress achieved including publications

The objectives for the current reporting period have been achieved. A protocol has been developed and agreed by all four investigators responsible for data collection. A draft coding schedule has also been constructed and is ready for testing.

Head of Project 6: Prof. Jacobi

II Objectives for the reporting period (May 1990-May 1991)

The BEIR IV committee has reviewed and analysed the data from the four most important cohorts of Western miners, mostly uranium miners In this work the important data from Czekoslovakia had not been included.
Objective of our work is, in cooperation with the Institute of Hygiene and Epidemiology in Prag, to analyse the Czekoslovakian data with methods similar to those in the BEIR IV report in order to reach comparability of the results.

III Objectives for next period are

To apply the non-parametric analysis to the different cohorts of Czekoslovakian miners. A joint publication describing the various cohorts and giving the results of this first analysis is planned for the coming period. It will be dedicated to J. Svec who recently died and whose life work had been to follow the U-miners. Under his leadership the measurements of exposures and health records of the individual miners have been collected over several decennies. Next step will be to analyse the data with models similar to those used in the BEIR IV analysis. This will be done with the AMFIT program package in its last version.

IV Progress achieved including publications

The BEIR IV committee has used in its analysis a variety of models, and it did not appear that any was clearly better than the others. It seems therefore necessary to first analyse the Czekoslavakian data non-parametrically. Advantage of such a first step is that almost no assumptions are required in the analysis. The results should help in the selection of the analytical models which will be used in the next step. For a non-parametric analysis we use the socalled double isotonic regression. It gives an estimate of a function (here the tumor incidence) depending on two variables (here cumulated exposure and age) with the only constraint that the function be monotonically increasing with exposure. Although the isotonic regression is not much used, at least in the field of epidemiology, it has been shown that it is a maximum likelihood estimator. The computer program to calculate the isotonic regression for a given data set is adapted from a program published in the mathematical literature.
We have applied the method of double isotonic regression to the data set of 1800 miners from the older cohorts of miners, kindly provided to us by our colleagues in Prague.

Project : **CASE CONTROL STUDY IN BRITTANY (France) EVALUATING THE RISK OF LUNG CANCER LINKED TO DOMESTIC RADON EXPOSURE**

Head of Project 8: **Dr. TIRMARCHE**

II Objectives for the reporting period **(may 1990 - may 1991)**

1) Elaboration of a protocol comparable to that of the Ardennes-Eifel Study.

2) Construction of the questionnaire adapted to the Britanny and Vendee situation.

3) Test of this questionnaire in a pilot sutdy at the University Hospital of BREST (pneumologist : Professeur CLAVIER).

III Objectives for next period **(→ may 1992)**

- Extension of this study to the whole region of Brittany and Vendee : contact with local and regional hospitals, university hospitals of BREST, NANTES, RENNES, and anti-cancer centers, in order to have a correct representation of the lung cancer incidence of the whole geographical region in our study (we estimate 10 hospitals to be included).

- Training of the interviewers recruted in the different hospitals.

- Interviewing of cases and controls on a larger scale.

IV Progress achieved including publications

This period has to evaluate the feasability of this case-control study in Brittany and Vendee. Our first contacts in the different pneumology services show :

1) A great interest manifested by the pneumologists for the Radon problem linked to lung cancer, especially if we can take in account the part of Radon in presence of tobacco.

2) The ignorance of the Radon problem by the patients. The mention of a radioactive gas occuring naturally in this region and that has to be measured in the dwellings, is often linked to a refusal of collaboration. In consequence, we decide to present the study as estimating the health risks linked to environmental factors.

3) Patients, essentially lung cancer patients, have to be interviewed as soon as possible after diagnosis, when they do not feel too tired, in order to be able to answer correctly the whole questionnaire (duration : 30 minutes)).

Publications : Presentation of the protocol and the study at CEC and DOE Meeting in Alexandria (22-23 July 1991) and at the 5th Int. Symposium on the Natural Radiation Environment in SALZBURG, Austria (September 22-28, 1991).

Progress Report

Contract: Bi6-295 Sector: C14

Title: Investigation of the relationship between lung cancer and radon in houses

1 O'Riordan NRPB

I. Summary of Project and Global Objectives

The project is a case-control study of exposure to radon in the home in Cornwall and Devon and lung cancer. The objective is to provide a more precise estimate of the risk of lung cancer from exposure in the home. Detailed residential histories and information about cigarette smoking and other risk factors for lung cancer will be collected for incident cases of lung cancer and also hospital and community controls. Attempts will be made to measure radon concentrations in the current homes of all subjects, and also in their previous homes in Cornwall and Devon during the last 35 years.

Head of Project 1: Dr O'Riordan

II. Objectives for the reporting period

The objectives were the recruitment of all newly diagnosed cases of suspected lung cancer in Cornwall and Devon, and inclusion in the study of all those which met the residence requirement and whose final diagnosis was lung cancer. In addition, the recruitment of hospital and community controls matched to the lung cancer cases, also subject to residence and diagnosis criteria. The measurement of radon concentrations in the present and past homes of subjects.

III. Objectives for the next period

In the next reporting period collection of data will continue as in the present reporting period. Lung cancer cases, hospital controls and community controls will be recruited and their suitability for inclusion in the study will be reviewed on the basis of the established criteria concerning their past residence and the confirmed diagnosis of their diseases. Measurements of radon concentrations will be made in their present and previous residences. In order to ensure recruitment of sufficient community controls in Devon, action will be taken through the Department of Health if necessary.

IV. Progress achieved including publications

1. Study Centres

Since the last progress report data collection at all five study centres (Truro, Plymouth, Barnstaple, Torquay, and Exeter) has proceeded smoothly.

2. Cases and Hospital Controls

A total of 1839 patients with suspected lung cancer have been identified to date (see Table 1). One hundred and seventy eight patients (9.7%) were too ill to be approached for interview and, of the remainder, 60 (3.6%) refused to take part. Seven hundred and forty one patients had not lived in Devon or Cornwall for long enough to satisfy our residence requirements, and 859 patients received a full interview.

Review of hospital discharge diagnoses has been carried out for 715 of the 859 patients with suspected lung cancer who received a full interview. For 475 (66.4%) the final diagnosis was lung cancer. The pathological review has continued smoothly. In about 96% of those for whom the final diagnosis was lung cancer an attempt was made to obtain a microscopic diagnosis. For 67% the diagnosis of lung cancer was confirmed by histology, and for a further 11% the lung cancer was confirmed by cytology alone.

A total of 1318 potential hospital controls have been identified (see Table 1). Forty nine patients (3.7%) were too ill to be interviewed, and 21 further (1.7%) refused to take part in the study. Of the remainder 476 did not meet the residence criteria, and 772 received full interviews.

Review of the hospital discharge diagnoses has been carried out for 689 of the 772 hospital controls with full interview. For 21 patients the final diagnosis rendered them ineligible as controls, as they turned out to have diseases strongly related to smoking (cancer of bladder (6), cancer of pancreas (2), other lung diseases (2), arterial embolism or thrombosis (2), ischaemic heart disease (1), cervical cancer (1), aneurysm of iliac artery (1), duodenal ulcer (1), pulmonary tb (1), transient cerebral ischaemia (1), cerebrovascular disease (1), pneumonia (1), chronic skin ulcer due to peripheral vascular disease (1)).

3. Community Controls

Interviewing of community controls started in late 1989 in Devon, and in the first quarter of 1990 in Cornwall. During the second half of 1990 interviewing of community controls has proceeded smoothly at all 5 centres. To date 1018 potential community controls have been selected from the Family Health Services Authority (FHSA) lists, and processing is complete for 905.

The first step in obtaining an interview from a community control is to seek approval from his or her General Practitioner (GP). At this stage 40 of those selected from the FHSA lists were found to have

died while a further 46 had moved and were no longer registered with the Practice. For 64 of the remaining subjects (7.8%) the GP recommended that it would be inadvisable to contact the patient, usually on grounds of psychiatric illness or subnormality, 6 further patients were found by our interviewers to be too ill to take part in the study, and a further 44 (5.9%) refused to take part. Three hundred and one community controls had not lived in Devon or Cornwall long enough to satisfy our residence requirements, and 404 have received a full interview.

The extension of the study to include community controls is now running very smoothly. How its continued operation depends on the receipt of further lists of names from the FHSAs. At the present time Devon FHSA have refused to supply any further names on the grounds that to do so would be a breach of confidentiality, and this poses a serious threat to the continued smooth operation of the study.

4. Measurements in Current Homes

Of the 2035 cases, hospital controls and community controls who have received a full interview, the interviewers have reported that a detector has been successfully installed in the homes of 1806 (94.8%). Forty three subjects (11 cases, 31 hospital controls and 1 community control) have refused to have a detector installed in their current home, 7 returned the detectors after less than two months, and detectors have not been installed in the homes of 14 patients who have moved recently, or died before a measurement could be made. A further 36 patients lived alone and either died before a measurement could be made or did not return to their original home after discharge from hospital. These addresses have been added to the list of 'Past Addresses' (see next section) and further attempts will be made to obtain measurements for them in due course. For the remaining 129 subjects we are awaiting a report from our interviewer or data processing is not yet complete.

The interviewers have reported that 1392 pairs of detectors have been retrieved after approximately 6 months measurement, and for 1173 of these measurements have been provided by NRPB.

5. Measurements in Past Addresses

Six batches of addresses of previous houses in Devon and Cornwall have been passed to NRPB for an initial postal approach, with those who do not reply being visited by our local interviewer.

CASES OF SUSPECTED LUNG CANCER

Year - half	Full interview	Short residence	Patient refused	Patient too ill	Total
1988 - 2	67	71	13	16	168[a]
1989 - 1	100	79	14	20	213
2	193	171	13	32	409
1990 - 1	207	190	10	37	444
2	155	137	7	48	347
1991 - 1[b]	137	93	3	25	258
Total	859[c,d]	741	60	178	1839

HOSPITAL CONTROLS

Year - half	Full interview	Short residence	Patient refused	Patient too ill	Total
1988 - 2	53	19	4	1	77[a]
1989 - 1	66	41	2	1	110
2	160	104	4	18	286
1990 - 1	208	122	6	13	349
2	172	105	4	10	291
1991 1[b]	113	85	1	6	205
Total	772[c,e]	476	21	49	1318

COMMUNITY CONTROLS

Year - half	Full interview	Short residence	Subject refused	Subject too ill	GP refused	Ineligible	Total
1989-2	5	5	-	-	2	4	16
1990-1	103	94	12	2	12	21	244
2[b]	141						
1991-1[b]	155	202	32	4	50	61	645
Total	404[c]	301	44	6	64	86[f]	905

a, b, c, d, e, f: Please see overleaf for notes.

Table 1. Numbers of patients with suspected lung cancer, patients with other diseases suitable for comparison purposes and community controls identified in Devon and Cornwall.

Notes for Table 1

a. Total for cases of suspected lung cancer includes 1 patient for whom available resources did not permit an interview, but for whom the final diagnosis was TB, and 30 patients identified in the first half of 1988. Total for hospital controls includes 1 patient identified in the first half of 1988.

b. Data for this period are not yet complete.

c. Totals include 11 cases of suspected lung cancer, 31 hospital controls, and 1 community control with full interview who refused to have a detector in their current home.

d. Hospital discharge diagnoses have been reviewed for 715 cases of suspected lung cancer with a full interview. For 475 the final diagnosis was lung cancer.

e. Includes 21 controls with full interview, but ineligible final diagnosis.

f. Includes 40 subjects found to have died, and 46 who had moved away.

Progress Report

Contract: Bi6-324 Sector: C21
Title: Development of fundamental data for radiation protection

1 Smith ICRP

I. Summary of Project and Global Objectives

Evaluating the biological basis of radiation-induced effects and the metabolism and dosimetry of incorporated radionuclides are integral parts of the recommendations in radiation protection. In this respect, the Main Commission requires an input from its Committees, aided by task groups, who critically evaluate all available data at the request of the Main Commission. Reports prepared by committees are considered by the Main Commission and, if adopted, are published by the Secretariat. The head of the project plays a coordinating role in this process.

New data and new interpretation of earlier information now indicate with reasonable certainty that some risks associated with ionising radiation are about three times higher than they were estimated to be a decade ago.

This increase called for some quantitative changes in the Commission's recommendations. One such change is a reduction of the dose limit for occupational exposure. The previous limit of 50 millisievert (mSv) in a year has been reduced to 20 mSv per year, averaged over five years, with the further provision that the dose should not exceed 50 mSv in any single year. The limit for public exposure is 1 mSv in a year. In general, these dose limits do not apply to natural sources of radiation or to the medical exposure of patients.

The Commission has maintained and strengthened its system of radiation protection. The basic requirements are; (a) that any practice and its protective system should do more good than harm (justification of a practice); (b) that the protective system should be optimised to maximise the net benefit, while providing adequate protection for the individuals exposed as a result of that practice (optimisation of protection); and (c) that the combined effect of all relevant practices on any individual should be suitably limited (individual dose or risk limits). Similarly, any intervention in the case of accidents should do more good than harm; should be optimised to maximise the good it does; and should prevent seriously damaging effects on any individual.

The new recommendations emphasise the difference between the practices causing exposure and the situations where accidents or existing exposures require decisions on remedial actions.

Head of Project 1: Dr. Smith

II Objectives for the reporting period

To produce new recommendations in radiological protection, based upon a sound scientific basis. This has involved commissioning task groups to evaluate risks of radiation induced cancer and severe hereditary effects at the low doses of radiation associated with normal practices involving the use of ionising radiation.

III Objectives for next period

To continue the process of reviewing and updating the evidence on radiation-induced risks and to consider the application of the basic principles to protect patients in radiodiagnosis and nuclear medicine, workers and members of the public.

IV Progress achieved including publications

During the period under review the following topics have been studied and reports prepared, or are under preparation, for adoption by the Main Commission.

Annual Limits on Intake of Radionuclides by Workers based on the 1990 Recommendations

Adoption of the 1990 Recommendations (ICRP Publication 60) necessitates a revision of the Commission's secondary limits contained in Limits for Intake of Radionuclides by Workers (ICRP Publication 30). In order to permit immediate application of these recommendations, new values of Annual Limits on Intake which incorporate the new dose limits, radiation weighting factors and tissue weighting factors, but retaining the metabolic and biokinetic data from Publication 30, have been calculated. These new values were adopted by the Main Commission in November and will be published as ICRP Publication 61. Eventually a complete revision of Publication 30 will be issued taking into account a new respiratory tract model, a new edition of Reference Man (ICRP Publication 23), and where appropriate, new biokinetic models.

Biological Basis for Dose Limitation in the Skin

The report discusses radiation effects in the skin, the experimental and epidemiological evidence for radiation induced skin cancer and deterministic effects. It defines the cells at risk and their spatial distribution in relation to different types and energies of radiation. In particular, it addresses the "hot particle" question. The draft seen by the Main Commission in November was sufficiently advanced to allow some of the data to be incorporated in the 1990 Recommendations.

Specified limits are needed for the skin since this tissue will not necessarily be protected against deterministic effects by the limit on effective dose. The Commission will recommend an annual limit for workers of 500 mSv for the skin averaged over any one square centimetre, regardless of the area exposed. This annual equivalent dose is reduced by a factor of 10 for members of the public. The report will be presented to the Main Commission for adoption at the 1991 Vienna meeting.

Age-Dependent Doses to Members of the Public from Intake of Radionuclides - Part 2

This report will include twelve elements not included in Part 1 (ICRP Publication 56) but only data for limiting intake by ingestion will be submitted to the Main Commission for adoption at the 1991 Vienna meeting. The new tissue weighting factor values and a new generic model for the alkaline earth elements and lead will be used.

During 1991, work will proceed as rapidly as practicable on the computation of doses following inhalation of radionuclides, based upon the new tissue weighting factor values and the new respiratory tract model.

Human Respiratory Tract Models

The new model was designed to permit biologically meaningful radiation doses to be estimated, taking into account the effects of smoking, air pollutants and respiratory diseases. Separate parameters are included for deposition, and clearance is considered as two competing processes, viz., mechanical clearance in mucus from the airways and translocation through lung tissue to blood and thence to organs of deposition. The report will address the relative radiosensitivity of different regions of the respiratory tract and regional doses within the respiratory tract will be calculated. This approach may necessitate a subdivision of the tissue weighting factor value for lung as used in the 1990 Recommendations. A new dosimetric model will be used to calculate doses to adults, children and infants. A final report is anticipated at the 1991 Vienna meeting.

Reference Man

The expanded update of ICRP Publication 23 for western man will include an appendix on data for non-western man. It will give details on elemental and cellular composition of body tissues, and anatomical and physiological parameters for all the major body systems. In particular, data on the fetus and children will be included. A draft of part of this report is likely to be available at the 1991 Vienna meeting.

Probabilistic Events

The report, which will explain the basic considerations for control of exposures not intended to occur (potential exposures), is intended for use by international agencies and national authorities when developing procedures applied to particular practices. The importance of a proper interaction with experts in nuclear and general safety has been recognised in the preparation of the report.

Planning for Intervention in the Event of a Nuclear Accident or a Radiological Emergency

The report will set out the general principles for planning intervention including guidance on intervention levels, both for the introduction and withdrawal of countermeasures. It deals not only with the short term and near field, but also with the longer term context of a gradual return to normal and at distances remote from the immediate environs of the accident. Annexes containing worked examples will be included.

Measures for Protection Against Radon

The report will exclude advice on protection against radon in mines, but will address the application of the system of protection in buildings. Guidance will be given on intervention levels in homes and in the workplace, the remedial actions available and the cost and effectiveness of the remedial measures. A particular issue will be the definition of the level of annual exposure to radon at work that leads to workers being considered occupationally exposed.

Updating of ICRP Publication 53 (Radiation Dose to Patients from Radiopharmaceuticals)

The objectives are to calculate organ and effective doses from new radiopharmaceuticals that are firmly established in medical practice. It is anticipated that the number of such new products will be of the order of a few each year.

PUBLICATIONS

ICRP Publication 60. 1990 Recommendations of the International Commission on Radiological Protection. Annals of the ICRP 21 (1/3) 1991.

ICRP Publication 61. Annual Limits on Intake of Radionuclides by Workers Based on the 1990 Recommendations. Annals of the ICRP 21 (4) 1991.

Progress Report

Contract: Bi6-347i Sector: C21

Title: Application of ALARA in complex decision-making situations (NRPB Association)

1	Wrixon	NRPB
2	Lochard	CEPN
3	Meggitt	SRD AEA

I. Summary of Project and Global Objectives

The objectives of this project are:

(a) to develop appropriate software and tools for undertaking optimisation of protection;

(b) to develop appropriate databases on incidence of failures;

(c) to investigate the application of ALARA to decision-making in complex situations and to propose a general methodology to aid decision-making in such situations.

Progress achieved to date

(a) Agreement has been reached over the basic structure of the software for cost-effectiveness and cost-benefit analyses.

(b) and (c) A first draft of a procedural guide has been prepared and used on the CEC funded training course on ALARA in November 1990.

(d) Preliminary discussions have started on how task specific dosimetry systems could be used for the practical implementation of ALARA at the operational level.

(e) An initial study has been undertaken of the data on accidents and incidents that are available in the UK.

(f) A start has been made on defining the elements of a generic optimisation and the interaction of generic optimisation with dose constraints.

(g) A review of approaches to decision-making in radiological protection in an ALARA context has been undertaken and a case study involving the use of multicriteria analysis has been started.

(h) Meetings have taken place in which the concepts of risk aversion have been discussed.

Head of Project 1: Dr Wrixon

II. Objectives for the reporting period

(a) To review the use of procedural guides for training in optimisation of protection;

(b) To define and start the development of a software package to carry out simple and extended cost benefit analysis;

(c) To outline the specifications for task specific dosimetry;

(d) To initiate a feasibility study of setting up an accident and incident database in the UK;

(e) To identify the component elements of a generic optimisation, prior to defining a case study.

III. Objectives for next period

(a) Further development of procedural guides.

(b) Complete initial programming, test software and write supporting material.

(c) Completion of a UK feasibility study on an accident and incident database, leading to proposals for extending the study to other Member States.

(d) Continue the development of a general structure for generic optimisation and initiate case studies.

IV. Progress achieved including publications

This work is being undertaken in collaboration with CEPN (France). During the period two joint meetings have taken place to define work programmes and to review progress. One outcome was to redefine the objectives of the project.

The development of further procedural guides is a wide ranging objective to which the other objectives will provide an input. A spin off from this objective and the precursor project has been the CEC training course, Optimisation of Radiological Protection in the Design and Operation of Nuclear and Industrial Facilities, held at Saclay 19-23 November 1990. The preparation of the lecture material was jointly funded by DGXI and DGXII under a separate contract. However, complementary work has also been undertaken under this contract and the course is a valuable focus for the development of procedural guides.

CEPN have taken the lead in developing the software package (see report of J Lochard). Specifications for software modules to take the user step by step through the ALARA procedure and to carry out simple and extended cost benefit analysis have been agreed and a major part of the initial programming has been completed.

The Saclay training course was used as a discussion forum on the need for and specification of task specific dosimetry. This confirmed earlier ideas that there would be value in having a CEC 2 day meeting/workshop on this subject.

The current availability of accident and incident data in the UK is being reviewed. Initial findings indicate that the quality and quantity of data varies significantly from one use sector to another. In many sectors the data is restricted to legally reportable incidents and is not easily accessible. Failures of equipment and procedures do not always result in legally reportable incidents but may be relevant to optimisation studies. Therefore, as a pilot scheme a system is being set up to collect incident reports (on a voluntary basis) from the 700 organisations for which NRPB acts as Radiation Protection Adviser.

Work has started on defining the elements of a generic optimisation and how these elements interact. A tentative framework for generic optimisations has been constructed and work is progressing. Consideration is being given to whether generic optimisation has a role in setting dose constraints.

Publications covering work of reporting period

None.

Head of Project 2: Dr Lochard

II. Objectives for the reporting period

- Design and specification of a decision aiding techniques software (cost-effectiveness and cost-benefit analysis).

- Preparation of a generic procedure guide for the implementation of the ALARA principle into radiation protection programme for the operation and maintenance of nuclear installations.

III. Objectives for next period

a) Decision aiding techniques software

The objective is to make the software available for external users by the end of 1991. In order to optimise cooperation of CEPN and NRPB on this topic, a member of CEPN team will spend two months at Chilton, to test the version already developed and write a user's guide.

b) ALARA procedure guides

Synthesis of work previously done both concerning ALARA in design and operation of nuclear installations will be presented first during the Guernsey meeting and secondly at the second ALARA training course to be held in June 1991. Some new elements will be added, as far as on going studies (such as preparation of next steam generator replacements, optimisation of protection of the radiological other buildings of the MELOX plant) will allow new developments. A final report will be prepared for the end of 1991.

IV. Progress achieved including publications

a) Decision aiding techniques software

An agreement has been reached about the methodology and the basic structure of a software for implementing simple cost-effectiveness and cost-benefit analysis both for occupational and public exposure reduction options. A specific NRPB/CEPN workshop on this topic has been organized in Paris (April 1990) to discuss the design the software. During the following months a report has been written describing the functions, algorithms and outputs (tables, graphs...) to be developed in the software (CEPN report no 174 - June 1990). Since then the software is in development using EXA-DATA base management system and C language.

b) ALARA procedure guide

b1 Maintenance and operation

In order to implement ALARA during maintenance and normal operations, CEPN has proposed to EDF, at the beginning of 1990, a complete ALARA programme including motivation

actions, ad hoc structures and procedures like reviews, check-list... These proposals have been largely adopted and tested at the occasion of the Steam Generator Replacement that took place at Dampierre 1 (February to May 1990) and led to a large success in terms of individual and collective dose saving. Based on the experience gained during that operation the objective of the project is to develop a more generic guide. First draft of such a guide has been presented during the Saclay ALARA training course in November 1990 (see later).

b2 Design of installations

In the course of 1990, CEPN has been actively involved in a project aiming at optimizing the radiological protection for the workers within a new fuel fabrication facility under construction in France (The MELOX plant). This project allowed to delineate the key issues about the implementation of the ALARA principle at the design stage. A Generic methodology and specific tools have been developed allowing a first generic reflexation about the role of ALARA in design.

c) ALARA training course

On behalf of the DGXI and DGXII of the CEC, a training course entitled "Optimisation of radiological protection in the design and operation of nuclear and industrial facilities" was organized jointly by NRPB and CEPN in close cooperation with the French Institute of Nuclear Sciences and Technologies (INSTN) and the Training Centre for the French Nuclear Industry (CETIC) in Saclay (France), 19-23 November, 1990. During this seminar the new developments achieved within the joint Project have been presented and largely discussed with the participants.

Publications covering work of reporting period

LOCHARD, J, LEFAURE, C. "Proposition d'organisation et procédure ALARA pour la préparation, le suivi et le retour d'expérience des chantiers de maintenance: application au RGV" Janv. 1990. Rapport CEPN no 166 bis.

SCHIEBER, C, LOMBARD, J, LEFAURE, C. "Propositions pour l'élaboration d'un logiciel d'analyses coût-efficacité et coût-bénéfice en vue de l'optimisation de la radioprotection" Juin 1990. Rapport CEPN no 174.

LEFRANCOIS, I, CROUAIL, P. "Analyse de la préparation et de la réalisation du replacement des générateurs de vapeur de Dampierre 1, en vue d'établir un rapport de retour d'expérience radioprotection", Mémoire de stage INSTN, Juin 1990.

LOCHARD, J. "ALARA programmes in operation: a generic framework" ALARA Course Saclay, Nov 90.

LEFAURE, C, CROFT, J. "Elements of designing ALARA programme for maintenance of nuclear facilities". To be presented at Conference on Occupational Radiation Protection, in Guernsey, 29 April - 3 May 1991.

PAGES, P, DEGRANGE, J P, LE BAIL-TASSEL, L, NIMAL, J C, DUCROUX, R, LORENZELLI, M. "ALARA at the design stage of nuclear installations". To be presented at Conference on Occupational Radiation Protection, in Guernsey, 29 April - 3 May 1991.

LOCHARD, J, LEFAURE, C, CROFT, J R, SAUNDERS, P. "Analytical tools for optimisation of radiological protection". To be presented at Conference on Occupational Radiation Protection, in Guernsey, 29 April - 3 May 1991.

Head of Project 2: Dr Lochard

II. Objectives for the reporting period

- Literature review of Insurance Economics on the concept of risk aversion.

- Introduction of multicriteria analysis in the case of a low and intermediate level radioactive waste disposal site.

III. Objectives for next period

a) Risk aversion

Based on the literature review, the objective is to explore the applicability of the previous concepts for risk management. A survey of past case studies will provide first results on:

- the difference between the man-Sievert values according to the level of occupational exposure;
- the limit values proposed for accidental situations (safety analysis of waste disposal...);
- the comparison of accepted values for public exposure and worker exposure.

b) Analysis of typical attitudes towards time, future events and integenerational problems, in various fields (law, economy, philosophy,... and sciences).

c) Multicriteria analysis

The objective is to perform a sensitivity analysis concerning the key parameters of the evaluation of the waste disposal site.

IV. Progress achieved including publications

A joint CEPN/SRD meeting was held in Paris (June 1990) to define the basic steps of the two years programme. It was decided to develop the project according the following steps:

1 Review of literature
2 Categorization of situations
3 Methodological developments
4 First applications

a) Risk aversion

The first part of the project for the CEPN, performed in collaboration with the University of Mons (Belgium), aims to clarify the risk-aversion concepts (meetings in: January, March, June, September, November). Starting from the theory of Insurance Economics, the basic concepts explored were:

- Criteria of evaluation: expected value, mean variance, expected utility.
- Definition of absolute risk-aversion: assumptions on utility function and graphical interpretation.
- Definition of relative and partial risk-aversion.

A report on these aspects is at the moment under development and will be ready in April 1991.

b) Use of multicriteria analysis

A case study is being performed, with the French Atomic Energy Commission (CEA) and the National Agency for Nuclear Wastes Management (ANDRA), to "optimize" the radiation protection measures of a low and intermediate level radioactive waste disposal site in France.

Taking into account the different categories of barriers acting as protection against the migration of radioactivity into the ground, a set of possible options has been identified.

The selection of an "optimum" solution is not easy because of the diversity of the factors to be taken into account as for example: the potential public radiation exposure several hundred years ahead, the occupational exposure during the monitoring period of the disposal site...

The study is composed of four steps:

- identification and analysis of options for the structure, allowing for different technical measures concerning the quality of concrete, temporary and definitive roofs, monitoring systems for the collected water;

- selection and estimation of the qualitative and quantitative criteria useful for the decision making process (eg, investment and maintenance costs, maximum individual dose for the public, separating short, medium and long term, collective dose for the workers during the monitoring period, maintenance capabilities, reliability and mechanical stability);

- determination of the "most effective" solutions using multicriteria analysis based on the (qualitative and quantitative) evaluation of the different criteria;

- sensitivity analysis and discussion of uncertainties related to the various assumptions.

Publications covering work of reporting period

PAGES, P, SCHNEIDER, T, LOMBARD, J. "Applying multi-criteria analysis to radiation protection optimisation of low and intermediate level radioactive waste disposal" to be presented at the International Symposium on Environmental Consequences of Hazardous Waste Disposal, in Stockholm, 27-31 May 1991.

SCHNEIDER, LOCHARD, J, PAGES, P. "Survey of the application of the risk aversion concept in the nuclear field" to be presented at the 18th Seminar of the European Group of Risk and Insurance Economics, Mons (Belgium), September 1991.

Head of Project 3: Dr Meggitt

II. Objectives for the reporting period

Aims

To investigate the application of ALARA to decision-making in complex situations and propose a general methodology to aid decision-making in such situations.

Objectives

1 Review the current approaches to decision-making in an ALARA context.

2 Propose a general framework for ALARA decisions and specific decision-aiding techniques.

3 Identify factors relevant to the decision process in various categories of problem (eg, routine exposure, probabilistic exposure, exposure in the future), how they interact with each other and the trade-offs involved in reaching a decision.

III. Objectives for next period

1 Investigate how these factors and trade-offs can be incorporated into the decision process.

2 Demonstrate the application of the methodology to selected case studies to illustrate its use. The case studies will be representative of the principal areas in which the methodology is likely to be applied.

Table 1: Four step programme agreed by SRD and CEPN

STEP	SRD	CEPN
Critical review of approaches to decision-making.		
Identification of attributes.	Report to HSE by April 1991	Assistance and review.
Incorporation of attributes into decision-process (methodology).		Report on "Risk Aversion" (Dec 1990). Report on "Irreversibility" (Dec 1991)
Demonstrate use.	Report to HSE by April 1992	Assistance with French case studies.

IV. Progress achieved including publications

This project is undertaken in collaboration with CEPN and a meeting was held in June 1990 to discuss the work programme. The work was provisionally divided into the four steps indicated in Table 1. These steps are reflected in the objectives detailed in Section 11.

The steps are rather arbitrary and it has not been sensible to consider them in isolation, so that, while the work performed conforms to the basic pattern set-out in Table 1 and Section 11, it inevitably overlaps several of the objectives.

A review of approaches to decision-making in radiological protection in an ALARA context has been undertaken. This has concentrated on ICRP's concept of ALARA and its implementation, the British concept of ALARP and its implementation, the approach of the Nuclear Installations Inspectorate and HSE, Public Inquiries into the granting of consent for nuclear power stations at Sizewell and Hinkley Point, and the general approach adopted by the nuclear industry in Britain to implement ALARP.

A tentative decision framework has been constructed which takes account of the recent work of ICRP and NRPB.

Various decision-aiding techniques have been described and their rational and relative merits discussed. The way in which factors are quantified and included in the various decision-aiding techniques has been described and discussed.

Work has begun on the quantification of detriment from probabilistic exposure.

Publications covering work of reporting period

None.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-F-299-P

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1. Dose assessment and quality assurance in diagnostic radiology.

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1. Dose assessment and quality assurance in diagnostic radiology.

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Dose assessment and quality assurance in diagnostic radiology

Head(s) of project:

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I. Objectives of the project:

1. Dose assessment and quality assurance in mammography, dental radiography and computer tomography.

2. Population dose assessment in radiodiagnostic in Portugal (NEXT Programme).

II. Objectives for the reporting period:

III. Progress achieved:

1. - Dose assessment and quality assurance in mammography, dental radiography and computer tomography

1.1 - Dose assessment and quality assurance in mammography

1.1.1 - Nationwide survey

A survey of mammographic centres was carried out in Portugal during 1988/89 covering 48 of the operational X-rays units (94%).

Assessment of image quality and patient doses were the main objective of this study. In each participant centre, statistical and technical information was collected concerned with the frequency of examinations, X-rays units, imaging systems, film processors and image viewing conditions.

Patient doses were estimated from exposure measurements carried out with TLD dosemeters on a 4 cm PMMA phantom with radiographic parameters in routine usage at every mammographic centre for a cranio caudal projection of a 5 cm average breast. In the same conditions image quality was assessed using appropriate phantoms and resolution patterns.

Results and conclusions

Equipment in use for mammography had, in general, up to date technical specifications, which fulfil those recommended in ECC document "Quality Criteria for Diagnostic Radiographic Images" (1). The survey shows that 90% of X-rays equipment are dedicated units. 96% of the tubes had Mo anodes and 56% were using moving grids. However defective and uncalibrated devices were frequently detected, and this ocurred with AEC units in 31% of the X-ray equipments.

Screen-film combinations and automatic film processors were beeing used in 100% of the participant centres. However only 15% were equiped with film processors exclusively dedicated to mammography.

Film processing is being carried out in many centres with large deviation from standard conditions as can be seen on Table 1.

Table 1

FILM PROCESSING

automa.film processing		
processing time (min)	.....	1.5 - 3.5
developing temperature	mean (° C)	34
	range.....	29 - 38
development control		
optical density of base + fog		
	mean	0.17
	range ...	0.12 - 0.24
speed index (ref.index 0.98)		
	mean	0.81
	range ...	0.20 - 1.40

Image viewing conditions were checked in every centre in what was concerned with ilumination level on viewing boxes, additional spotlight and viewing room and the measurements show that they were only acceptable in 35% of the centres.

Image quality was evaluated for a simulated cranio caudal projection with radiographic parameters in routine usage at every mammographic centre for a 5 cm average compressed breast. Results shown on Talle 2 are in general acceptable with mean values similar to values published in others european countries.

Table 2

IMAGE QUALITY

tube voltage (kV panel indication)	
mean	29
range	25 - 37
contrast at the top surface (OD)	
mean	0.31
range	0.08 - 0.65
film optical density (OD)	
mean	1.41
range	0.45 - 2.60
resolution in sternum-nipple direction(lp/mm)	
mean	10.6
range	6.0 - 15.0
resolution in transverse direction	
>10 lp/mm (%)	96
detectability of details	
round details <3 mm (%)	98
micro-calcifications < 0.2 mm (%)	79

The median glandular dose per image in participant centres was estimated as 1.1 mGy per image (mean was 1.5 mGy).
The corresponding values for centres with dedicated stands using nil grids was 0.8 mGy, with stationary grids 1.0 mGy, with moving grids 2.6 mGy. Entrance surface dose per image was calculated as 8.0 mGy (mean value) which is lower than the limit value of 10 mGy recommended by EEC (1).

Table 3

DOSES AT MAMMOGRAPHIC CENTRES

	(mGy)
entrance surface dose/image	
mean	6.0
median	4.0
range	0.29 - 23
exit dose/image	
mean	0.13
median	0.09
range	0.02 - 0.34
average glandular dose/image	
mean	1.5
median	1.1
range	0.10 - 5.3

The annual frequency of mammographies in the country was estimated in 16.5 per thousand inhabitants and in 3.4 mammographies per 100 X-rays examinations.

Taking into account statistical information on the frequency of examinations and the mean dose per image in every centre it was estimated the mean patient dose per image for the country as 1.4 mGy and the collective breast dose equivalent as 50 person-Sv per million inhabitants (Table 4).

Table 4

patient breast dose /image.....	1.4 mGy	PATIENT AND POPULATION
patient dose/breast.....	3.0 mGy	
collective breast dose equivalent per 10^6 inhabitants.....	50 person-sievert	DOSES FROM MAMMOGRAPHY

Glandular doses estimated in this survey were similar with others evaluated in others EEC countries. Doses values were strongly influenced by technical procedures whence the implementation of quality control will optimize mammographic systems

1.1.2 - Quality Control

A quality control programme was established in 10 mammographic centres, all over the country in hospitals, clinics and private offices, to be operated during 8 months. The programme was intended to evaluate the consistancy of the mammographic systems performance, checking periodically several equipment components that are crucial elements for image quality and patient doses. Participants were selected to represent whole types of technical capabilities in routine usage in the country.

The quality control programme was designed with the following features: (i) weekly the radiographer of the participant centre checked film processor by sensitometry and obtained a radiography of a LNETI phantom to test the automatic exposure control (AEC), radiation exposure and image quality; (ii) once in each three months a LNETI staff team visited every participant centre to more extensive and precise checking and to discuss with the radiologist improvements of the mammographic system to increase image quality and whenever possible, to reduce patient doses; (iii) staff time required from each participant centre was reduced to 15 min./week of a radiographist time and 1 h per 3 months of a radiologist time; (iv) equipment available at each participant centre to operate the programme was the LNETI phantom; (v) sensitometric film strips were exposed and analised as well as phantom radiographies at the control laboratory of LNETI; (vi) all the procedures were standardized and all the centres measurements and evaluations were compared with those obtained previously during the survey.

1.2 - Dose assessment and quality assurance in dental radiography

A national survey (1989-1990) was carried out in intraoral dental radiography all over the country in hospitals and private offices to evaluate patient doses, X-ray units performance, film processing and image quality.

The field study included about 250 dental X-ray units and was carried out by post using two sets of questionaries and devices for each X-ray unit. Through questionaries information was collected concerning X-rays units (manufacture , model, year of acquisition, cone length, etc.), number of radiographies performed per month, film used (manufacturer, type) and film processing. One of the devices included 6 TLD rods, aluminium filters, one sheet of radiographic film and was designed to measure entrance dose, HVL (half-value-layer), tube filtration and radiation field size. The other device included two dental films, one of them exposed on a dental phantom with a known entrance dose, to be processed in the participating installation, the other film would be exposed in the installation but developed in our laboratory under standard conditions, being this device designed to evaluate the film processing and image quality.

Results and conclusions

One thousand conventional units for intraoral radiography were estimated to be in operation all over the country and about 25% of them were checked during this study.

The age distribution of X-ray apparatus presents a mean of 8 years, having 70.6% of them less than 10 years.

Safety technical specifications are summarized on Table 5, where it can be seen that 48.9% of X-ray units have a cone lengh not greater than 10 cm, 72.6% a cable switch length greater than 1.5 m, 83.7% an electronic timer and 55% a radiation field size at cone extremity larger than 6 cm.

Table 5 - CHARACTERISTICS OF X-RAY EQUIPMENT SURVEYED.

Age	8 y (mean)		≤ 10 y	70.6 %
			≤ 5 y	49.4 %
Cone length	15.2 cm (mean)		≤ 10 cm	48.9 %
			]10 , 20] cm	40.0 %
			]20 , 30] cm	5.19 %
Field size	6.3 cm (mean)			
	] 4.7 , 9.3] cm		≤ 6 cm	45 %
Exposure switch	– cable	92.2 %	(>1.5 m	72.5 %)
	– temporizer	40.1 %		
Timer	– mechanical	16.3 %		
	– electronic	83.7 %		

The consistency of the sensitometric process with films processed until 1 month after exposure were satisfactory tested.

Results and Conclusions

Due to a large spread of technical capabilities and staff keen interest of participant centres, results could not be present in a global or statistical form. Results from each centre should be analized separately and recommendations would be applicable only to every centre.

As an example Figure 1 present some results of a mammographic centre located in a private office of radiology using a dedicated X-ray unit (with Mo anode tube, moving grid and AEC), appropriate screen-film combination and the automatic film processor was used also to process conventional radiographies.

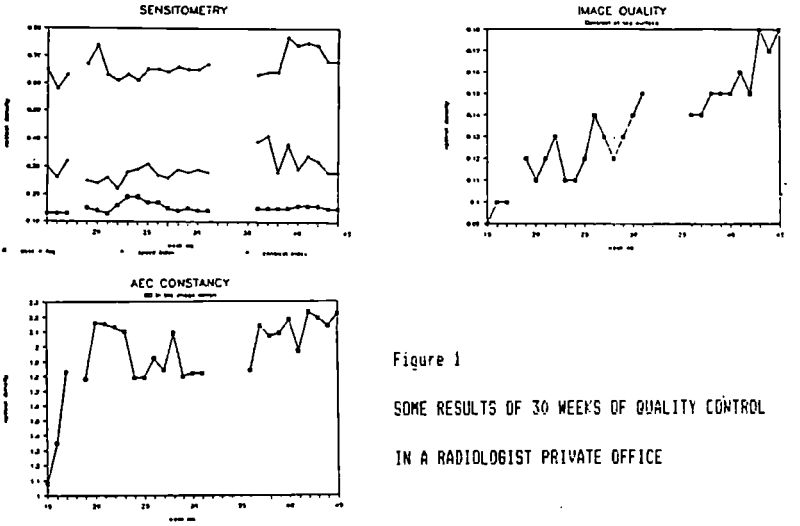

Figure 1

SOME RESULTS OF 30 WEEKS OF QUALITY CONTROL

IN A RADIOLOGIST PRIVATE OFFICE

In this centre, during the programme, image quality was improved with increase on image contrast and keeping nearly constant the patient glandular dose.

General conclusions of the programme were the following:

- a simplified quality control programme can contribute significantly to the optimization of mammographic systems;
- the availability of a dedicated film processor is a crucial element on the improvement of programme results;
- participation on quality control of specialists from outside of mammographic centre contribute to the sucess of the programme.

In at least 95% of the installations the film used is of type D speed and film processing is being carried out manualy in 69.1% of premises and in 56% of them without control of development time and temperature as is shown on Table 6.

The frequency of intra-oral radiography was evaluated in the survey in 77 films per month per X ray unit. A total of 845 X 10^3 films were estimated to have been performed in 1989.

Type	automatic	39.8 %
	manual	69.1 %
	both	8.9 %
Control	by sight	56.0 %
	time/temperature	48.6 %
	both	4.6 %

Table 6 - FILM PROCESSING.

Entrance doses were estimated from TLD measurements exposed in identical conditions to those used for an upper molar tooth radiography. Statistical data of technical factors used for that type of dental radiography are listed in Table 7 as well as the correspondent calculated values for tube filtration and half-value-layer (HVL) and in Figure 2 the distribution of X-ray units per dose interval.

The mean value for entrance dose for a molar tooth radiography was calculated to be 9.2 mGy,while median value was 6.3 mGy. This value is slightly higher than the expected entrance dose for a film speed D that was estimated in 4.35 mGy according to Adams (2) and for the tube potential distribution observed in the survey.

Table 7 - PHYSICAL PARAMETERS AND ENTRANCE DOSE ON A MOLAR TOOTH RADIOGRAPHY.

	sample average	median	lower quartile	upper quartile	minimum value	maximum value
Tube potential (kV_p)	59.5	60	50	65	46	75
Current x time (mA s)	11.2	6.2	4.5	8.0	0.30	88
Entrance dose (mGy)	9.2	6.3	4.4	13	0.66	48
H.V.L. (mm Al)	1.8	1.7	1.4	2.1	0.50	3.7
Filtration (mm Al)	2.0	2.0	1.6	2.5	0.90	3.0

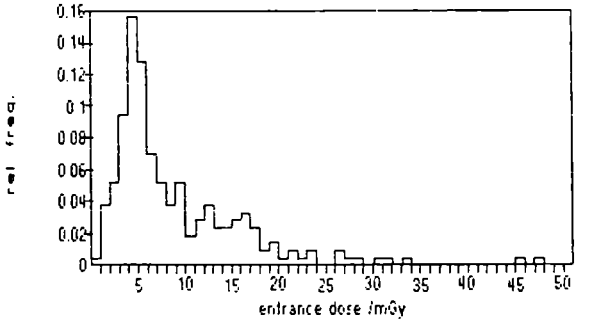

Figure 2 - DISTRIBUTION OF X-RAY UNITS per DOSE INTERVAL

2.3 - Dose assessment and quality assurance in computer tomography

A survey of CT installations was carried out in 1990 all over the country in hospitals, clinics and private offices.

CT scanners performance and image quality were assessed with a RMI-463 phantom for scanning parameters used for routine head examination. With the same scanning parameters, peak doses were evaluated in surface and centre of a PMMA head phantom with 16.5 cm diameter.

Doses free-in-air were measured per single scan on the axis of rotation with the scanning parameters used at every CT installation for routine examination of head, chest, abdomen and pelvis. These measurements were carried out with an array of 12 TLD's inside a PMMA capsule.

Results and conclusions

Statistical and technical data were collected in 36 CT scanners (90% of the operational units) participating on voluntary basis.

The majority of scanners (78%) have an age lower than 5 years, they were in average available for operation 71 hour/week (30 to 168 h/week), performing in average 112 examinations/week (25 to 300 exam./week) with a rate of retakes nearly nill.

Scanners maintenance and quality control were assured by manufacturer representatives in 97% of the installations with the following periodicity: 14% less than 1 month; 66% monthly; 20% more than 1 month.

Performance tests were carried out in every scanner unit: accuray of gantry position was better than ± 2 mm in 70% of units, accuracy of slice thickness was better than ± 0.5 mm in 91% of units when compared with value set on machine console.

Image quality was evaluated through measurements of CT numbers in several materials (water, air, PMMA, bone), noise, high contrast resolution, and detectability of low contrast, with results shown on Table 8.

Table 8 IMAGE QUALITY EVALUATION

		mean	median	lower and higher values	
CT numbers	bone	1376	1400	1023	1532
	acrylic	126.5	125.6	113.2	164.8
	air	-996.7	-1000	-1024	-964.9
	water	-0.77	1.9	-95.9	12.6
noise		4.9	4.5	3.2	12.6
low contrast					
	CT no.(high)	94.5	94.1	85.2	109
	CT no.(low)	89.0	87.9	79.6	103
high contrast resolution		0.8 mm	1.0 mm	1.25 mm	1.5 mm
		21 %	38 %	38 %	3 %
detection of low contrast		3 mm	4 mm	5 mm	20 mm
		12 %	55 %	18 %	15 %

Results of image quality present good agreement, better than 80%, with values adopted by manufacturers, since no generally accepted recommendations exist actually.

Peak doses per head routine examination at every scanner were measured in a head phantom with median value of 50.6 mGy (on surface) and 42 mGy (on centre).

Doses free-in-air on the isocentre for a single scan are presented on Table 9 and theirs distributions on Fig.3.

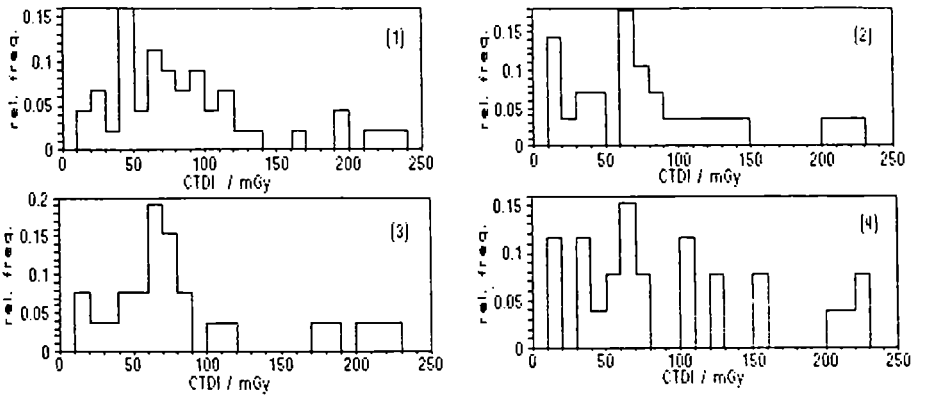

Fig. 3 - Distributions of CTDI values free-in-air on the isocentre for a single beam in routine head (1), thorax (2), abdomen (3) and pelvis (4) examinations.

Table 9 — CTDI values free-in-air on the isocentre for a single scan.

	dose (mGy)				
routine examination	mean	median	lower quartile	upper quartile	range
Head	67.1	71.4	47.6	108	19 – 231
Thorax	82.9	69.9	35.6	109	12 – 223
Abdomen	88.7	69.9	52.2	107	13 – 223
Pelvis	94.0	70.0	41.6	123	12 – 223

Mean doses estimated are higher than values published in others EEC countries. However dose range present closer values or even similar values (as routine head examination). This could be explained by a different distribution in Portugal of scanners by manufacturer and model, and it is also important to emphasize that the routine examination doses were obtained using scanning parameters pre-programmed by manufacturers.

Detailed results and analysis of this field study will be presented elsewhere.

2 - Population dose assessment

A survey of conventional radiodiagnostic installations was carried out in the country covering 75 premises considering the public hospitals, local and regional public health centers in a total of 175 X-ray tubes.

The survey was carried out by statistic sampling taking into account the number of examinations in the National Health Service, around 3 130 000 (3). A statistic approach was defined considering the film consumption in the different regions in the country. The representativeness of the sampling was statistically estimated to be around 97.5%, however it was take into account the remaining 2.5% in the data processed.

The methodology applied was based in the new NEXT (Nationwide Evaluation of X-ray Trends)programme developed by the American National Center for Devices and Radiological Health.

The information collected covers the premise data, tube and operator data and projection data. This information permited the evaluation of the physical parameters involved in each projection in the calculation of the organ doses. The projections studied were the chest, skull, abdomen, urography, thoracic spine, cervical spine, lumbo sacral spine, full spine, feet and dental (posterior and periapical).

Results and conclusions

Table 10 shows the average values of some technical parameters for all surveyed projections, while in Table 11 ranges of variations are shown.

The values presented in the above mentioned Table 10 and Table 11 can be compared with the data already recovered and published in Italy (4) and with the EEC document - "Quality Criteria for Diagnostic Radiographic Images".(1).

TABLE 10

Sample Average Values of Some Technical Parameters for All Surveyed Projections

Projection	Voltage kV	current. time mAc	HVL mm Al	Beam area/ film area	Source film dictance(cm)
Chest (PA)	76	16	2.4	1.66	163
Skull (L)	65	76	2.0	1.02	103
Abdomen (AP)	76	62	2.7	1.19	104
Urography (AP)	67	99	2.0	1.16	107
Thoracic spine (AP)	72	97	2.2	0.66	104
Cervical spine (AP)	59	48	1.9	1.20	107
Lumbo-sacral spine(AP)	76	90	2.2	0.85	104
Full spine (AP)	76	117	2.5	0.51	137
Feet	56	25	1.7	1.23	101
Dental (posterior)	62	--	1.5	3.34	27
Dental periapical	58	--	1.1	3.19	25

TABLE II

Ranges of Variation of Some Technical Parameters for All Surveyed Projections

Projection	Voltage kV	current. time mAs	HVL mm Al	Beam area/ film area	Source film distance(cm)
Chest (PA)	45-150	2-96	1.1-4.4	0.32-5.54	100-200
Skull (L)	52-85	20-300	1.2-3.2	0.04-1.86	85-140
Abdomen (AP)	60-96	16-150	1.6-4.3	0.42-3.12	90-120
Urography (AP)	47-84	3-200	1.3-4.1	0.43-2.14	90-130
Thoracic spine (AP)	63-90	74-160	1.3-3.0	0.42-0.93	100-115
Cervical spine (AP)	45-66	6-96	1.2-2.7	0.16-3.30	94-150
Lumbo-sacral spine(AP)	60-98	26-300	1.1-3.2	0.30-1.98	94-115
Full spine (AP)	70-85	24-200	1.5-3.1	0.12-0.87	100-180
Feet	40-70	1-100	0.9-2.8	0.46-4.55	90-115
Dental (posterior)	56-70	------	1.4-1.6	2.20-5.30	21-35
Dental periapical	50-65	------	0.8-1.5	1.64-7.22	16-44

As far as what concerns the "chest" (PA) projection, the used average voltage in Portugal (76 kV) is far lower then the recomended EEC's values (100 - 150 kV) and only 16% are within this range. Also the source to film distance (163 cm), although this figure is lower than the EEC's recomended value (180 cm) is within the acceptable range (140 - 200 cm). Comparing the mean values of the technical parameters for the chest projection with the same parameters of the cited italian study one can see that they are very similar (less than ± 20%) except for the beam area to film area ratio where the italian value is aproximately 30% greater than our value.

For the "skull" (L) projection the national mean voltage value is in the lower limit of the recomended EEC's values. In what concerns to the source to film distance recovered neverthless being lower than the recomended value it is witnin the recomended range of variation. For that projection poor radiological techniques procedures in Portugal were used.

Concerning the "abdomen" (AP) only one can compare the mean values of the technical parameters with the italian values because EEC's document don't refer that projection. Except for the voltage, the other parameters show differences greater than ± 20%.

For the "urography" (AP) and regarding the data collected the mean voltage is lower than the EEC's recomended values and the source to film distance besides being lower than the recommended value is in the suggested range of variation.

For the "lumbo-sacral spine" (AP) the voltage recovered is in the recommended EEC's range of variation and the source to film distance is lower than the recommended EEC's value. In comparison with the italian values it can be seen a very good agreement (less than ± 20%) except for the beam area to the film area ratio where the italian value is about 40% greater than our value.

Regarding the ranges of variation one can see that wider intervals have been obtained in our survey mainly in the upper limits.

In Table 12 and 13 it is shown the average skin entrance exposure and the ranges of variation for all surveyed projections. The mean values obtained in the cited italian study are higher than our study except for the skull where the value 86 μC kg-1 is really very low compared with the value 213 μC kg-1. The intervals of variation are very large in the two studies and for all projections.

The organ doses calculated through the computer code are shown in Table 12 and Table 13. The organ doses presented show for the projections abdomen and lumbo-sacral spine lower values except for the chest projection where there is a very good agreement with the italian values.

TABLE 12

Weighted Average Skin Entrance Exposure (μC.kg-1) and Doses (μGy) to Some Patient Organs for All Surveyed Projections

Projection	Skin entrance exposure	Whole body	Thyroid	Lung	Bone marrow	Ovarian	Testicular
Chest (PA)	9.2	47	11	134	35	<5	<5
Skull (L)	213.7	129	591	7	111	<5	<5
Abdomen (AP)	134.9	528	<5	82	211	1178	111
Urography (AP)	155.2	425	<5	99	129	824	214
Thoracic spine (AP)	291.5	219	496	374	84	<5	<5
Cervical spine (AP)	85.5	88	1699	78	32	<5	<5
Lumbo-sacral spine (AP)	174.9	261	<5	33	81	698	65
Full spine (AP)	140.0	477	640	1411	174	194	<5
Feet	18.3	*	*	*	*	*	*
Dental (posterior)	47.9	*	*	*	*	*	*
Dental periapical	236.5	*	*	*	*	*	*

* Negligible values

TABLE 13

Ranges of Variation of Skin Entrance Exposure (μC.kg-1) and Some Organ Doses (μGy) for All Surveyed Projections

Projection	Skin entrance exposure	Whole body	Thyroid	Lung	Bone marrow	Ovarian	Testicul
Chest (PA)	1.8-93.9	<5-620	<5-150	10-1930	<5-480	<5-40	<5
Skull (L)	14.4-618.4	<5-270	<5-1470	<5-30	<5-220	<5	<5
Abdomen (AP)	17.8-308.3	50-1260	<5	<5-360	20-440	130-2370	<5-1100
Urography (AP)	17.0-696.3	30-1820	<5	<5-500	10-620	100-3440	<5-2240
Thoracic spine (AP)	68.6-469.6	70-1210	20-5140	160-3960	30-490	<5-30	<5
Cervical spine (AP)	11.9-412.3	10-460	340-11830	<5-640	<5-190	<5	<5
Lumbo-sacral spine (AP)	40.5-682.1	50-1420	<5	<5-270	10-410	180-2230	<5-370
Full spine (AP)	21.7-315.3	60-720	<5-1640	170-2340	30-260	<5-450	<5-10
Feet	1.0-78.9	*	*	*	*	*	*
Dental (posterior)	25.5-53.7	*	*	*	*	*	*
Dental periapical	56.0-518.1	*	*	*	*	*	*

* Negligible values

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V. Publications:

- Carvalho, A.F.; Rocha, M.P.; Alves, J.G.; Carreiro, J.V.; Galvão, J.F., 1990, RADIATION DOSES IN MAMMOGRAPHY, presented in the Workshop on Statistics of Human Exposure to Ionizing Radiation, Oxford, April (to be published in Rad. Prot. Dosimetry).

- Carvalho, A.F.; Oliveira, A.D; Amaral, E.M.; Carreiro, J.V.; Galvão, J.P., 1991, DENTAL RADIOGRAPHIC EXPOSURES IN PORTUGAL (to be presented in the Seminar on Dosimetry in Diagnostic Radiology, Luxembourg, March).

- Carvalho, A.F.; Oliveira, A.D.; Amaral, E.M.; Carreiro, J.V; Galvão, J.F., 1991, ASSESSMENT OF PATIENT DOSES AND IMAGE QUALITY IN COMPUTED TOMOGRAPHY (to be presented on invited paper in the Seminar on Dosimetry in Diagnostic Radiology, Luxembourg, March).

- Carvalho, A.F.; Rocha, M.P.; Alves, J.G.; Carreiro, J.V.; Galvão, J.F., 1991, MAMMOGRAPHIC DOSES AND IMAGE QUALITY IN PORTUGAL (to be submitted to The Brit. J. of Radiology).

- Serro, R.; Carreiro, J.V.; Galvão, J.F.; Reis, R., 1991, POPULATION DOSE ASSESSMENT ON RADIODIAGNOSTIC IN PORTUGAL (to be presented in the Seminar on Dosimetry in Diagnostic Radiology, Luxembourg, March).

The weighted average dose values per projection and for the different organs obtained during the survey allowed to estimate the whole body per caput on 530 μGy considering to be 696 the number of examinations per 1000 inhabitants and 2.3 the mean number of films per examination. These figures were calculated based on the total films consumption, of 7 109 000 in 1986, in the total number of portuguese population (10.2 million) and assuming that the private sector for diagnostic radiology in Portugal is 56% of the country workload.

From the gonadal organ doses above mentioned it was estimated on 200 μGy the genetic significant dose (GSD). This calculation was based on the mean value of the relative frequency (25% for females and 35% for males) of the radiological examinations performed by portuguese population below the mean age of child bearing since there is not available in the country the gonadal dose for each type of examinations stratified per age group or sex.

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RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-F-317-DK

Aarhus University Hospital
Nørrebrogade 44
DK-8000 Aarhus C

Head(s) of research team(s) [name(s) and address(es)]:

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Title of the research contract:

The impact from quality assurance on dose reduction in computerized tomography in Denmark.

List of projects:

1. The impact from quality assurance on dose reduction in computerized tomography in Denmark.

Title of the project no.:

The impact from quality assurance on dose reduction in computerized tomography in Denmark

Head(s) of project:

Dr. K. A. Jessen

Scientific staff:

K.A.Jessen, J.Juul Christensen, J.Jørgensen, J.Petersen and E.W.Sørensen

I. Objectives of the project:

An assessment of the collective effective dose equivalent from computerized tomography (CT) imaging techniques to the whole Danish population investigated by a complete periodically registration of the use of CT examinations.
Evaluating the relation between the usage of dose and the scanner performance as a base for formulating quality criteria for CT images and for optimizing the use of CT.

II. Objectives for the reporting period:

Questinnaires adapted from the running programme are sent to all CT installations in Denmark to obtain data on frequency and scanning parameters for all examinations for a given period. The associated quality assurance performed are also registered together with dose measurements for the most commonly used sets of parameters. This information allow calculation of collective effective dose equivalent from CT in Denmark. Site visits are planned for performing identical quality control measurements of physical image parameters on standard phantoms as a base for future proposals of quality criteria for CT examinations.

1. METHODOLOGY

A nationwide survey has been conducted in Denmark to establish frequency and technical parameters adapted in each type of CT examination. Questionnaires were sent to all the 24 facilities in operation primo 1989. They are all located in diagnostic departments in hospitals under the Danish Public Health Care System. Relevant information including kilovoltage, mAs, number of slices, slice thickness, couch increment, the use of i.v. contrast and the duration of the examinations were collected. The questionnaires have been used for two periods, each of three weeks in order to obtain a valid documentation by extrapolation of the actual use of CT in the year 1989 in Denmark.

Dose measurements with TL dosimeter chips have been collected by a postal service. Measurements of doses free-in-air at the axis of rotation for a single slice for the most commonly used sets of scanning parameters have been performed in accordance with the method used by other contractors, giving the axial dose profile and the Computed Tomography Dose Index (CTDI)

$$CTDI = \frac{1}{T} \int_{i=1}^{i=n} D_i \cdot t$$

where D_i is the dose in i'th TL chip, t the thickness and n the number of the TL chips and T the nominal slice thickness.

Dose measurements free-in-air and in phantoms have also been performed for the same scanning parameters with a pencil shaped ionization chamber giving alternative and independent dose measurements. Measurements on the surface of the phantom with the ionization chamber give the Multiple Scan Average Dose (MSAD) - i.e., the average dose across the central slices from several contiguous slices.

In order to obtain information about the beam quality on the central axis, some dosemeters have been covered by 5 mm Al for filtration. To get information on the pre-patient filtration, dose profiles in a phantom have been measured.

Results from the dose measurements were matched with data from the examinations performed during the survey and extrapolated for the whole of 1989. Conversion factors given by GSF (ref. GSF-Bericht S-1026, 1985) relating organ doses to the free-in-air dose on the axis of rotation of the scanner, the CTDI value measured by TLD and/or ionization chamber, were used for calculation of organ doses and the collective Effective Dose Equivalent (EDE) from CT in Denmark. Assessment of the collective EDE from conventional X-ray examinations has been performed in order to determine the relative impact from CT.

A site visit to 92% of the systems has been performed to carry out identical dose and quality control measurements of physical image parameters (especially noise and resolution) using phantoms for normal sets of scanning parameters. From systems where the digital image could be exported in a suitable format, the edge response function between water and perspex was calculated. These results, together with the surface dose (MSAD) provide enough information to calculate the Q value defined by

$$Q = (1000/(R^3 \cdot Z \cdot D \cdot S^2))^{1/4} \ mm^{-2} \ Gy^{-1/4}$$

where R is the spatial resolution in mm, Z the nominal slice width in mm, D the MSAD surface dose and S the normalized standard deviation. The higher Q values imply higher image quality.
A special test on the linearity of CT numbers for a given system was performed when the size, shape and position of the object in the gantry aperture varied. This test is important for the judgement of the suitability of a system for quantitative use of image information. For example as used for tissue characteristics, bone densitometry and CT based dose planning in radiotherapy.

2. RESULTS AND DISCUSSIONS

A. The survey on CT examinations

The total number of reported CT examinations in Denmark in 1989 was 72.452, which give 14.5 exam./1000 inhabitants. In the survey the examinations were divided into 29 different types. These types were further aggregated to form 3 larger groups: Spine, Body plus Head and Neck.

Examination type	Percent	Group	Percent
Columna lumbalis	7.8	Spine	10.4
Columna cervicalis	2.1		
Columna thoracalis	0.5		
Abdomen	13.8	Body	26.6
Thorax / Axil	6.3		
Pelvis	4.1		
Hepar/kidney/Gall./Pancreas	1.1		
Arms/legs	1.3		
Skull/Brain	57.5	Head and Neck	61.6
Neck	1.2		
Orbita	1.1		
Ear/Parotis/Sella turcica	1.8		
Unclassified	1.4		1.4
Total	100.0	Total	100.0

Table 1.The distribution of the main types of CT examinations
(Examinations representing less than 1% are combined)

Nearly two third of all examinations are still neuroradiological and about a quarter are body scans. Many of the examinations are performed with standard procedures, with pre-coded programs containing different sets of scan parameters. Standard procedures may be necessary for a department in order to handle a large number of patients, but constructors of these programs have to be very careful that the procedures are optimized for the intended use.

The Danish Health Care hospitals are classified in 2 categories: University hospitals and County hospitals. A University hospital performs specialized functions for a whole province or the country. The County hospital has only service of the local area or region.

Hospital category	No.of units	Total examinations percent	Exams/unit /year	% of examinations			
				Body	Head/Neck	Spine	Others
University	15	65.7	3174	34.5	58.7	5.3	1.5
County	9	34.3	2760	16.0	65.5	17.3	1.2

Table 2.

In Table 2 the number of units, the distribution of total examinations, the total mean workload per unit for 1989 and the distribution of examinations into the four groups for both category of hospitals are given. The units does not have the same capacity or age, furthermore there are 3 brain scanners all located at University hospitals. The head and neck group clearly dominate examinations at the County hospitals.

The median number of slices, slice thickness and mAs per examination for the spine, body and the skull and brain examinations are given in Table 3 for all units together. 8 mm slice thickness dominate in the body group because 57% of all wholebody units are SOMATOM's.

Group	Number of slices/examination			Slice thickness			mAs used in examination		
	1 Quartile	Median	3 Quartile	1 Quartile	Median	3 Quartile	1 Quartile	Median	3 Quartile
Spine	20.0	25.0	31.0	2.0	3.0	4.0	460.0	480.0	520.0
Body	20.0	28.0	39.0	8.0	8.0	9.0	350.0	350.0	350.0
Skull/ Brain	9.0	12.0	16.0	4.0	8.0	10.0	450.0	460.0	520.0

Table 3.

From the survey it can further be concluded, that in two third of the examinations only one slice thickness is used. However, especially in the head and neck group, more than one slice thickness may be used. In 28% of all the examinations i.v. contrast was used. 80% of these i.v. examinations fall in the head/neck group.

B. Dosimetric investigations in computed tomography

Dose profiles for a single slice at the centre of rotation have been measured at 21 units (88%). A total of 200 profiles were obtained for different conditions of irradiation. As seen by other contractors the measured profiles are significantly wider than the nominal slice width. Especially for narrow slices, there will be an overlap of the dose profiles for series of nominally contiguous slices. Therefore an enhancement of the dose will result in adjacent slices due to construction of the collimating system or misadjustments. The beam is, in some systems, collimated after the patient and in front of the detector for the very narrow slices. All TL dosimeter chips used in this investigation have been calibrated individually before and after use. They have a thickness of 0.8 mm each. As an example of some of the main results of this dosimetric survey, which are going to be published elsewhere, some figures are presented in Table 4.

Number of units	Nominal slice thickness mm	Measured FWHM mm	CTDI mGy	Corrected "CTDI" mGy	CTDI/mAs mGy/mAs	"CTDI"/mAs mGy/mAs	Max Value mGy	f_e= CTDI/Max	I. C. *) mGy
3	1	2.5	110.3	44.6	0.212	0.086	41.1	2.69	101.9
3	2	2.5	66.0	53.3	0.127	0.101	47.0	1.40	53.5
4	4	4.3	47.6	44.1	0.092	0.085	38.1	1.25	44.6
4	8	8.6	37.3	34.2	0.110	0.101	32.6	1.14	34.4

*) not measured for all units

Table 4

The results are mean values obtained under similar conditions for 3-4 units. The measured slice thickness as Full Width Half Maximum (FWHM) of the dose profiles illustrate the problems with thin slices. Therefore CTDI values based on nominal slice thickness deviate considerably. If the measured FWHM values are used in this calculation, similar values for the corrected CTDI's are obtained for different nominal slice thicknesses. The same result is seen for CTDI/mAs values. The enhancement factor f_e is defined as the ratio between the CTDI value and the maximum dose value for a single slice obtained from the profile distribution. The last column gives the results from the free-in-air measurements with the ionization chamber for some of the units. There is a reasonable agreement with the calculated CTDI values for these independent dosimetric measurements.

Radial dose profile measurements in a phantom clearly divide the units into three groups depending on the pre-patient filtration. In Table 5 the ratio between the dose to the center and to the surface of a phantom of 22 cm diameter measured with the ionization chamber is given. The SOMATOM systems have a flat filter and the GE9800 the most shaped filter. In order to determine the beam quality on the central axis of rotation, doses were measured with an ionization chamber free-in-air and covered with 5 mm Al. These ratios are also given in Table 5 and indicate similar filtration for the SOMATOM and TOMOSCAN systems at the center. The GE9800 however is different, with a more pronounced shaped filter at the edge but has softer radiation at the center. This may be investigated further in order to perform a beam quality corrected calibration of the TL dosimeter chips.

CT scanner model	No. of units	Surface/center ratio	IC / IC + 5 mm Al
Siemens Somatom	9	1.61 +/- 0.05	1.24 +/- 0.06
Philips Tomoscan	3	1.38 +/- 0.06	1.25 +/- 0.02
General Electric 9800	2	1.25 +/- 0.01	1.58 +/- 0.04

Table 5

C. Organ doses and effective dose equivalent

A selection of some of the calculated average organ doses for radiation sensitive organs are shown in Table 6 for the most frequently used examination types, averaged over sex. The numbers in brackets are the organ contribution to the EDE taking the new weight factors from ICRP (draft 90) into account.

Organ	Examination			
	Thorax	Abdomen	Skull	Pelvis
Eyelenses			41.5	
Thyroid	5.6 (2)		24.2 (33)	
Breast	16.2 (11)	2.9 (1)		
Lungs	34.0 (62)	10.3 (12)	1.2 (11)	1.3
Stomach wall	3.7 (8)	45.0 (62)		1.3 (4)
Colon		20.7 (16)		28.0 (51)
Gonads		1.3 (1)		10.3 (27)
Red bone marrow	8.3 (14)	6.3 (7)	5.1 (42)	6.4 (16)
Bone	23.5 (3)	16.1 (1)	13.5 (9)	6.9 (1)

Table 6. Average organ doses (mGy) and % contribution to the EDE (in brackets).

Examination	EDE mSv	Collective EDE man Sv
Hepar/Spleen/Gall-Bladder	22.1	6.1
Sacrum	17.5	
Abdomen	11.7	127.0
Kidney	10.3	4.4
Pancreas	8.8	
Thorax/Axil	7.2	28.9
Upper Arm	6.3	
Columna Thoracalis	5.9	
Columna Spinalis/Lumbalis	5.4	29.1
Pelvis	4.9	14.0
Hip	4.4	
Sternum	2.2	
Scull	1.4	56.2
Parotis/Sinus/Maxil	1.1	
Sella Turcica	1.1	

Table 7 gives the average EDE for a subset of the examination types recorded and the average EDE by examination types.

The EDE per examination are listed in Table 7 together with the collective EDE for the seven largest contributing examination types. As seen from Table 7 more than 40% of the collective EDE comes from examinations of the abdomen. The collective EDE from more than 72.000 CT scans performed in Denmark in 1989 has been evaluated to be 300 man Sv giving 0.06 mSv collective EDE per caput per year or 6-10% of the collective EDE from conventional X-ray examinations.

Compared to EDE values from conventional X-ray examinations found in the literature (Maccia et al. Health Physics 54 (1988)) the corresponding EDE values from the most commonly used CT examinations is a factor of one to six times greater. This is half the order of magnitude often used in the literature, which can be explained by the fact that CT has overtaken the most complex examination types. In the last decade (1979-1989) the number of conventional X-ray examinations in Denmark has remained almost constant at 2.5 millions (500 exam./1000 inhabitants), while the number of CT examinations has grown almost linearly from 14.500 (1979) to 72.500 (1989) - in 1989 3% of all X-ray examinations. Assuming each CT examination has replaced a corresponding conventional X-ray examination, introduction of CT has increased the collective EDE by approximately 200 man Sv per year (1989) or 0.04 mSv collective EDE per caput.

D. Quality assurance in computed tomography

In all, 18 of the 24 installations responded to the inquiry concerning quality assurance of 15 models from 5 different firms. The primary responsibility for the QA program rested chiefly on the manufacturer's representatives (70%) rather than on the facility staff (12%). Only one fifth of the departments have access to local technical/physical expertise. 45% of the systems are under full service contract and 45% have a part contract. A surprisingly high number of the facilities do not perform full QA testing as indicated in the Table 8. Many systems are only controlled monthly as a consequence of the typical regular service one day every month.

Frequency % / Parameter	Not done	Daily	Weekly	Monthly	As needed	Other
Sensitivity	17	0	0	44	22	17
Reproducibility	11	17	11	56	0	5
Contrast Scale	17	6	0	39	33	5
Noise	0	17	6	67	5	5
Resolution	22	0	0	28	39	11
Uniformity	0	17	0	67	5	11
Alignement	17	0	0	39	44	0

Table 8

The spread in Q values for a given system when varying only the reconstruction algorithm were comparable with the spread of Q values between similar CT systems obtained with similar scanning parameters.

Conditions	Q(mm^{-2} $Gy^{-1/2}$)
1 unit /10 algorithms	1.97 ± 0.43
6 units/ 1 algorithm	2.00 ± 0.59

Table 9

This indicates that the Q values should not be considered as the most suitable parameter for judgement of image quality. Comparisons between systems are only possible when identical phantoms and data analysing procedures are used together with comparable scanning parameters and reconstruction algorithms. Such tests are however essential when accepting a new unit in the department and for more detailed follow up of the unit after major changes such as exchange of X-ray tube or detector arc, collimator adjustments or introduction of new reconstruction software.

The image quality measurements have shown that the high EDE seen for certain systems in some of the examinations is not optimal. F.ex. the GE9800 and the SOMATOM DRH have equally Q-values, but in the calculation of the EDE the value for GE9800 is a factor of 2 to more than 4 times higher for thorax, abdomen and kidney examinations. These represent more than 20% of all examinations. The calculations are based on conversion factors for CT scanners with a flat pre-patient filter which may slightly overestimate the doses from the GE9800, but not to the observed levels.

The dose dependent image parameters such as noise and resolution do not give valid information about the linearity and stability of CT numbers. Under the site visits special tests performed as reported in the literature(publ. 1,2) demonstrate great differences between systems. These tests underline the fact, that if a system is going to be used for quantitative CT the influence on CT numbers of size, shape and position in the gantry aperture of the object has to be investigated.

3. CONCLUSION

The number of CT examinations are still increasing and since this project was started in January 1989 5 new CT installations have been established in Denmark. Besides this an exchange of the older units to new faster units with higher capacity is now in progress. The relatively high dose contribution from CT is therefore increasing so reliable dose estimates are needed. The calculation of organ doses and of the collective EDE in this report are based on conversion factors for units with a flat pre-patient filter. Because more than two third of the scanners are of this type the approach used may not affect the results severely, but new calculations will be performed as soon as conversion factors for shaped filters are available to test this approximation. The accurate determination of the CTDI values also need to be investigated further.

One of the main conclusions is that for some examinations the doses seem to be considerably higher than can be justified by the demand for an acceptable image quality. It may simply be caused by the extended use of not optimized standard procedures. Therefore image quality criteria for CT examinations are urgently required similar to those recently set up by the CEC and now in use for some common conventional X-ray examinations.

The image quality depends on many parameters but primarly on dose which again depend on the scan parameters. In daily pratice it is essential that simple and quick tests of the CT images can be performed by the radiologist or technician. The image noise which depend on dose in a welknown fashion represents one of these parameters. A protocol for simple measurements should be established taking special requirements into consideration. These requirements typically depend on the contrast level within the actual anatomic region. It would be possible to perform such measurements at the start of each examination.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Barry Wall & Paul Shrimpton, NRPB, Chilton, U.K.
W.Panzer, GSF, Münich, FRG.
A. Ferro de Carvalho, LNETI, Portugal.

V. Publications:

1. K.A.Jessen and J.Jørgensen:
Quality control in quantitative computed tomography. BIR Report 18, British Institute of Radiology, London 1989, p.84.

2. K.A.Jessen and J.Jørgensen:
The influence of patient size and shape on absolute CT numbers for different scanner systems. BIR Report 20, British Institute of Radiology, London, 1989, p.158.

3. K.A.Jessen, J.Juul Christensen, J.Jørgensen and E.W.Sørensen:
Dosimetric Quality Control of CT scanners. (In preparation).

4. K.A.Jessen, J.Juul Christensen, J.Jørgensen, J.Petersen and E.W.Sørensen:
Report on computed tomography in Denmark. Quality assurance and patient doses. (In preparation).

Progress Report

Contract: Bi6-342 Sector: C22

Title: Establishment of a common protocol for the use of a whole-body counter.

1. Dr A. Schmitt-Hannig	Bundesamt für Strahlenschutz, Neuherberg
2. Dr C. Proukakis	University of Athens/School of Medicine, Athens
3. Dr V. Barbina	Centro di Recerca Appl. e Document., Udine
4. Dr J.D. Cunningham	Nuclear Energy Board, Dublin

1. Summary of Project and Objectives

One aim of the Commission's Radiation Protection Programme is to provide the scientific basis and the technical knowledge necessary for the development and improvement of procedures and instrumentation to determine internal radiation exposure by individual monitoring.

There are basically two methods for assessing the amount of radioactive substances in the whole body or in specific tissues: direct (in vivo) and indirect (in vitro) monitoring. Especially after the Chernobyl accident direct individual monitoring by means of whole-body counters was carried out in most European countries. In order to compare the results, calibration techniques and measurement procedures have to be equivalent and the same methods for interpreting the measured data, e.g. for assessing the dose, have to be used.

The CEC therefore asked the Institute of Radiation Hygiene of the Federal Office of Radiation Protection in the Federal Republic of Germany

1. to develop guidance for the calibration of whole-body counters and the interpretation of measured data and

2. to assist the staff of the Hippokration Hospital of the University of Athens in the implementation of calibration procedures.

Actions to achieve point 1:

For the development of guidance a Technical Working Group Meeting has been organized at the Institute of Radiation Hygiene in Neuherberg, in which, among others, experts from Ireland, Italy, Greece and Germany participated. The group discussed and reviewed a draft report on a common protocol for the calibration of whole body counters to be used in the medical area, for occupational monitoring, for monitoring of the population and for assessment of incorporated radioactive material after accidents and the interpretation of measured data. At the same time the Meeting served as a forum to exchange experience with different whole-body counting systems for a variety of applications as well as to discuss problems involved in the calibration of such systems.

As a result of the Meeting a final report was prepared. The report is a practical guide to the selection of suitable calibration techniques and to their implementation. The document also gives guidance on the interpretation of the whole-body counts in the light of international recommendations. There is so far no other guide on a national or international level combining the calibration procedures (measurements) and the sometimes rather high-sophisticated calculation methods (interpretation of measured results).

The Meeting participants found this type of meeting stimulating for their work and suggested that a WBC User Group should meet on a regular basis to exchange scientific and technical information on specific subjects.

The Meeting participants also felt it necessary to prepare a similar report on WBC calibration for specific organ counting (lung, thyroid).

The Meeting report could serve as input for the WBC intercomparison project which the CEC is going to initiate 92/93 (DG XI) and in which as many WBC systems in the countries of the European Communities as possible should participate.

The international Atomic Energy Agency is very interested in these activities. They sent comments on the draft report and would like to be informed on further actions.

There were also requests from Switzerland, Austria, Sweden and Finland, as well as from Portugal, Spain and the United Kingdom, asking for being involved in further activities in this field.

Actions to achieve point 2:

Mr König, who is in charge of WBC measurements at the Institute of Radiation Hygiene of the Federal Office of Radiation Protection, has been at the Hippokration Hospital from 23-27 June 1990. The situation he found was as follows.

The WBC used at Hippokration Hospital (type ACCUSCAN from CANBERRA with ABADOS II software) is a high-tech devise for incorporation monitoring of radiation workers. The system is designed for rapid and easy routine measurement of potentially incorporated radionuclides, i.e. detection of low activities. By means of the ABADOS II software the system is able to indicate whether the calculated dose is in compliance with the annual limits of intake or not.

The knowledge of the staff concerning the interaction of components like detector, spectrum, evaluation, dose calculation, is not profound enough to change the system configuration. To use this type of WBC for the detection of high activities (e.g. accident monitoring, nuclear medicine applications) major reconstruction work and different software would be necessary. In other words, the use of this type of high-tech equipment is rather limited and cannot be extended easily. For a wider range of applications a more simple type of machine and application-oriented self-made software would be better.

Besides this the phantom delivered by CANBERRA is only for quality control measurements and not for calibration purposes involving strongly deviating geometries. Also the use of radionuclides other than those of the supplied test sources will not be without problems.

It was proposed that the staff should be trained again by the supplier how to make better use of the hard and software components and that staff members should visit other WBC, especially in hospitals, in order to exchange scientific and technical experience.

Following these recommendations, Ms Louizi who is in charge of operating the WBC at the Hippokration Hospital in Athens has been in Germany for 2 scientific visits: 6-20 December 1990 and 27 January to 6 February 1991, the second time together with Mr Niagasas. They visited WBC installations in Munich, Neuherberg and Frankfurt and studied the different calibration methods which these institutions use for different applications in the medical area, for occupational monitoring, for monitoring of the population and for assessment of incorporated activities after accidents and discussed the interpretation of measured data.

Ms Louizi and Mr Niagasas participated in the Meeting on the Development of a Common Protocol for the Calibration of Whole-Body Counters, 30 January - 1 February 1991 in Neuherberg, where they had the opportunity to exchange experience with other European colleagues and to discuss their problems with Mr Bronson, the vice-president of CANBERRA and at the same time one of the best experts in the field worldwide, who was also invited to the meeting.

Associated laboratories:

- **Nuclear Medicine Department at the Hippokration Hospital, Athens (GR)**
  Head of Project: Prof. C. Proukakis

II. Objectives of the reporting period

During this period we performed:

1. Efficiency calibration of the whole body counter system using the Canberra RMC REMCAL TRANSFER PHANTOM;

2. Design study and construction of the shielding for the NaI detector unit, in order to achieve the required measurement accuracy, which will allow high Tl-201 activity measurements to be performed.

III. Objectives for the next period

Tl-201 and Tl-202 metabolism including some kinetics. The study will be carried out in patients requiring the Tl test for medical indications.

IV. Progress achieved including publications

A. Louizi, Ch. Proukakis, S. Simopoulos and M. Angelopoulos: Effect of Dietary Intake on the Cs-137 Retention Model (submitted for publication).

- **Centro di Ricerce Applicata e Documentazione, Udine (I)**
  Head of Project: Dr V. Barbina

II. Objectives for the reporting period

Organization of an Italian Group of WBC users, under the auspices of ENEA, whose work programs shall be consistent with CEC protocols and recommendations.

III. Objectives for next period

Preliminary calibration program (i.e. initially limited to radiation protection applications) of Italian WBCs, by means of a simple bottle phantom and uniformly-distributed radionuclides.

IV. Progress achieved including publications

At local level: Calibration of the WBC ND chair at CRAD, Udine; intercomparison within the analogues facilities of Genua and Bologna by means of the ND phantoms. At national level: First meeting of WBC responsibles at ENEA, Bologna, 21 May 1991: definition of overall objectives and close work programmes.

Nuclear Energy Board, Dublin (IRL)
Head of Project Dr J. Cunningham

II. Objectives for the reporting period

Preparation of draft list of guidelines for a Common Protocol on the Calibration of WBCs

III.

IV. Progress achieved including publications

The Technical Working Group Meeting organized by the Institute for Radiation Hygiene was instructive because it allowed us access to wide-ranging expertise in the area of whole-body phantoms and calibration techniques. It also gave us an opportunity to identify a number of areas of clinical application for our WBC.

Progress Report

Contract: Bi6-347g Sector: C22

Title: Reduction of patient exposure in medical diagnostic radiology. Dosimetry and risk (NRPB Association)

1	Wall	NRPB
2	Drexler	GSF Neuherberg
3	Kramer	PTB
4	Broerse	TNO-ITRI

I. Summary of Project and Global Objectives

All participants met at a contractors meeting in Brussels on 23 and 24 April 1990. Partners in other CEC co-ordinated contracts concerned with the reduction of patient doses were also present, since collaboration between the contracts was seen to be as important as collaboration within each contract.

Advice was provided by the co-ordinator to participants in other contracts some of whom visited NRPB to discuss how best to adopt a common dosimetric approach in their projects. Discussions were held with Prof Van Loon (Belgium), Dr Theissen (Netherlands), and with Drs Dance and Faulkner of the UK. Inter-calibration of TLDs was arranged with the latter.

Progress by the four participants in this contract was satisfactory despite one of them (TNO) not officially signing the Agreement until November. NRPB have provided dosimetric support to trials of the CEC quality criteria for diagnostic radiographic images and have completed organ dose calculations for CT examinations with a wide range of modern scanners. GSF have carried out detailed spectral measurements of x-ray beams passing through patient phantoms and antiscatter grids which provide essential information for establishing revised protocols for measuring film screen sensitivity. They have also made extensive Monte Carlo calculations of radiation spectra inside phantoms. PTB have assessed the performance of one out of a planned three AEC systems used in mammography and have identified an important effect of scattered radiation on AEC operation. TNO have calculated the large displacement correction factors for ionisation chambers used in mammography and will use them to compare measured and calculated depth dose distributions in breast phantoms.

Global Objectives

(i) Provision of patient dosimetry services and advice for the further development of the CEC "Quality Criteria for Diagnostic Radiographic Images".

Development of Monte Carlo programs for calculating organ doses from CT examinations and from conventional paediatric x-ray examinations.

Estimation of age and sex specific radiation risks, detriment and QALYs for x-ray patient.

(ii) Survey on the speed of film-screen combinations commonly used in x-ray diagnosis.

Construction of further realistic (voxel) phantoms for persons of different ages and sizes using CT data.

Calculation of organ doses from typical x-ray examinations using geometric (MIRD) and voxel phantoms.

Calculation of photon spectra inside tissue equivalent phantoms and their dependence on the position inside the phantom.

(iii) Assessment of the ability of automatic exposure control (AEC) systems in mammography x-ray units to produce a given wanted optical density of the exposed and developed film under a wide range of practically occurring conditions.

(iv) The dosimetric aspects of the project include investigations of absolute dosimetry, studies on monitoring of dose to the breast in actual mammography and dose specification. The technical aspects concern studies on determination of physical image quality as well as optimisation of technical conditions (eg, film/screen combination, film processing, use of antiscatter grids, tube voltage and automatic exposure control units) to achieve maximum physical image quality at minimum absorbed dose. Quality Control is essential to maintain optimum conditions.

Head of Project 1: Mr Wall

II. Objectives for the reporting period

To provide advice and dosimetry services as required for the development of the "Quality Criteria for Diagnostic Radiographic Images" that are being developed by CEC study groups for both adult and paediatric radiology.

To extend Monte Carlo organ dose calculations to cover x-ray examinations with recent models of CT scanner.

To study methods for estimating age and sex specific risk factors for radiation carcinogenesis, and radiation detriment in terms of years of life lost and QALYs.

III. Objectives for next period

Dosimetric support for further trials of adult CEC quality criteria. Extension of Monte Carlo organ dose calculations for conventional x-ray examinations to cover paediatric patients.

Application of latest radiation health effects models to medical exposures to derive age and sex specific risk factors and measures of detriment based on years of life and QALYs lost.

IV. Progress achieved including publications

Support for CEC Quality Criteria documents:

Comments were made on the draft of the second edition of the adult "Quality Criteria Document" and on the report of the European trial of the earlier edition (EUR 12952). Some aspects of the results presented in the trial report appeared to conflict with information included in the second edition of the criteria. The possible implementation of the Quality Criteria Document in the UK has been discussed with representatives of the College of Radiographers, the Institute of Physical Sciences in Medicine and the Department of Health.

The calcium fluoride TLDs used for patient dosimetry in the trials of the paediatric quality criteria were all recalibrated prior to issue in December for the second stage of the trial. On average their sensitivity was within 1% of that determined previously.

Monte Carlo Organ Dose Calculations for CT:

A mathematical anthropomorphic phantom and Monte Carlo computational techniques were used to calculate organ doses normalised to the free-in-air axial dose for CT scans through any transverse slice of the body. Patient doses for particular examinations can then be estimated knowing the axial dose for the scanner and the local examination techniques.

The predicted dose distributions within the phantom vary between types of CT scanner owing to differences in both the quantity and the quality of radiation emerging at different angles across the fan beam and the different distances between the x-ray tube focus and the axis of rotation of the scanner. Information was sought from CT manufacturers relating to the conditions of irradiation for their range of scanners, in particular concerning the use of beam shaping filters. Sufficient information for dose modelling purposes was finally obtained for 27 models of CT scanner from five manufacturers which between them account for 85% of scanners currently in use in the UK.

Doses to 30 different organs, normalised to the free-in-air axial dose, were calculated for CT scans through each of the 200 5 mm transverse slabs of the phantom from 10 cm below the base of the trunk to the top of the head. The organ doses can be suitably combined to provide estimates of, for example, the effective dose equivalent (HE) and Fig 1 shows how normalised values of HE vary with the location of the scanned slice in the phantom for four types of scanner. Peaks in the normalised doses correspond to the position of the radiosensitive organs which feature in the calculation of HE, such as testes (-5 cm) and breast (55 cm).

Differences in HE of up to a factor of 5 occur between the different scanners for some individual scanned slices, although differences nearer a factor of 2 are appropriate when doses are averaged over larger sections of the phantom. The Siemens DRH scanner delivers relatively high normalised doses to the breast compared to the other scanners because it does not have a beam shaping filter that preferentially attenuates the edges of the fan beam.

The results of these calculations were made available on floppy discs to all interested CEC contractors working in this area of the Radiation Protection Programme. An accompanying document ("Organ Doses from CT Examinations - the NRPB Method") was supplied with each set of floppy discs explaining how the data could be used to derive patient doses for any type of CT examination on any of the 27 models of scanner covered. They are being used for the estimation of patient doses from CT in at least 5 member states, including the UK, in conjunction with national or local surveys of CT practice. A comprehensive report embracing many aspects of CT practice in the UK, including frequency of use, patient doses and quality assurance procedures is in preparation.

Study of radiation risks, detriment and QALYs:

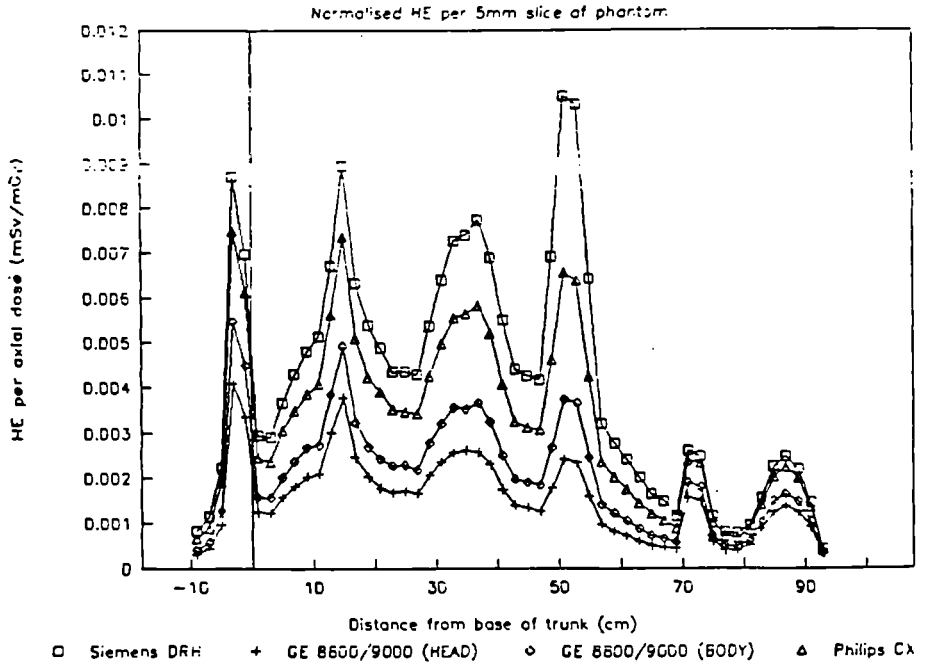
Age and sex specific radiation risk models have been developed at NRPB based on the recent reports of UNSCEAR and BEIR. A system of spreadsheets is available for analysing and presenting information on health detriment from exposure to radiation (SPIDER). A preliminary study of these spreadsheets has been made which confirms their usefulness for predicting radiation risks and detriment in terms of both lifetime probability of cancer induction and years of life lost for x-ray exposure of patients at different ages. A start has been made on studying the problems of refining estimates of years of life lost into Quality Adjusted Life Years (QALYs) lost, by consultation with staff of the Centre for Health Economics at the University of York who have been involved in the development of this concept.

Publications covering work of reporting period

Maccia, C., Moores, B.M., Nahrstedt, U., Padovani, R. and Wall B. CEC Quality Criteria for Diagnostic Radiographic Images and Patient Exposure Trial. CEC XII/268/90, EUR 12952. (1990).

NRPB and Royal College of Radiologists. Patient Dose Reduction in Diagnostic Radiology. Documents of the NRPB, Vol 1. No.3, 1990.

Fig 1 Monte Carlo Results for CT



Head of Project 2: Dr Drexler

II. Objectives for the reporting period

Measurement of X-ray spectra behind phantom and antiscatter grid. Calculation of photon spectra inside water (or lung) phantoms.

III. Objectives for next period

Continuation of spectral measurements behind phantom and grid. Simulation of radiation behind phantom by means of filtered radiation.

Construction of a realistic phantom of an adult person. Evaluation of the influence of patient size on organ doses in diagnostic radiology.

Compilation of a catalogue of organ doses resulting from CT examinations.

IV. Progress achieved including publications

Measurement of X-ray spectra behind phantom and antiscatter grid:

The existing national (DIN) and international (ISO) standards for measuring the sensitivity of film screen systems for X-ray diagnosis are going to be revised. This was not foreseen at the time the project was issued. For this revision it is essential to know the spectral distribution of the radiation impinging out of all directions on a point in the plane of the imaging system behind a patientlike phantom and antiscatter grid. Because of the complicated and fine structure of grid these spectra cannot be calculated by Monte Carlo methods but must be determined experimentally.

Spectra were measured with a germanium detector behind a perspex phantom (thickness: 20 cm; field size: 30 cm x 30 cm) and a grid (grid ratio: 8; strips/cm: 40; focus to grid distance: 115 cm) for tube voltages of 70 kV, 90 kV and 110 kV by applying a scanning procedure which allows for the detection of beams out of all angles up to 52 degrees to the central beam. The spectra were found to be significantly different from those achieved without a grid.

Calculation of photon spectra inside water (or lung) phantoms:

The alteration of several spectra relevant in diagnostic radiology inside cubic or cuboid water phantoms was evaluated using the Monte Carlo programme KASTENSPEC.

Firstly, the photon spectra as they emerge from the X-ray tube were calculated by a theoretical method which allows for the target material, the inherent filtration, added filtration and air path, target angle and tube voltage. Those spectra were then used as incident beams on the phantom and the photon transport in depth and width was simulated using Monte Carlo techniques. Spectra were calculated at several depths (including the exit surface) and off-axis distances together with their mean photon energy.

A cubic phantom of 30 x 30 x 30 cm^3 and a cuboid of 40 x 40 x 20 cm^3 were chosen as the phantoms most commonly used. The material was water in most cases, in some cases lung tissue was simulated. The incident spectra considered are summarised in the following table:

Table 1:

X-ray beams whose spectra were calculated inside water (or lung) phantoms:

a) For diagnostic X-rays:
Spectra for 50, 60, 70, 80, 90, 100, 110, 125, 130, 140 kVp, tungsten target and additional filtration of 2.5 mm Al.

b) For radioactive sources:
Co-60, Cs-137, I-131, I-123, I-125, Mo-99, Tc-99m, Ir-192, Ra-226.

c) To compose arbitrary spectra:
0.050, 0.100, 0.150, 0.200, 0.300, 0.400, 0.500, 1.0, 1.2, 1.3, 1.5 MeV monoenergetic beams.

A catalogue of spectra resulting from the above incident beams at several depths in the phantoms, on the central axis as well as at several off-axis distances, was compiled.

Publications covering work of reporting period

Drexler, G., Panzer, W., Widenmann, L., Williams, G., Zankl, M. : The Calculation of Dose from External Photon Exposures Using Reference Human Phantoms and Monte Carlo Methods. Part III: Organ Doses in X-ray Diagnosis. Revised and amended. GSF-Bericht 11/90 (1990).

Petoussi, N., Zankl, M., Panzer, W., Drexler, G. : A catalogue of photon spectra inside water phantoms. GSF-Bericht (1990) (in print).

Zankl, M., Petoussi, N., Veit, R., Saito, K. : "The calculation of dose from external photon exposures using human phantoms and Monte Carlo methods, "in Proceedings of the First International Conference on Supercomputing in Nuclear Applications (SNA'90), March 12-16 1990, Mito City, Japan, 227-232 (1990).

Petoussi, N., Zankl, M., Panzer, W., Drexler, G. : Calculation of photon spectra in water phantoms using Monte Carlo methods. Paper presented at "Gemeinsame Jahrestagung 1990", Göttingen, 19-22 Sept. 1990, submitted for publication in "Strahlenschutz in Forschung und Praxis".

Veit, R., Panzer, W., Zankl, M., Scheurer, C. : Vergleich berechneter und gemessener Dosen an einem anthropomorphen Phantom. Paper presented at "Gemeinsame Jahrestagung 1990", Göttingen, 19-22 Sept. 1990, submitted for publication in "Strahlenschutz in Forschung und Praxis".

Zankl, M., Petoussi, N., Veit, R., Saito, K. : The calculation of dose from external photon exposures using human phantoms and Monte Carlo methods. Paper presented at "Gemeinsame Jahrestagung 1990", Göttingen, 19-22 Sept. 1990, submitted for publication in "Strahlenschutz in Forschung und Praxis".

Head of Project 3: Dr Kramer

II. Objectives for the reporting period

Experimental investigation of the AEC system of the first of three mammography units. Determination of characteristic parameters of the radiation field behind phantoms.

III. Objectives for next period

The measurements conducted so far require still a comprehensive evaluation. On the basis of this evaluation the investigations will be extended to the AEC systems of two further mammography units. The results obtained so far seem to suggest that the various film screen combinations have rather similar properties. If this finding can be substantiated in the future, the number of film screen combinations examined will be reduced and greater emphasis will be given to working conditions of the AEC systems for which they may not have been designed initially, which however may allow conclusions on possible improvements of the AEC system as was shown for the unit examined so far.

IV. Progress achieved including publications

The activities in the period from 30 June to the end of 1990 can be divided into two groups. Those of the first group were directed at a quantitative description of the radiation field behind mammographic phantoms of various materials and thicknesses for the range of usually employed radiation qualities The other activities focused on the properties of the AEC system of the first mammography unit put at the disposal of PTB.

The quantitative description of the radiation field behind mammographic phantoms was investigated both by means of Monte Carlo (MC) simulations and experimentally. The investigations had the objective to determine for a number of tube voltages and phantom thicknesses the relative air kerma contributions of the direct and the scattered radiations as a function of distance behind the phantom. Experimentally this was achieved for perspex as phantom material by measuring under narrow beam conditions the dose rate of the direct radiation and in a wide beam geometry the sum of direct and scattered radiation. The MC simulations were first carried out for the geometries used in the experiments. As the agreement between the two techniques was within a few percent, as demonstrated in fig. 1, further conditions were covered only by MC simulation. By means of MC also a more realistic breast substitute material (H:11.1%, C:51.0%, N:1.5%, O:36.1%; taken from D R White et al, B J R 60 (1987) 907) was examined with the result that the attenuating properties referred to identical area specific mass coverage were slightly greater for the realistic breast substitute than for perspex; the ratio of scattered to direct radiation is however essentially identical for the two materials. The calculations have now been completed for a wide range of conditions.

One important parameter in view of the field properties behind a phantom is the scattered radiation generated in the cassette. So far quantitative measurements have been made under conditions in which no unattenuated radiation is incident on the cassette. Even in this case a substantial enhancement of scattered radiation was found. If the whole of the cassette apart from

that part behind the phantom is irradiated by the unattenuated beam a much greater amount of scattered radiation is produced, which will, if not properly shielded between cassette and detector for the AEC system, affect the AEC in a non favourable way as a relatively constant amount of scattered radiation reaches the detector irrespective of the conditions of the individual exposure. In the following part, devoted to the functioning of the AEC system of a commercial mammography unit this effect was found to be of importance.

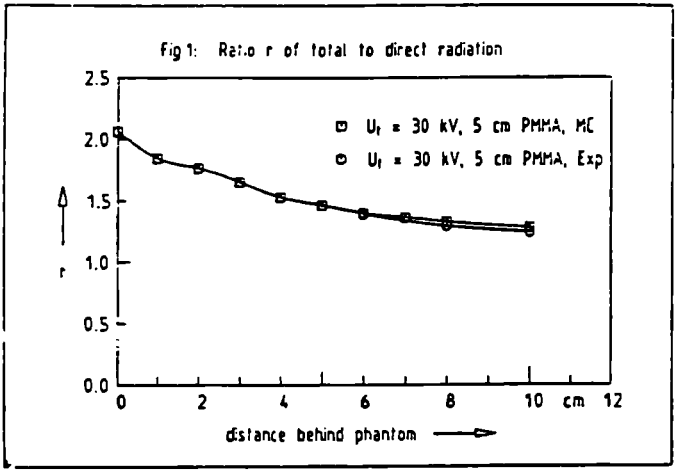
The first of the three mammography X-ray units to be examined in the course of this project was installed at PTB on 21 August. After preliminary measurements aimed at familiarizing with the various modes of operation of the machine systematic investigations were carried out for the complete range of accessible high voltages, for perspex phantoms between 2 and 6 cm in thickness and for film screen combinations of Kodak, Agfa, Fuji and Dupont. Great care was taken to ensure that the film processing was stable throughout the experiments. Fig. 2 shows for the example of the Kodak film screen combination a typical example of the results obtained. In each of the four curves the fourth symbol from the bottom denotes the optical density obtained when the AEC is left at its standard setting. The symbols upwards and downwards on each line refer to correction steps as selected with the correction device built into the machine. If the AEC worked ideally all symbols belonging to a given setting of the correction would lie on a horizontal line, implying that the optical density of the film is not influenced by phantom thickness. From fig. 2 it can be taken however, that for the neutral setting of the correction, variations in optical densities between +1.3 and -1.0 are observed. Fractional values of one step were obtained by interpolation in graphs of the type given in fig. 2. When the whole range of tube voltages and phantom thicknesses is considered corrections between about +1.5 and -3.0 steps are necessary for securing the desired optical density of 1.5 under all conditions. In the above considerations one correction step corresponds to a nominal variation of tube output of 12.5%, which for the film screen combination under consideration means a difference in optical density of about 0.2.

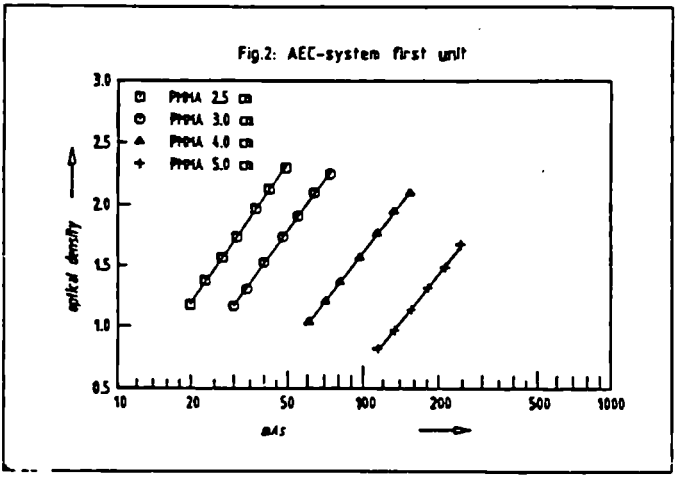
Additional examinations were carried out in view of the scattering properties of the cassette. To avoid scattered radiation as much as possible the radiation field size was restricted to that of the phantom by placing a lead shield of 1 mm thickness around the phantom. Leaving the AEC setting unchanged with respect to the situation without the lead shield a substantially greater mAs product and a correspondingly greater optical density was observed, which implies that the detector of the AEC is significantly influenced by the scattered radiation escaping from the cassette. The overall performance of the AEC system was then also examined in the presence of the lead shield with the result that the system was substantially improved in view of independence of optical density from phantom thickness. After the correct ground state for the AEC with the lead shield in place was established it was found for the example of a tube voltage of 28 kV that a variation of phantom thickness between 2.5 and 4 cm had no influence at all on the optical density and an increase from 4 to 5 cm required a correction of only 0.3 steps to achieve constant optical density. The corresponding figures for the situation without lead were +1.3 and -1.0, respectively. While the introduction of the lead represents an important improvement in view of independence from phantom thickness this is hardly the case in view of independence from tube voltage, a behaviour which after closer examination of the system also would be anticipated.

It is of course realised that a shield in front of the film is not realistic in practical cases. Therefore further investigations are currently under way with the objective to introduce a shield between cassette and detector of the AEC system, an arrangement which is hoped to produce a similar improvement as the shield in front of the cassette, which is however applicable to practical cases.

Publications covering work of reporting period

A Publication of the results concerning the first X-ray unit is under way.





Head of Project 4: Dr Broerse

II. Objectives for the reporting period

Comparison between dose measurements with ionisation chambers in phantoms of various materials and calculation of dose distributions in-phantom. Displacement correction factors of ionisation chambers in-phantom to be investigated for various materials at a radiation quality relevant for mammography.

III. Objectives for next period

Continuation of calculations of depth-dose distributions in homogeneous PMMA phantoms employing different published energy spectra. Comparison of the results from calculations with depth-dose measurements. Determination of image quality employing a contrast detail phantom for various technical conditions (including screen combination, film processing, use of antiscatter grids). Continuation of quality control and improvements of quality control protocol.

IV. Progress achieved including publications

For photons with energies in excess of that of about 100 kV X-rays, displacement corrections for ionisation chambers commonly applied in photon dosimetry are generally only a few per cent different from unity. For photons employed in mammography, however, experimental displacement correction factors obtained by linear extrapolation to zero cavity radius differ considerably from unity, e.g. K_d, is about 0.7 for a 0.6 cm^3 Baldwin-Farmer (Nuclear Enterprises Ltd, UK, type BF2571) ionisation chamber (Zoetelief et al, 1989). Non-linear extrapolation to zero cavity radius for mammography radiation qualities would lead to different displacement correction factors and their associated error. Some of the experimental data at different cavity radii (Zoetelief et al, 1989) suggest a non-linear dependence of the reading on cavity size. There is, however, a lack of extrapolation models. In addition, for photons as employed in mammography, information is not available for phantom materials other than polymethyl-methacrylate (PMMA) and is restricted to cylindrical and spherical ionisation chambers. There is only limited knowledge of the dependence of displacement correction factors on depth in-phantom.

Consequently transport calculations for photons were made with the Monte Carlo Neutron Photon (MCNP) code version 3 developed at Los Alamos (Briesmeister, 1986). For the calculations, the fluence spectrum data from Panzer et al, (1978) for 28 kV X-rays produced in a Mo anode applying a 0.025 mm Mo filter (first half value layer (HVL) of 0.31 mm Al) were used.

Phantoms, with an area of 10 x 10 cm and a thickness of 6 cm (in the beam direction) were placed at a source-to-surface distance (SSD) of 54.0 cm. Various phantom materials were employed for the calculations, i.e. materials relevant for the average breast composition (PMMA and BR-12), EVA/28 simulating fat, and water and A-150 plastic simulating water. Cavities that were spherical, cylindrical and disc-like in shape in the various phantom materials were defined reducing the density inside the cavity by a factor of a thousand relative to the phantom. To derive

displacement correction factors, calculations of absorbed dose were performed inside the homogeneous phantoms as well as at various positions in the cavities of different shapes and sizes.

The results of calculations of dose in the cavities relative to dose at 30 mm depth in the homogeneous phantoms (D(r)/D(0)), for spherical cavities of different radii placed with their centres at 30 mm depth inside the PMMA, A-150 plastic and EVA/28 phantoms are shown in Table 1 for various cavity radii.

Table 1; Calculations for spherical cavities placed with their geometrical centres at 30 mm depth inside the PMMA phantom and for 30 mm depth inside the homogenous phantom (cavity radius zero). Given are averages and relative standard deviations for the dose values per unit fluence in the centre of the cavities, $D_{centre,}$ the lateral correction factors, fr the ratios of doses in the cavity relative to the value in the homogeneous phantom and the displacement correction factors, $K_{d(r)}$, which are the inverse of D(r)/D(0).

cavity radius, r	$D_{centre}(r)$			
(mm)	(Gy cm^2 x 10^{-18})	fr	D(r)/D(0)	$K_d(r)$
0	6.98 (0.035)	-	1.00	1.00
4	8.85 (0.02)	0.97 (0.02)	1.23 (0.03)	0.81 (0.03)
8	11.1 (0.02)	0.89 (0.02)	1.42 (0.03)	0.70 (0.03)
12	14.2 (0.015)	0.85 (0.02)	1.73 (0.03)	0.58 (0.03)
16	18.4 (0.006)	0.78 (0.02)	2.05 (0.02)	0.49 (0.02)

Also shown in the table are the displacement correction factors. The results of calculations for different cavity shapes and phantom and chamber wall materials are presented elsewhere (Zoetelief et al, 1990).

Conclusions drawn from these studies are that calculation of displacement corrections for photons as employed in mammography can be made with a Monte Carlo code, since the displacement corrections are relatively large. The use of point detectors provides accurate results for points inside cavities of different shapes as well as for homogeneous phantoms for depths up to about 30 mm. For larger depths in-phantom the number of histories employed (4.10^5) is too small to provide accurate results for the homogeneous phantom.

The results of calculations for various shapes of cavities in PMMA and for spherical cavities in various phantom materials indicate that extrapolation to zero cavity radius should be made with an exponential instead of a linear function. This is compatible with linear extrapolation in the case of displacement correction factors not much different from unity as found in experimental studies for

neutrons and higher energy photons. Exponential fits to experimental data for spherical cavities of different radii for mammography will, however, change the experimental displacement correction factors significantly.

Displacement correction factors are dependent on the shape and size of the cavity and on phantom material. For non-exponential depth-dose distributions, as observed in the calculations, variations in displacement correction factors with depth are found.

The influence of the wall material of the chamber is related to differences in attenuation between wall material and phantom material.

The results from application of the Monte Carlo code indicate that, for the mammography quality used, the calculations of the displacement correction are compatible with radial displacements based on geometrical considerations.

Depth-dose distributions as obtained from calculations and those measured with spherical ionisation chambers differ beyond the experimental and calculation uncertainties; the rapid decrease followed by a slower decrease found in the calculations is not observed in experiments. This might be related to differences in the actual spectra at mammography installations, but requires further investigation.

The best estimate of k_d for 0.6 cm^3 thimble type graphite NE2571 Baldwin Farmer ionisation chamber based on experiments and calculations is 0.79 ± 0.04 for measurements in PMMA phantoms.

References

Briesmeister, J F (1986). MCNP, a general Monte Carlo Code for neutron and photon transport. Los Alamos National Laboratory. Report LA-7396-M, version 3A, rev. 2.

Zoetelief, J., De Wit, N J P., and Broerse, J J (1989). Dosimetrical aspects of film/screen mammography : in-phantom dosimetry with thimble-type ionisation chambers. Phys. Med. Biol. 34, 1169-1177.

Publications covering work of reporting period

Zoetelief, J., Eisenhauer, C M and Coyne, J J (1990). Calculations of displacement corrections for in-phantom measurements with ionisation chambers for mammography. Phys. Med. Biol. 35, 1287-1299.

Zoetelief, J., and Broerse, J J (1990). Radiation hygiene in diagnostic radiology (in Dutch). IKR Bulletin 14, 83-86.

Progress Report

Contract: Bi7-014 Sector: C22

Title: Quality criteria, tolerances, limiting values, dosimetry and optimization in a number of fluoroscopic, digital fluoroscopic, DSA and digital radiological systems.

1	Malone	St James Hospital
2	Boddy	General Hospital
3	Busch	Univ. Heidelberg Klinikum Mannheim

I. Summary of Project and Global Objectives

This contract has initiated work on bridging a gap which has opened up between major advances in fluoroscopic and digital medical imaging techniques on the one hand, and the lack of corresponding developments in Quality Assurance, Patient and Staff Dosimetry and the optimization process. The areas of work involved are clear from the technical reports which follow from each partner in the co-ordination group. However these are somewhat limited, due to resource limitations, when compared with the original proposals. The work presented is broadly concentrated on AEC/AGC studies; identification of important parameters and the limitations on them; and dosimetry/ optimization studies.

The co-ordination group was established with a view to creating a project with a strong collaboration and complimentary role between the partners. A successful methodology to foster this has been established and involves three working meetings of the partners per year. The work of the group has stimulated each of the partners and the overall results produced are greater than the sum of the individual components. In addition the group has taken into consideration the associated continued contract.

The group has paid special attention to ensuring standardization in dosimetry studies through participation in two international intercalibrations. In addition it has standardized or devised protocols for patient and staff dosimetry methods, reporting of units, AEC/AGC studies. It has also availed of expertise in other groups and where relevant taken due account of activities elsewhere in its work programme. This for example was the case with respect to the AEC work being performed in mamography, which though essentially different, has a useful lateral bearing on the groups work. The group reviewed the potential of expert systems on a number of occasions. Finally in association with the Commission the group is contributing to the organisation of a meeting of experts in 1992 in its areas of activity.

Head of Project 1: Dr. Malone

II Objectives for the reporting period

1. Study of the operation of AEC/AGC systems with a view to establishing the basis for their operation.
2. Establishment of standard dosimetry methods and cross calibration with partners.
3. Initiation of Dosimetry and Optimization Studies.
4. Retrospective identification and critique of basis for writing off a number of Fluoroscopy Rooms.

III Objectives for next period

1. Continuation of characterization of AEC/AGC and Factor Selection Systems in Digital Units.
2. Development of Criteria for Optimization of Image Quality viz a viz exposures selected by automatic systems.
3. Standardization of definition and methods of assessment of SNR.
4. Participation in various aspects of staff and patient dosimetry.
5. Further evolution of criteria for acceptance/rejection of equipment.

IV Progress achieved including publications

1. Distribution of Q.A. and Optimization Effort:

A critical review of the work in a large Imaging Department in a general hospital was undertaken with a view to establishing how the effort employed in QA related to the frequency of examinations; the cost of the equipment; the dose to patients and the staff employed in the examination; and the extent to which digital technology is employed in the examinations. It was found that most QA effort is employed on the common examinations (84%), but that these involved a limited range of equipment (~20%) when cost is the criterion. Thus these are not the examinations that employ very expensive equipment, involve high doses to patients/staff; or employ digital technology. For example very little QA/Optimization is used with over 70% of the equipment when the assessment is on the basis of cost. Likewise very little of the QA effort is biased towards examinations with high individual patient or staff doses. This project is concerned with these types of equipment and examination and thus it is contributing to improving the overall balance of effort.

2. Automatic Exposure Controls:

Automatic Exposure Control (AEC) and Automatic Gain Control (AGC) investigations have been undertaken. Studies involve both Conventional and Digital Fluoroscopy Systems. The studies have looked at the influence of these automatic controls on a number

of different factors, including: (a) Entrance dose rate to the image intensifier; (b) Light output from the image intensifier, and (c) Signal to noise ratio (SNR) in the image. In addition the influence of one automatic control on the other has been investigated. Finally functional diagrams of the systems have been derived from service manuals and the suppliers.

For conventional fluoroscopy, light output levels were measured on three different image intensifiers, for entrance exposures set with the automatic controls, as used clinically (i.e. AEC and AGC both in operation). In all cases the output was found to remain constant regardless of the x-ray beam attenuation level. However when the image brightness was measured with the digital image processor, one system showed a fall off in brightness with increasing attenuation. With the removal of the AGC in the camera electronics this fall off was eliminated. In addition, this system had a measured SNR typically 6dB lower than the others. With the removal of AGC this 6dB descrepancy was also eliminated, with an improvement in the measured SNR to 40dB, matching the other systems. Thus AEC would appear to achieve its design purpose: constant light output from the image intensifier, constant image brightness and relatively good SNR with little deterioration over the attenuation range. The introduction of AGC may need to be investigated to observe any influence on the image brightness level over the attenuation range and descrepancies in the measured SNR. The manufacturers of the three systems were requested to supply details of the AEC and AGC facilities used with their equipment. Details supplied amounted, in the main, to circuit diagrams. It is felt that the data should be more specific, especially in relation to the operation of the AGC circuit and its correct set-up and adjustment, given that AGC has been shown to significantly reduce the measured SNR.

3. AEC/AGC and Optimization:

Dose rate investigations have revealed a wide range of image intensifier entrance exposure levels. A study has been undertaken to determine the optimum value that should be employed for differing circumstances. The end points used include SNR in the image and resolution. The variables involved include the purpose and type of examination as well as the specification of the image matrix. It has been found, for example, that there is no improvement in the SNR of 256 x 256 x 8 bit images when the air kerma in the entrance plain is greater than 0.25μGy per frame. Likewise the resolution in 512 x 512 fluorographic images is optimized at a nominal value of 1μGy per frame. Further work in this area is in progress.

4. SNR:

Critical evaluation of the work undertaken in the group, and the method of specification of SNR in equipment, reveal difficulties arising from inconsistent definitions applied to this Quantity. This problem has been identified and is being addressed.

5. Dosimetry:

An international study of the calibration and performance of dose area product meters (Diamentor, PTW Freiburg) has been undertaken. Assessment of the diamentors was performed for a range of radiographic and fluoroscopic exposures. The readings from the diamentors were compared with those calculated from measurements made using a dosemeter (MDH 2025) placed in the measured x-ray field at the time of exposure and with a calibration traceable to a national standards laboratory. The results have been expressed as the ratio of the dose area product calculated from the dosemeter reading to that given by the diamentor. This ratio is called the calibration factor. The only significant variation observed in the calibration factor was with x-ray tube potential. Between systems there was significant variation in the calibration factor indicating the importance of calibrating the diamentor for the x-ray tube to which it is connected.

6. Equipment Write Off:

A detailed study of the records and correspondence leading up to the write off of over 10 Fluoroscopy units was undertaken with a view to identifying the features that regularly appeared and were regarded as important. The conclusions will be presented in detail elsewhere. Briefly it was evident that excessive input dose requirements at the image receptor featured frequently, and that image quality criterial featured less frequently than might have been expected.

7. Publications:

1. Cooney P., Malone LA. and Malone JF., 1989. Exposure Rate and Noise Level Evaluation of Automatic Exposure Control Systems. In "Optimization of Image Quality and Patient Exposure in Diagnostic Radiology". BIR Report 20.

2. Cooney P., van der Putten WJM., Crean P. and Malone JF., 1990. An Evaluation of the Specification and Imaging Performance of a Digital Cardiac Imaging System. In

3. Cooney P., van der Putten WJM. and Malone JF., 1990. An Assessment of Automatic Exposure and Gain Control in X-Ray Image Intensifier-TV Systems using a Digital Image Processor. IPSM, Annual Conference, Oxford. Book of Abstracts.

4. Faulkner K., Busch HP., Cooney P., Malone JF., Marshall NW. and Rawlings DJ., 1991. An International Intercomparison of Dose-Area Product Meters. CEC Seminar on Dosimetry in Diagnostic Radiology, Luxembourg. Book of Abstracts.

5. Malone JF., Busch HP., Faulkner K., Cooney P. and Kotre CJ., 1991. Automatic Exposure Control and Digital Systems: Their Impact on Patient Dose and Image Quality. IPSM, Patient Dose and Image Quality, Birmingham. Book of Abstracts.

6. Faulkner K., Malone JF., Busch HP., Cooney P. and Kotre CJ., 1991. The Impact of Image Quality and Patient Dose Measurement in Writing Off Old Fluoroscopy Equipment. IPSM, Patient Dose and Image Quality, Birmingham. Book of Abstracts.

7. van der Putten WJM., Cooney P., Hamilton D., King G., Crean P., Walsh M. and Malone JF., 1990. Ejection Fraction Measurement: A Comparison between Digital Imaging Modalities and Conventional Cine Angiography. IPSM, Numerical Measurements in Diagnostic Radiology, Newcastle-Upon-Tyne. Book of Abstracts.

Head of Project 2: Prof. Beddy/Dr. Faulkner.

II Objectives for the reporting period

- Investigate the use of automatic exposure control (AEC) ... automatic brightness control (ABC) systems in fluoroscopy and digital imaging.
- Design and develop quantitative techniques for image quality assessment.
- Commence patient dosimetry studies on selected examinations and treatments involving fluoroscopy and digital imaging. Study staff doses and protection in fluoroscopy and digital imaging.

III Objectives for next period

- Further investigation of the use of AEC and ABC systems for particular examinations.
- Extend patient and staff dosimetry measurements.
- Commence performance evaluation of fluoroscopy and digital imaging systems using quantitative test objects.
- Review the application and usefulness of available test objects in fluoroscopy and digital imaging.
- Start development of automated quantitative quality assurance measurements in fluoroscopy and digital imaging.

IV Progress achieved including publications

Automatic Exposure Control (AEC) Automatic Brightness Control (ABC) Systems:

A survey of the performance of AEC and ABC systems has been undertaken. Two series of measurements were performed; one using perspex as a scattering medium and the other with the effects of scattered radiation at the image receptor minimised. The results of this survey are being analysed with a view to the development of a test protocol which will include tolerances and limiting values.

A theoretical model to predict the energy absorption in the image intensifier input surface relative to the dose/air kerma monitored by an ionisation chamber has been developed. Predictions of the energy absorbed have been made using this model for image intensifier input phosphors of various thicknesses and photostimulable phosphor receptors.

The effect of magnification on patient doses in fluoroscopy whilst using an AEC system has been studied. It was concluded that the use of magnified fields of view did not change the total energy imparted/unit time, provided the image intensifier had an AEC system and automated collimation. An experimental study into the role of AEC systems in selecting the optimum dose/image on a range of digital imaging devices has been initiated.

Quantitative Test Objects:

A widely used technique for assessing the performance of imaging systems is the use of contrast detail test objects, in which circular discs varying in contrast and diameter are imaged. Observers are requested to record the number of discs of a particular diameter they can perceive. The most common test objects for contrast detail measurements are, in general, limited to one set of operating conditions. Consequently, it was necessary to design a set of contrast detail test objects for optimisation studies in fluoroscopy and digital imaging. A set of test objects have been developed for use at a wide range of operating conditions, using a contrast prediction program written in Pascal. These optimisation test objects have been manufactured and subsequently used in a preliminary series of contrast detail measurements during fluoroscopy and in digital fluorography. In addition, a theoretical model to predict contrast detail performance from a limited set of performance measurements is being developed. It is proposed to experimentally verify the accuracy of this model using the quantitative set of contrast detail test objects.

Patient Dosimetry:

Radiation dose levels are particularly high for procedures involving extended periods of fluoroscopy or cine fluorography. It is therefore important to perform patient dosimetry in fluoroscopy and in digital imaging; firstly to quantify patient dose levels and secondly as a basis for dose reduction and optimisation studies. A patient dosimetry protocol has been developed, after discussion with other contractors in the radiation protection programme. International studies into patient dose levels in fluoroscopy and digital imaging techniques have been initiated using dose-area product meters supplemented by thermoluminescent dosimetry (TLFD). Measurements have been performed at the various centres involved in this survey to determine the calibration of the dose-area product meters. The performance of the TLD used for both patient and staff dosimetry has been verified using a calibration facility (NRPB). A number of fluoroscopic examinations will be videotaped to deduce typical screening procedures. A Rando phantom, loaded with TLD will be irradiated in accord with the typical screening procedures in an attempt to quantify organ doses during fluoroscopy examinations.

Staff Dosimetry:

The increasing use of interventional radiology has served to re-emphasise the general concern over staff exposures. The longer screening times involved imply a large dose to the staff. Consequently, staff dose measurement surveys in procedures with potentially high doses to personnel have commenced, using a protocol agreed by the three contractors concerned. An investigation into the effect of various protective devices is almost complete.

A simplified method of assessing scattered radiation doses to personnel in diagnostic radiology, based on normalised dose-rate measurements has been investigated. The initial results of this study imply that it is possible to predict staff doses from a relatively limited set of measured data.

Publications covering work of reporting period:

1. H.P. Busch, K.J. Lehmann, M. Georgi, K. Faulkner. (in press) Strahlenexposition des Patienten bei digitalen Bildaufnahmeverfahren. Strahlenschutz in Forschung und Praxis.

2. IR Chambers, K. Faulkner, N.W. Marshall, (in press). Recording dose-area product information using an electronic personal organiser. Journal of Radiological Protection.

3. C.J. Kotre, N.W. Marshall, K. Faulkner. (in press) Energy imparted to the patient during fluoroscopy using magnified fields of view. British Journal of Radiology.

4. K. Faulkner, H.P. Busch, P. Cooney, J.F. Malone, N.W. Marshall, D.J. Rawlings. An international intercomparision of dose-area product meters. Paper presented at CEC meeting, Luxembourg, 19th-21st March 1991.

5. N.W. Marshall, K. Faulkner. Theoretical investigations into the energy absorption characteristics of image intensifier phosphors. Presented at the IPSM/HPA Annual Conference/ Second European Congress of Medical Physics, Oxford, 13th-15th September 1990.

6. R.M. Harrison. (in press) Digital radiography - a review of detector design. Nuclear Instrumentation Methods.

Head of Project 3: Dr. Busch

II Objectives for the reporting period

Digital Image Intensifier (II) Radiography:

- Study of imaging capabilities in comparison to conventional film/screen radiography (spatial resolution, contrast, dose).
- Optimization of exposure parameters for imaging of the gastrointestinal tract by phantom, specimen and patient studies.
- Constancy tests for digital image intensifier radiography (single exposure).
- Digital II radiography and storage phosphor radiography: Comparison of different digital and analog imaging methods for the chest by phantom and patient studies.

III Objectives for next period

Digital II Radiography:

- Patient/staff dosimetry for different kinds of examinations with comparison to conventional film/screen radiography.
- Optimization of parameters of low dose examinations (e.g. paediatrics, pelvimetry).
- Constancy tests (including DSA).

Storage Phosphor Radiography:

- Comparison of different digital and analog imaging methods of the chest including newest film/screen combination (KODAK: InSide-System).

IV Progress achieved including publications

In the Institute of Radiology in Mannheim advantages and disadvantages of digital imaging methods are under evaluation for two years. Methods for digital imaging are digital image intensifier radiography and storage phosphor radiography. Digital II-radiography can be used for DSA and single shot exposures instead of conventional film/screen radiographs. As a part of our study imaging capabilities of digital II radiography were compared with conventional film/screen radiography. Depending on the preselected dose and the image intensifier diameter the parameters spatial resolution, contrast and entrance dose (abdomen phantom) were measured. Depending on the dose spatial resolution decreased significantly below a certain level for each image intensifier diameter. Our aim was to lower the dose as much as possible. So we had to answer the question: What dose and image quality is necessary for special diagnostic questions.

Indications for digital II-radiography can be divided into three groups:

- Highest image quality is necessary (e.g. examinations of the gastrointestinal tract in double contrast).
- Lower image quality (and dose) is possible (e.g. phlebography, hysterosalpinography, examinations of the gastrointestinal tract in monocontrast).

- Lowest dose is necessary (e.g. functional examinations of the gastrointestinal tract in paediatrics, pelvimetry).

By measuring physical parameters (spatial resolution, contrast) and comparing images of specimen and patients with different preselected dose values we gave recommendations for dose selection for these three classes of examinations. For examinations of the class 1 the entrance dose, measured by an abdomen phantom was in the range of 25% - 50%, for examinations of the class 3 the entrance dose was 5% compared to a film/screen combination (speed class 200).

For imaging of the chest different digital and analog methods are available. We compared digital II radiography and digital storage phosphor radiography with spotfilm-, film/screen-, and slot (AMBER) techniques. Estimation of image quality was done by nodule detection phantoms and patient examinations. As a result digital II radiography cannot be recommended for imaging of the lung, because the spatial resolution for large entrance fields is not sufficient (0.5 Lp/mm). Storage phosphor radiographs demonstrated a high image quality, especially in the mediastinal and retrocardial areas of the chest, but gave only limited additional diagnostic information compared to film/screen radiographs. Dose could be lowered to 75% - 50% of film/screen combination (speed class 200). Dose values under 50% were connected to a significant decrease of image quality. Best imaging capabilities for chest at the wall stand were demonstrated by the slot (AMBER) technique.

One of the main areas of work is the development of constancy tests for digital radiography. Weekly since November 1990 we make constancy tests for digital II radiography. Especially digital values like signal/noise ratio, dynamic range and mean grey value were measured for all image intensifier entrance fields. Spatial resolution was determined semi-automatically. After a period of one year we will decide which parameters are necessary to characterise image quality of a digital image intensifier system.

For the next period we will continue with these constancy tests including new Phantoms for tests of the DSA mode.

After a prestudy we will start patient/staff dosimetry for different kinds of examinations with digital II radiography.

Comparison of different analog and digital imaging methods will be continued, including the newest film/screen system (KODAK: InSide-System).

Publications:

1. H.P. Busch, K. J. Lehmann, M. Georgi, K. Faulkner: Strahlenexposition des Patienten bei digitalen Bildaufnahmeverfahren. Strahlenschutz in Forschung und Lehre, (1991) Band 32.

2. H.P. Busch, M. Georgi (Hrsq.): Digitale Projektionsradiographie - Erfahrung und Heinung. Blackwell Ueberreuter Verlag, Berlin 1990.

3. H.P. Busch, M. Georgi (Hrsq.): Digitale Thoraxaufnahmen - Fortschritt der radiologischen Diagnostik? Schnetztor-Verlag Gmbg. Konstanz 1991.

4. H.P. Busch, J. Hartmann, M.C. Freund, K.J. Lehmann, M. Georgi: Thoraxaufnahmen mit dem AMBER-System-Ein Vergleich mit Klassischen Film/Folien-und Speicherfolien-Aufnahmen - Fortschr, Rontgenstr, (1991) (under review).

5. H.P. Busch, M. Georgi: Digitale Radiographie - Illusion oder Zukunftspersbektive?. In: R.W. Gunther, H.P. Gockel: Jahrbuch der Radiologie 1991. Biermann Verlac, Zulpich 1991, 47 - 66.

6. H.P. Busch: Qualitatskontrolle bei der digitalen Radiographie, Biomedical Journal, Heft 27, (1990).

7. H.P. Busch, A. Winter-Nossek, U. Bethke, P. Kohler, M. Georgi: Lungenaufnahmen in der Intensivmedizin - Ein Vergleich von digitalen Speicherfolien - und konventionellen Film/Folien-Aufnahmen. Fortschr, Rontgenstr. 152.4 (1990) 412-416.

8. K.J. Lehmann, H.P. Busch, A. Sommer, M. Georgi: Die Wertigkeit digitaler Bildaufnahmeverfahren bei der Skelettdiagnostik. Fortschr, Rontgenstr. 154.3(1991) 286-291.

Contract: Bi6-343 Sector: C22

Title: The effective dose equivalent due to X-ray diagnostic examinations and the impact of quality control on medical exposure.

1 Schmidt Klinikum der Stadt Nürnberg

I. Summary of Project and Global Objectives

The general goal of the project is to assess the relationship between radiation exposure of Interventional Radiology and the risks of alternative methods. The contribution of interventional examinations to the collective radiation exposure in radiodiagnostic shall be determined.

Dose equivalent of patients and staff will be measured and calculated, considering physical parameters, like time of flouroscopy, number of images, type of x-ray units, and clinical aspects, like nature, localization and method of interventions.

The second aim of this projekt is to investigate the suitability of storage phosphor foils for mammography. Considering image quality and dose, digital and conventional mammograms will be compared. For that reason physical parameters were evaluated and ROC-curves will be analysed.

Head of Project 1: Prof. Schmidt

II Objectives for the reporting period

- To start a central German documentation on Interventional Radiology.
- To evaluate first results, for instance of the Klinikum Nürnberg.
- To measure the dose equivalent of fingers of staff in Interventional Radiology and Angiography.
- To analyse the dependence of personnel exposure and to relate it with exposure limits.
- To compare conventional and digital luminescence mammography, using the method of ROC-Analysis.

III Objectives for next period

The project will be continued.

IV Progress achieved including publications

Introduction

The reduction of exposure in medical diagnostic radiology is aimed at by the optimization of radiological equipment and techniques. Two major advances in radiology are digital imaging systems, to improve diagnostic quality, and Interventional Radiology (IR), to treat diseases with perhaps better results and less risk than conventional therapy.

Interventional Radiology

Patient dose

Considering the realitively high exposure per examination and the increasing extent of interventions to other organs, it is justified to direct closer attention on angiographic procedures and interventional radiology. For this reason it is surprising, that the NRPB-document (vol.1, no.3, 1990) did not include these examination procedures into the list of those which make a major contribution to the medical exposure.

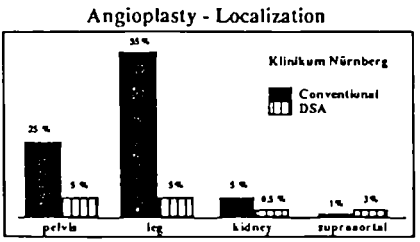
In order to obtain a general survey of the frequencies and thus of the collective exposure, a central documentation - in which more than 100 institutes will participate - was started in Germany conducted by the Klinikum Nürnberg. More than 3.500 interventions have been recorded within the first 6 months of 1990. The results represented in this report have only been obtained from the Klinikum Nürnberg itself during the first year. The most frequent by applied interventional procedure (see tab. 1) is the angioplasty. The localisation of angioplasties is represented in fig. 1. All

Table 1

Interventions Klinikum Nürnberg 1990 Total = 579

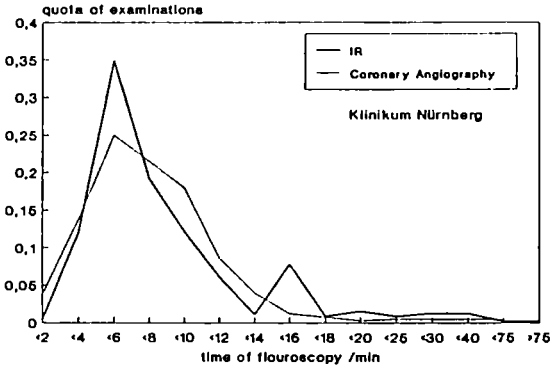
Angioplasty	458	Genito - urinary system	26
Embolisation	11	Venal and portal drainage disorder	6
Extraction of foreign bodies	1	Broncho-pulmonary system	0
Billiary system	29	Pain therapy	6
Gastro - Intestinal tract	1	Biopsies und drainages	41

Figure 1



over Germany, approx. 40 % of the angioplasties are made by means of DSA-units (pain therapy by means of CT).

Figure 2



Decisive physical - technical parameters for the patient exposure are not only the type of the x-ray unit and the sensitivity of the imaging system, but also the time of flouroscopy and the number of images per examination. Fig. 2 represents the frequency distribution of the times of flouroscopy in IR as well as that of coronary angiographies. It shows that there is no substantial difference between them.

The assessment of patient exposure in flouroscopy is difficult, because an examination like IR or angiography is dynamic. In addition to the length of the examination, data of field dimensions, positions, x-ray energies and intensities are taken into account to calculate the effective dose and imparted energy. A large number of such data is necessary to calculate an average of radiation induced cancer risk and to compare it with the risk of conventional therapy like surgical risk. The lethal risk of the patient, caused by the sickness without therapy is 10^{-1}, caused by surgery 10^{-2} and caused by radiation perhaps 10^{-4} for IR.

Staff dose

As for the IR and the angiography, it is even more difficult to representatively determine the staff exposure in medical diagnostic radiology. The hands are particularly subject to high dose during the different examinations, so that the legal dose limits for application in occupational exposure are probably achieved. A multitude of factors, to some extent entirely independent, indicate the complexity of the problem:

- sensitivity of image receiving systems
- arrangement of the radiation system
- time of flouroscopy and number of images
- experience and method of the examiner
- number of x-ray exposures to the hand (complications during the examination)
- kind of examination
- patient parameters
- protective devices.

On the basis of previous experience in person dosimetry, the exposure of the hands of the radiologist and the assistant was raised anonymously in this study. For this reason, the distribution of the ring dosemeters was not person-orientated but unit-orientated. Monthly 2 ring-dosemeters (radiologist and assistant) at each of the 4 x-ray units were evaluated by an official measuring station at Neuherberg.

Table 2

x-ray unit	1	2	3	4
tube position	over head	over head	under couch	under couch
flouroscopy	DSA	conv.	DSA	conv.
images	image int	film	100 mm	image int.
time of flouroscopy per exam.	6.6 min	6.6 min	9 9 min	5 3 min
number of images per examination	7.5	6 5	21 7	13.2
investigations per month	55	15	45	85
rel frequency of IR	0 04	0 35	0 96	0 12
dose equivalent on fingers per examination				
examiner /mSv	0 25	0 26	0 07	0 29
assistant /mSv	0 03	0 03	0 04	0.02

The following data were collected by means of a questionary:

- intervention, and if so, which one?
- examination area
- injection entry by artery or vein
- DSA or conventional flouroscopy
- time of flouroscopy
- number of images
- identification of radiologist and assistant
- subjective estimate of the frequency of hand exposure to the x-ray field.

The study was carried out within a period of approx. 6 months. Table 2 shows the mean value of the times of flouroscopy, the number of images and the dose equivalent to the fingers during one examination, as well as the number of examinations per month and the relative quota of interventions The finger dose of the assistant personnel was quasi the same, namely 0.03 mSv (per month in the range of 0.02 to 0.06 mSv) for every unit. The average dose equivalent to the hand of the radiologist, which results from all the measuring values is approx. 0.25 mSv per investigation. The average extent of the variation of the dose values established each single month is in the order of 0.05 mSv to 60 mSv. The undercouch tube with conventional flouroscopy has the lowest exposure on the fingers of the examiners. As to the overhead tubes, it is nearly 4 times as high. The relatively high exposure of the hands at the DSA-unit with the undercouch tube can be explained on the one hand by a dose intensity set relatively high at the image intensifier input screen, but mainly by the examination technique, which does not use long holes for the injection of contrast media.
The result of an average finger dose per unit and month is a value of 10 mSv. This value is apportioned to four radiologists respectively. Previous person orientated measurements in the same teams of doctors indicated monthly doses absorbed by the hands of 0.2 mSv up to 80 mSv. The monthly values of the dose per person of the radiologist measured by means of a radiation survey meter on the chest under the protective apron were also widely spread (0.1 mSv to 3 mSv). Summing up it may be said:

- usually, the exposure of the hands with IR is not higher but even lower than that with angiographies,
- the mean value of the dose equivalent to the hand is 0.25 mSv per examination.
- an excess of the dose limit of 500 mSv p.a. determined by the German x-ray act is normally not to be expected,
- considering the variation of the individual doses, correlations between exposures and parameters such as time of fluoroscopy, number of images and nature of examination cannot be proved.
- undercouch tubes substantially reduce the exposure of the examiner s hands.
- the individual examination technique is an important factor of exposure.
- an individual finger dosimetry is recommended to each examiner.
- additional protections from radiations are recommended.

Digital radiography

The digital storage phosphor foils offer a new imaging with the possibility of reducing the patient exposure in medical diagnostic radiology. In this connection it must also be examined whether it is suitable for the mammography. A comparison was made to evaluate on the one hand the quality of the image of storage phosphor foils (Digiscan, Siemens) and the conventional mammography film screen combination (Microvision, DuPont, Orthex Mamma-foil, speed class 25) by finding out physical parameters and by analysing ROC.
The examination of physical parameters was presented in the previous report.
58 mammograms were made with the conventional as well as with the digital technique by using the same dose. Approx. 20 patients had a tumor.
The digital images existed in a standard version elaborated by the Digiscan system as well as in a version individually optimized by the doctor.
The temporary results of the ROC-analysis are the following:
The advantages of the DLR are found in the bigger object latitude giving a general good image impression. The subcutane regions and the envolvement of the skin can be judged much better. The individually elaborated image is subjectively better. The image shows a good blackening irrespective of the exposure.
Opposed to these, there are disadvantages of the DLR. The recognition of micro calcereous deposits is less clear in the digital image. A black halo appears around bigger calcifications which seems very unnatural. Brightenings in the honeycomb-structured graphs of fatty tissue do slightly disturb the image impression Underexposed images have a clearly intensified noise.
Today the study shows already, that a reduction of the exposure in the digital image does obviously entaile inferior image quality. This means that with the storage phosphor mammography a reduction of the exposure cannot be realized at the present time

Contract: Bi7-019 **Sector:** C22
Title: Quality assurance and reduction of patient exposure.

1	Fagnani	CAATS - INSERM
2	Moores	Integr.Radiological Services Ltd
3	Alm Carlsson	Univ. Linköping
4	Dance	The Royal Marsden Hospital
5	Proimos	Univ. Patras
6	Flioni-Vyza	Greek Anti-cancer Institute
7	Rimondi	Univ. Ferrara

I. Summary of Project and Global Objectives

The first year of the coordinated project was characterised by the reinforcement of exchanges among laboratories already involved in the previous CEC radiation protection programme, and, on the other hand, by the establishment of positive collaborations with laboratories which have recently joined the project.

Two main observations have emerged from this year's work :

1) All the laboratories involved in the project have made considerable efforts in order to respect and achieve their own project objectives ;

2) The participating laboratories constitute an homogeneous group and represent a coherent example of European cooperation.

The first steps towards the realisation of the coordinated programme's final objectives -- practical implementation of QA in diagnostic radiology and design of ES for QA -- have encountered certain difficulties, differing according to the specific country concerned. These problems include the availability of radiological and computer equipment, institutional and governmental approval, compatibility of computers, etc.

The two primary groups of laboratories experienced unusually satisfactory cooperation in their work throughout the last year. The first group consists of those laboratories involved in the theoretical aspects of computing codes, mathematical simulations, and the design of physical phantoms (Project n°3, n°4, n°7). The second group includes those laboratories concerned by the pratical implementation of QA and by the interpretation of results (Project n°1, n°2, n°5, n°6, contract Bi6-214, contract Bi6-211, contract Bi6-236). The confrontation of these two approaches was particularly effective during the design process for the Expert System for Quality Assurance

Therefore, the process of harmonising the QA and QC protocols in diagnostic radiology has commenced under positive conditions of cooperation among the CEC member states. Next year's work will extend and consolidate these efforts.

Head of Project 1: Dr. Fagnani

II Objectives for the reporting period

The main objectives of the first year of the contract were :
- to establish for the first time in France a comprehensive quality assurance protocol in the framework of a breast cancer screening campaign (Bas-Rhin region) taking into account the particularities of the local organisation of medical services involved in such an initiative (radiological private practices exclusively) ;
- to run such a quality assurance protocol ;
- to contribute to the design of an Expert System for a Quality Assurance prototype by providing relevant data on parameters influencing both image quality and patient dose.

III Objectives for next period

The objective for the next period are :
- to devote more efforts to the improvement of observed current screening practices in the Bas-Rhin breast cancer campaign, in order to proceed towards a process which optimises image quality in mammography ;
- to finalize, in close collaboration with the other involved laboratories, the expert system prototype ;
- to further develop parts 2 and 4 of the original project, namely "Risk analysis for medical exposure" and "Quality criteria in digital radiology".

IV Progress achieved including publications

Screening for breast screening campaign

In France there are ten mammography screening campaigns officially recognized by the National Social Security System as "Pilot experiences" devoted to the early detection of breast cancer. All these campaigns are organized on a regional scale and almost exclusively involve private practitioners.

Among these regional experiences, only one is at present concerned with Quality Assurance : the Bas-Rhin. This campaign includes 46 screening centres spread over a triangle of 300 Km in the eastern part of France, next to the German border. This campaign started in June 1989 without either previous technical evaluation nor precise technical requirements concerning the radiological equipment involved.

In such a context, characterised by a total absence of information or training on QA and QC, and considering the total lack of QC equipment in the 46 centres (phantoms, measuring instruments, test tools), a QC protocol specific to this screening campaign and local technical conditions was defined in early 1990. The elements included in this protocol were chosen in order to identify the "status quo" of local radiological equipment and technical practices. The protocol addressed the four aspects of the mammography chain, including :

- production of the image (X-ray generator) ;
- image recording (X-ray film, cassettes) ;
- image process (processor, dark room conditions) ;
- image visualisation (viewing boxes).

The data analysis is still underway; a set of preliminary results have been presented to the radiologists involved.

These results, including image quality evaluation and dosimetry, clearly demonstrated the absolute necessity for a well-established quality control program within the context of a screening program and for strict compliance with its technical requirements.
By way of illustration, the preliminary image quality results obtained from Leed TORMAS test phantom expressed in terms of high contrast limiting resolution performance varied from 5.6 lp/mm to 8.9 lp/mm with a mean value of 7.4 lp/mm.

As far as dosimetry is concerned, entrance surface dose assessment for a 5 cm tick perspex phantom was performed in each radiological centre by using specifically calibrated for mammography TLDs. As one might have expected a very large range of doses was found for all the centres : average entrance dose value of 16 mGy with a minimum value of 4.5 mGy and a maximum value of 34 mGy.

In a more general way, problems were identified at different levels of the entire mammography chain : kVp consistency, dark-room safelight, film-screen contact, developer temperature, film-screen sensitivity, focal spot size etc.

In conclusion this first evaluation enabled us : to set up a management framework to facilitate higher quality screening; to identify technical corrective actions; to stimulate and improve multidisciplinary team work and to disseminate information to all those who are responsible for training and educational initiatives.

Expert system for QA in diagnostic radiology.

Data collected through the ongoing survey of radiological equipment used within the context of the Bas-Rhin screening initiative, together with the daily results obtained in a Parisian X-ray hospital department (4 months of follow-up), represent the starting point upon which the multi-partners collaboration on QA Expert System (ES) designing was established.

Initially, the CAATS-INSERM's task was to focus on the analysis of available information concerning film processing equipment.

A fairly comprehensive database was therefore created, and a first attempt was made to identify the influence of x-ray processors' parameter variations on image quality through data analysis. For the purposes of this analysis, image quality was defined with the aid of phantom or measuring instruments (limiting resolution performance, contrast, film-speed, optical density). Such an approach enabled us to better understand how to correlate QC test results with the corrective actions to be taken in order to infer abnormal situations.

In order to take advantage of concurrent research in the field of breast cancer screening, application of this first-generation expert system prototype will be limited to mammography technique.

Publications

Maccia C.; Castellano S. Preliminary results of a Quality Assurance programme in mammography. 1991, Proc. of the CEC Seminar on Dosimetry in Diagnostic Radiology, Luxembourg, 19-21 March 1991 (to be published).:

Head of Project 2 : Dr M.B. Moores

Objectives for the reporting period.

i) To review and assess all previously published work on risk evaluation in mammography.

ii) To develop a framework for the analysis of objective image quality test phantom measurements in relation to the radiographic factors employed and establish mechanisms for data collection and evaluation.

Objectives for the next period.

To implement routine quality control data analysis techniques and assess the relevance and importance of quality control measurements in predicting objective image quality, as measured from test phantom images, and the x-ray exposure requirements. To implement this management regime into a prototype expert system and expand the data base of quality control measurements.

To develop a localized model for assessment of risk in mammography.

IV Progress achieved.

A comprehensive technical quality control programme has been established within the framework of the Mersey Region's breast screening programme which adheres closely to the structure outlined within the Pritchard Report published in the United Kingdom. The programme involves 10 breast screening centres and data collected at each is transferred to a central location for assessment and data analysis.

Data analysis protocols have been established so that multi centre, multi parameter analysis can be performed on the results. To date work has concentrated on establishing the mechanism for this analysis and a preliminary evaluation of the results obtained. Also some basic scientific investigation of factors which underpin such a programme have been performed.

The quality control performance involves daily assessment of imaging performance using a Leeds TOR MAS phantom and automatic processor performance using sensitometric strip measurements.

Performance of the automatic exposure control (AEC) device, and entrance surface dose assessment for a 4 cm thick perspex phantom are performed weekly as well as x-ray tube output consistency. More detailed assessment of the performance of the tube and generator and AEC device are performed every 6 months.

To date a preliminary detailed analysis of the image quality results obtained from the Leeds TOR MAS test phantom have been undertaken in order to relate this performance to the radiographic conditions. Effort has concentrated on the following measurements:-

i) high contrast resolution.

ii) large detail threshold contrast (1 cm diameter).

iii) small detail threshold contrast (0.25 mm diameter).

The high contrast limiting resolution performance was assessed for each breast screening centre and the mean and standard deviation formed from approximately 200 measurements in each centre. The mean value for resolution varied from 8.9 lp/mm to 12.5 lp/mm with standard deviations from 0.9 lp/mm to 2.0 lp/mm.

These results corresponded to measurements performed on a daily basis by staff employed in each breast screening centre. To assess the possible effect of observer variation a sample of 5 test phantom images were selected from each centre and assessed by a separate group of seven observers. The results of the 50 images assessed by 7 observers is shown in Figure 1 where the

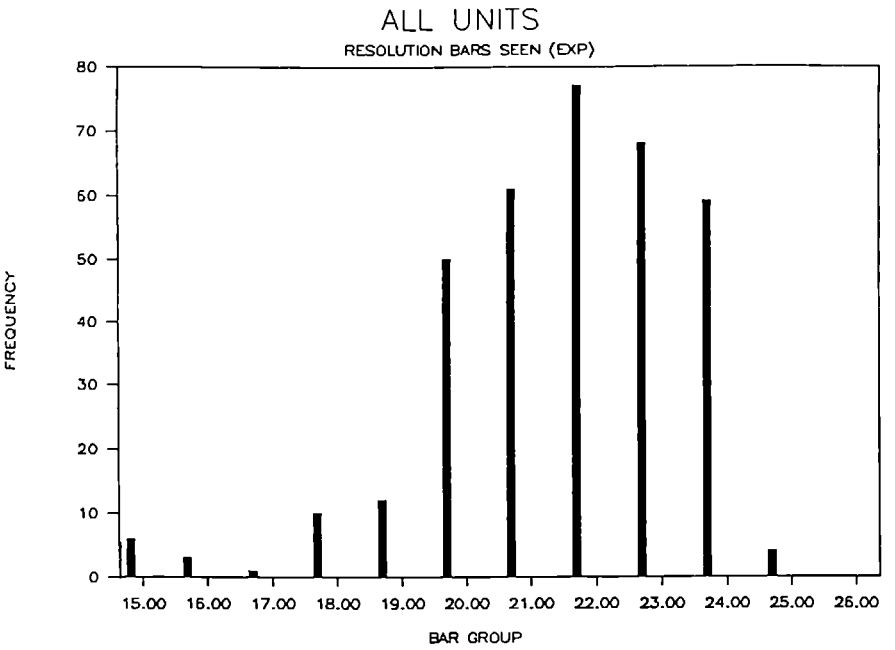


Figure 1. Variation in measured bar group resolution for 7 observers and 10 screening centres.

abscisa is expressed in bar groups for the Leeds phantom.The spatial frequency corresponding to each bar group corresponds to 8.0 lp/mm for bar group 19, 10.00 lp/mm bar group 21 and 12.5 lp/mm for bar group 23. The histogram of results shown in Figure 1 agreed closely with the histogram of data resulting from measurements performed in each centre. This indicates that observer variations play a small role in the observed variation in resolution.

Since a different test phantom was employed at each screening centre variations in measurements due to possible phantom variations were also assessed. All phantoms were imaged at a single location and the results demonstrated that phantom to phantom variations were minimal.

In order to assess possible causes of variations in resolution at different locations the high contrast resolution both parallel to and perpendicular to the anode cathode axis was investigated in relation to focal spot size. The focal spot size perpendicular to the anode axis is usually less than that parallel due to the tighter focussing of electrons in the x-ray tube in the former direction. Focal spot sizes perpendicular to the anode cathode axis ranged from 0.28 mm up to 0.5 mm whereas parallel to the anode cathode axis sizes ranged from 0.5 mm up to 1.0 mm.

High contrast resolution corresponding to focal spot dimensions perpendicular to the anode cathode axis was always higher and always more than 12.5 lp/mm. Resolution corresponding to focal dimensions along the anode cathode axis were less than 12.5 lp/mm ranging from 8.0 lp/mm up to 12.5 lp/mm.

These results leads one to conclude that for focal spots greater than approximately 0.5 mm, high contrast resolution is governed by focal spot size for the geometry normally employed in mammography. For focal spot sizes less than 0.5 mm resolution is to a large extent governed by

screen/film unsharpness, which was demonstrated by performing measurements with the test phantom in contact with the screen/film combination.

The high contrast resolution was also assessed as a function of film density at a single screening centre and thus screen/film combination. The results indicated a clear relationship between film density and observed resolution with results varying from 11.1 lp/mm at density 0.4 to 14.3 lp/mm at density 1.2 followed by a slight fall to 12.0 at higher densities. These results indicate that film density does play some part in the high contrast resolution measurement.

A similar analysis of the results for large area threshold contrast and small area threshold contrast detectability is presently underway.

Head of Project 3: Dr Alm Carlsson

II Objectives for the reporting period

The main objective was to establish the collaboration with the Royal Marsden Hospital and to develop a Monte Carlo computer code which combines essential features of codes developed seperately in the past at the two centres. The combined code should enable the calculation of primary and scattered photon fluences at points on the exit side of a simple homogeneous (body) phantom, the energy imparted to the phantom (used as risk indicator) and to the image receptor (correlated to image quality) as well as the influence of an anti-scatter grid (or airgap) on phantom dose and image quality: contrast (conventional imaging) and signal-to-noise ratio (digital imaging). The program should allow easy variation of relevant parameters: irradiation geometry, atomic composition and thickness of the contrasting detail and detector, grid parameters. Data on commersially available grids and fluorescent screens should be collected.

III Objectives for the next period.

The main objectives is to run the program for a variety of situations representative to examinations of the chest, abdomen, skeleton, and paediatric radiology. To decrease computing time, the program will be implemented and run at the super computer in Linköping. The results should be systematized as to facilitate the optimal choice (with respect to patient risk and image quality) of grid and X-ray spectrum in each case. The program should be expanded to incorporate an estimation of the effective dose equivalent as a risk parameter using published values of the energy imparted to the patient and organ doses. Some experiments should be designed and performed to check the validity of the calculated results. Strategies to expand the computer code to include simulation of crossed anti-scatter grids and inhomogeneous phantoms (in particular incorporation of low density "lung" regions) should be tested.

IV Progress achieved

1. Organisation of work

The PhD student from Linköping University visited the Royal Marsden Hospital during 3 months (october-december 1990) in which period the computer code was written combining the most efficient parts of the codes developed in the past at both institutions. In particular, the collision density estimator developed in Linköping (Persliden and Alm Carlsson 1986, Persliden 1986) was used to make calulations of scatter fluences at points in the image plane feasible and was combined with the grid code developed in London by Dance and Day 1983. The grid code was extended to treat the generation of secondary photons in the grid. The program was extensively tested against previous codes from the two departments as well as against results from the literature. After the PhD student returned to Linköping the program has been further tested and some preliminary results achieved (reported below).

2. Code performance and testing

The code calculates

- contrast (conventional imaging) and signal-to-noise ratio (digital imaging) at a specified position in the image plane (detector) and for a given contrasting detail.
- the mean absorbed dose (energy imparted per unit mass) in the phantom.
- contrast improvement factor (CIF), signal-to-noise ratio improvement factor (SIF) and dose increase factor (DIF) using an antiscatter grid or an airgap and for a particular contrasting detail.

The program has been made flexible to allow easy variation of input parameters such as: photon energy spectrum, beam filtration, phantom size and atomic composition, focal distance, entrance field area, position of interest in the image plane, grid parameters (including cover and interspace material), airgap length, thickness and composition of contrasting detail and detector.

Data about commersially available grids have been obtained from the grid manufacturers Siemens, Philips, Mitaya, and Toshiba. In all, data on about 100 grids are available, some containing reports about measurements. The code has been tested against some of the measurements as well as against some measurements in the literature using airgaps. The results are summarized below.

- The fraction of incident energy imparted to a water slab and the conversion factor, $\varepsilon / \int K_{c,air}\, dA$ agrees, within two standard deviations, with the results from Alm Carlsson et al 1984.

- Scatter fractions, F_S, without grid were compared to the results of Kalender 1979. F_S is compared while varying photon energy, field size, position of interest in the detector plane and airgap length. Agreement within two standard deviations were found in most cases.

- Good agreement is also found with published results by Chan et al 1985 with regard to grid transmission, dose increase (Bucky) factor and contrast improvement factor.

- Comparison was also made with results from measurements obtained by a grid manufacturer, Stargardt and Angerstein 1975 and with Nielsen1986 as shown in the tables below.

Comparison with measurements reported by a grid manufacturer(SMIT röntgen)

Grid	Ratio	Dose increase (Bucky) factor		Contrast improvement factor	
		Measurement	Monte Carlo	Measurement	Monte Carlo
Smit	8:1	3.49	3.40 +/- 2%	2.68	2.70 +/- 2%
Smit	10:1	4.23	4.14 +/- 2%	3.18	3.23 +/- 2%
Lysholm	8:1	4.44	4.25 +/- 2%	2.90	2.98 +/- 2%

The measurements were performed with the geometry suggested in IEC627 1978 i.e. 100 kV, FSD=100 cm, 20 cm water slab thickness, 900 cm^2 field size and a 40 mg/cm^2 $CaWO_4$ screen. The calculations were corrected for the fact that the measurements do not include the degradation in primary contrast by the grid interspace and cover. The precision in the calculations is given by +/- one standard deviation.

Comparison with measurements by Nielsen 1986

Slab thickness	Tube potential	Scatter-to-primary ratio , S/P	
		Measurement	Monte Carlo
10 cm lucite	140 kV	0.94 +/- 4%	0.94 +/- 1%
20 cm lucite	100 kV	2.66 +/- 4%	2.78 +/- 1%

A 30 cm airgap was used between the slab and the 40 mg/cm^2 $CaWO_4$ screen.

Comparison with measurements by Stargardt and Angerstein 1975

Airgap length	Slab thickness	Field size	Scatter fraction	
			Measurement	Monte Carlo
2 cm	18.5 cm	900 cm^2	0.81 +/- 10%	0.843 +/- 0.5%
10 cm	18.5 cm	900 cm^2	0.75 +/- 10%	0.756 +/- 0.3%
30 cm	18.5 cm	900 cm^2	0.59 +/- 10%	0.531 +/- 0.6%

105 kV, FSD=400 cm, 900 cm^2 field size and 40 mg/cm^2 $CaWO_4$ screen were used.

3. Preliminary results - paediatric radiology

A first preliminary run of the code has been made in a simulated paediatric procedure. Three grids were compared. The figure below shows an example of the results which can be obtained. The signal-to-noise ratio improvement factor, SIF, is shown as function of the tube potential in geometries with small (child) and large (adult) scattering volumes. With the large scattering volume (adult), the signal-to-noise ratio increases significantly using the grid due to removed scattered radiation. In the child geometry, SIF<1 using the same grid. Scattered radiation is removed but the primary radiation is also attenuated contributing to reduced signal-to-noise ratio. The low ratio grid with cover and interspace material of carbon fiber has little effect on primary transmission and contributes to SIF>1 also in the child geometry.

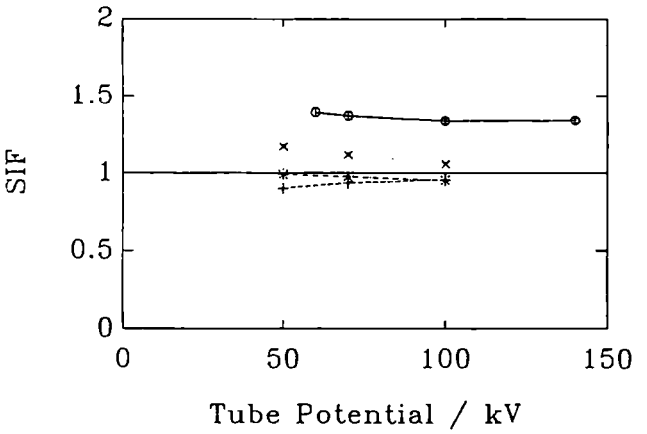


Figure . Signal-to-noise improvement factor SIF as function of tube potential for three different grids used in geometries with small (child) and large (adult) scattering volumes. Figure legends: o___o: adult geometry, grid ratio 12:1, (Al-interspace and cover), + - - +: child geometry, ratio 12:1 (Al), *-.-*: child geometry, ratio 6:1 (Al) and x.....x: child geometry, ratio 6:1 (carbon fiber interspace and cover).

4. Conclusion

The collaboration between the groups in Linköping and London has been established and fruitful results have eminated. The code has been worked out and shown to perform well. The objectives of the first reporting period have been achieved.

References

Alm Carlsson et al 1984. Phys Med Biol 29: 1329-1341
Chan and Doi 1985. Med Phys 12: 449-452
Day and Dance 1983. Phys Med Biol 28: 1429-1433
IEC627 1978. International Electrotechnical Commission Publication 627
Kalender 1979. Thesis. University of Wisconsin.
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Persliden and Alm Carlsson 1986. Med Phys 13: 19-24
Stargardt and Angerstein 1975. Fortschr. Röntgenstr. 123: 364-369
Walraven and Reth, SMIT röntgen, Postbox 218, 5600 MD Eindhoven, The Nederlands

HEAD OF PROJECT 4: Dr D R Dance

II OBJECTIVES FOR THE REPORTING PERIOD

The principal objective for the first reporting period was to participate in and advise on the development and exhaustive checking of a computer program which could be used to study the properties of anti-scatter grids in terms of the important physical parameters dose (as energy imparted to the patient), contrast and signal to noise ratio. The program was to be a combination of codes developed at the University Hospital Linköping and the Royal Marsden Hospital, London extended to cover the diagnostic energy range up to 150 kV and to include modelling of photon interactions within the grid. The program and its associated data were to be sufficiently flexible that a wide range of grid parameters, contrast details and patient (phantom) and field sizes could be explored.

III OBJECTIVES FOR THE NEXT PERIOD

The principal objective for the next reporting period is to advise on the use the computer program to study the performance of anti-scatter grids under a wide range of scatter conditions and to optimise the choice of grid and X-ray spectrum. The conditions studied should be representative of examinations of the chest, abdomen and skeleton as well as paediatric examinations. Dependence on field size should be studied. The optimisation should be made in terms of the contrast (conventional imaging) or signal to noise ratio (digital imaging) obtained using the energy imparted to the patient as a measure of detriment. Preliminary computer studies should be made of the use of crossed anti-scatter grids, and the effect of tissue inhomogeneities on scatter and advice given on the design of the experimental studies on grid performance which are to be made in Linköping.

IV PROGRESS ACHIEVED

INTRODUCTION AND ORGANISATIONAL DETAILS

The development of the computer program which models the use of anti-scatter grids in diagnostic radiology has been done jointly with the Department of Radiation Physics, University Hospital Linköping. Much of the code has been written by a student in Linköping, but the supervision of this student, the theoretical background for the computer algorithms adopted and the design of the computer model been shared between the two Departments. To facilitate this collaboration, both project leaders have spent time visiting the other Institution and the student himself has worked in London for three months to facilitate the addition of the grid code to the computer program. Care has been taken to ensure that the computer codes developed can be run in either institution.

Both institutions have contributed to the various aspects of the computer software, but the major contribution of the London group has been the addition of the grid to the software, and accordingly more attention is given in the discussion below to this aspect.

THE COLLISION DENSITY ESTIMATOR AND THE COMPUTER MODEL

The calculation of contrast, scatter or dose at a point is difficult using standard Monte Carlo techniques because of the large amount of computer time required to achieve adequate statistical precision. The computer program we have developed reduces this problem by making use of the collision density estimator previously developed in Linköping (Persliden and Alm Carlsson, 1986), extended to allow for the calculation of scatter at a point (Dance, Persliden and Alm Carlsson, 1991).

Careful consideration was given to the choice of differential scattering cross sections. It proved to be unnecessary to use cross sections which allow for inter-atomic interactions

(for which little data is available), the use of free atom cross sections allowing adequate accuracy in all cases.

The resulting computer program can estimate the scatter, contrast and signal to noise ratio at any point in the image plane for the use of a grid or airgap as anti-scatter technique. The program also calculates the energy imparted to the patient, simulated as a rectangular block of tissue whose size and composition can be readily varied. The programs have been structured so that all input parameters are easily varied including the test detail used for the contrast calculation, the grid parameters and the X-ray tube potential and filter.

MODELLING THE ANTI-SCATTER GRID

Anti-scatter grids remove both primary and secondary photons from the radiation field which exposes the image receptor. We have modelled this using the method developed by Day and Dance (1983) which facilitates the calculation of the transmission through a focussed grid, (averaged to allow for grid movement), for any photon direction and position of incidence on the grid. The method uses an analytic rather than stochastic approach to calculate the average transmission and has important advantages in the computation time required to achieve a given statistical precision on any calculated quantity.

A second effect which has to be considered is the generation of secondary particles within the grid and grid covers, which contribute to the field exposing the image receptor. These secondary particles arise from scattering processes and from fluorescent X-rays produced within the lead lamellae of the grid. For the low photon energies used in mammography, these can be neglected (Dance, Persliden and Alm Carlsson, 1991) but for the photon energies used in the present work , they must be included. We have therefore developed software which calculates the effects of these secondary particles by direct analogue simulation. The computer code was based on earlier techniques developed by Dance and Day (unpublished) and treated the grid as a series of *unit cells*, each comprising a lead strip and an interspace region together with top and bottom covers. Photons are traced through the unit cell into the next region of the model. If photons leaving the unit cell remained within the grid, then the code for the unit cell was simply used again with appropriate entry point parameters for the photon.

Grid performance is estimated by the program in terms of primary and secondary photon transmissions, contrast improvement and dose increase (Bucky) factors together with the contrast, noise and dose related quantities mentioned earlier.

PROGRAM CHECKING & COMPARISON WITH OTHER WORK

There are many possible pathways through Monte Carlo computer programs and it is important to apply careful checks to all code developed. A strong feature of the present collaboration is the ability to check quantities for some special cases using codes developed independently in each Hospital and we have made use of this wherever possible.

As a further check the methods developed for the analogue simulation of secondary particles produced within the grid have been used to calculate the grid transmission and excellent agreement was found with the analytic method.

Comparison has also been made with the results in the published literature. For example, good agreement was been obtained with the primary grid transmissions, total grid transmissions, Bucky factors and contrast improvement factors published by Chan et al. (1985).

Our results show that the effect of secondary photons produced in the grid is to increase the secondary transmission for grids with an aluminium interspace by about 22%. Most of this contribution arises from scattered photons, the contribution of L-fluorescent photons from lead being negligible and the contribution of K-fluorescent photons from lead amounting to about 5% for photon energies well above the K-edge.

Calculations of the reduction of contrast due to beam hardening by the grid show a loss of 2-8% depending on grid design and photon energy.

CONCLUSION

The objectives of the first reporting period have been achieved. Both institutions feel that the collaboration has been symbiotic.

REFERENCES

Chan H-P, Higashida Y and Doi K 1985
Performance of antiscatter grids in diagnostic radiology : experimental measurements and Monte Carlo simulation studies.
Med. Phys. 12 449-454

Day G J and Dance D R 1983
X-ray transmission formula for anti-scatter grids. Phys. Med. Biol. 28 1429-1433

Dance D R, Persliden J and Alm Carlsson G 1991
Calculations of dose and contrast fortwo mammographic grids.
Submitted to Phys. Med. Biol.

Persliden J and Alm Carlsson G 1986
Calculation of the small-angle distribution of scattered photons in diagnostic radiology using a Monte Carlo collision density estimator.
Med. Phys. 13 19-24.

Head of Project 6: Prof. B. Proimos

II The main objectives for the reporting period, were:
a) Localizing the mammographic units installed in hospital belonging to National Health Service in Greece.
b) Oral and written briefing of the departments concerned, in order to get permission to check their mammographic installations.
c) Administrative procedures for approval and release of the necessary funds for the purchase of the needed equipment.
d) Defining the required technical specifications for the above equipment.

III Objectives for next period, conmencing May 1991, we plan:
a) To complete the checking and measurements of mammographic installations in hospitals of the region of Peloponnesos and West Greece.
b) Analysis and evaluation of the final results.
c) Preparation of the final report.

IV Progress achieved including publications

After the presentation of our proposed Protocol during our meeting in Brussels, in April 1990, we started the realization of the program. Our first step was to localize and record the hospitals which have installed and use mammographic units. In trying to do so, we faced with a number of difficulties because this information was not available from a central authority. In order to overcome this problem, we had to contact the local hospitals, machine manufactures, film companies, etc.

As a result, we have been able to locate approximately thirty mammographic units altogether. About one fourth of the above units will be checked by our team, and the rest by the Athens team.

Following the above, an official letter was sent to each hospital, in order to inform them about the CEC program in progress and ask for permission to check their mammographic installations.

Concerning the purchase of the necessary equipment, we must note that the whole procedure was very time consuming due to the administrative procedures followed in our country (approval of the expenses by the Board of Trustees of our University) and long delivery times of the equipments. Because of the above all the required equipment is not yet available and a number of measurements will have to be carried out at a later stage (e.g. image quality with a phantom, kVp meter).

Up to now, we have checked 4 installations, 2 of them in Patras, 1 in Ioannina (300 km far away from Patras) and 1 in Argos (220 km far away from Patras). Checks and measurements were carried out following our Protocol, and the results are summarized in Table 1. Measurements concerning high voltage, time, field uniformity and film sensitometric checks are not included in this Table.

Presentations.

1. A. Flioni-Vyza, S. Xenofos, E. Giacoumakis, G.Panayiotakis, B. Proimos: Quality Control of Mammographic Units in Greece.
8th Panhellenic Congress in Radiology.
Athens, 10-13 of September 1990.

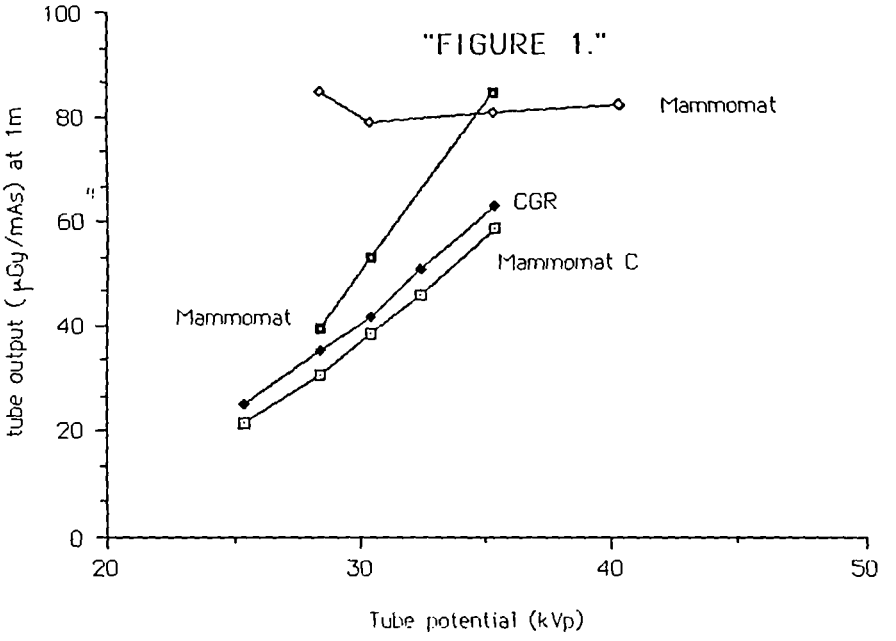
2. A. Flioni-Vyza, S. Xenofos, E. Giacoumakis, G. Panayiotakis, B. Proimos: A Protocol of Quality Control of Mammographic Units.
1st Panhellenic Congress in Radiation Protection.
Athens, 24-26 of October 1990.

Table 1

Type of unit	Senographe 500T (CGR) [1]* Mammomat (Siemens) [2] Mammomat C (Siemens) [1]
Year of installation	'80[1], '86[2], '90[1]
Users manual (Y/N)	[2]/[2]
Service manual (Y/N)	[2]/[2]
Quality Control (Y/N)	[3]/[1]
Workload (patients/week)	10-50 pat./week
Target material-Filter	Mo-Mo[1], Mo-Al/Mo[3]
Users	Radiologist [3] Radiographer [1]
Compression cones	Manual [3] Automatic [1]
Focal Spot Size	0.3mm[1], 0.4mm[3]
Grid (Y/N)	[3]/[1]
Light/X-ray field congruence	Perfect
Cassette format	18x24cm [4]
Magnification system (Y/N)	[2]/[2]
Automatic exposure control (Y/N)	[4]/[0], one out of order
Xerorgaphic system (Y/N)	[0]/[4]
Biopsy system (Y/N)	[0]/[4]
SID	60cm [4]
Total filtration	0.5mmAl [1], 0.6mmAl [3]
A.E.C checks	
a) reproducibility	<4% [4]
b) responce vs kVp (28-35kVp)	<20% [3]
c) responce vs thickness (2-4-6cm perspex)	<20% [3]
Output	see figure 1.
Entrance dose (4cm perspex)	28kV: 3.2-8.9mGy 30kV: 2.0-7.1mGy
Leakage radiation 18cmx24cm (35kVp, 50mAs)	<150mR/h at 1m
Cassette/Screen/Film	Kodak Min-R [4]/Kodak Min-R [4]/ Kodak Min-R [2], Konica CM [1], Agfa Mammoray MR3-II [1]

Automatic processor	Dedicated [1]
Processing time	1.5-2.0min
Special warning signs (Y/N)	[2]/[2]
Protective clothing (Y/N)	[0]/[4]
Protective panel for user (Y/N)	[4]/[0]

* Nr in brackets=Nr of units


"FIGURE 1."
100
80
60
40
20
0
20
30
40
50
tube output (µGy/mAs) at 1m
Tube potential (kVp)
Mammomat
CGR
Mammomat C
Mammomat

Head of Project 6: Dr. Flioni-Vyza

II Objectives for the reporting period

The main objectives for the above period, were:

a) Localizing the mammographic units installed in hospitals belonging to national health service in Greece
b) Oral and written briefing of the depertments concerned, in order to get permission to check their mammographic installations.
c) Administrative procedures for approval and release of the necessary funds for the purchase of the needed equipment.
d) Defining the required technical specifications for the above equipment.

III Objectives for next period

For next period conmencing May 1991 we plan

a) To complete the checking and measurements of mammographic installations in hospitals of the region of Athens and advance with the application of our program in the installations of North Greece and islands.
b) Analysis and evaluation of the final results.
c) Preparation of the final report.

IV Progress achieved including publications

After the presentation of our proposed Protocol during our meeting in Brussels, in April 1990, we started the realization of the program. Our first step was to localize and record the hospitals which have installed and use mamographic units. In trying to do so, we faced with a number of difficulties because this information was not available from a central authority. In order to overcome this problem we had to contact the local hospitals, machine manufactures, film companies, etc.

As a result we have been able to locate approximately thirty mammographic units altogether. About one fourth of the above units will be checked by the Patras University team.

Following the above, an official letter was sent to each hospital, in order to inform them about the CEC program in progress and ask for permission to check their mammographic installations.

Concerning the purchase of the necessary equimpment we must note that the whole procedure was very time consuming due to the administrative procedures followed in our country (approval of the expenses by the Board of Trustees of our hospital, approval by the State Controller) and long delivery times of the equimpents. Because of the above all the required equipment is not yet available and a number of measurements will have to be carried out at a later stage (e.g. image quality with a phantom).

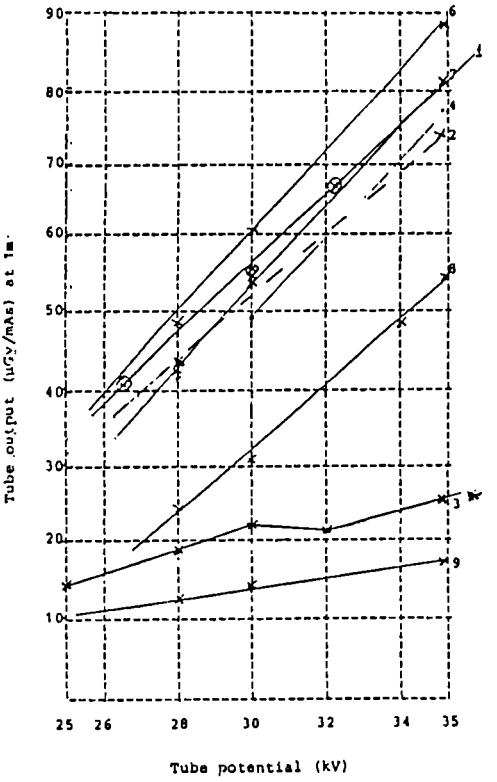
Up to now, we have checked 9 installations, all of them, except one, in Athens. Checks and measurements were carried out following our Protocol, which is mainly based on the Report No 59 of the IPSM and the results are summarized in Table I. Our measurements concerning leakage radiation, field congruence and uniformity, minimum response time and film sensitometric checks are not incluede in this table.

TABLE 1

Type of unit	GE(2)* - SIEMENS(5) Toshiba(1) Picker(1)
Year of installation	1968(1),1975(1),1987-1990(7)
Users manual (Y/N)	4/5
Service manual (Y/N)	1/8
Quality Control (Y/N)	2/7
Workload (patients/wk)	10-200
Target Material- Filter	Mo-Mo(8), W-Mo, Rh, Al(1)
Focal spot sizes	0.1-0.4 (4), 0.5-1.0 (5)
Compression cones (Manual/Aut)	6/3
Source to film-distance in cm	45(2),60(4),65(1),72(1)
Grid /Grid removalbe	5/3
No Grid	4
Automatic exposure control (Y/N)	8/1
kVp reproducibility	± 1% (all units)
kVp accuracy	≤10%(9), ≤7%(5)
Total filtration	0.5 - 0,68mm Al equiv.
Exposure time reproducibility	± 2% (6), ± 10%(3)
AEC checks a) reproducibility b) response VS KV (28-35 kVp) c) Response VS thickness (2-4-6cm perspex)	 <± 2% (8), ± 10% (1) ± 10%(3), ± 20%(2), >30%(2) ± 10%(3), ± 25%(2), >40%(2)
Output	See Figure 1
Entrance dose with backscatter (4 cm pesrspex)	28kV: from 1,4mGy to 15mGy 30kV: from 1.3mGy to 12mGy
Film Processors	dedicated (5)
Processing time	1.5 - 2.2min(8), 4min(1)
Cassette/screen/films	Kodak MinR 5 units Agfa Mammory 1 unit In the 3 remaining installations, at least one component was of different manufacturer.

*Nr in brackets = Nr of units

FIGURE 1



* Automatic change of
filter at 31kV in unit 3

Presentations

1. A.Flioni-Vyza, S.Xenofos, E.Giacoumakis, G.Pannagiotakis, B.Proimos: Ouality Control of mammographic units in Greece.
   8th Panhellenic Congress in Radiology.
   Athens, 10-13 of September 1990.

2. A.Flioni-Vyza, S.Xenofos, E.Giacoumakis, G.Pannagiotakis, B.Proimos: A Protocol of Quality Control of mammographic units.
   1st Panhellenic Congress in Radiation Protection.
   Athens, 24-26 of October 1990.

Head of Project 7: Prof. Rimondi

II Objectives for the reporting period

Project of an electronic instrument for measuring waveform, kVp, ripple, HVL, exposure time and exposure in the mammography energy range.
Choice of detectors having such an efficiency that the signal-to-noise ratio has an acceptable value for proper filtration conditions.
Mechanical and electronic design of the probe and of the pre-amplifier.
Component construction and assembly.
Probe testing using an X-ray beam for mammography.

III Objectives for next period

The probe will be interfaced to an A/D converter add-in card for a personal computer. The software required to measure (in a single shot) kVp, HVL, exposure and exposure time will also be implemented.

IV Progress achieved including publications

A probe with two couples of Si PIN detectors having a sensitive surface of 7.34 mm^2 and 100 mm^2 respectively was built.
The electronics needed to acquire the relevant signals was designed and implemented.
The four detectors in the probe have, in couples, different functions. Those having a wider area are meant for measuring kVp. The relevant underlying theory was the subject of our paper in Ref.(1).
The smaller detectors measure exposure above and under an 0.5 mm thick Al filter.
The tests for evaluating the performances of the device for the exposure measurement were carried out in two stages:
1) the output signal of the smallest unfiltered detector, time integrated, was compared with the response of an ionization chamber for mammography. This analysis pointed out that the device, without any correction, is able to measure exposure or air kerma within ± 7% in the mammography range 25-35 kVp.
2) The detector was then embedded in the surface layer of a phantom having an X-ray transmission close to that of a standard breast. The electronic equipment was made portable. In this way it was possible to test the device and the associated electronics in actual mammography units.

The results of these tests were presented at the seminar on "Dosimetry in Diagnostic Radiology", Luxemburg, 19-21 March 1991 (2).

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(2) M. Gambaccini, M. Marziani, L. Cristaudo, E. De Guglielmo, "A Performance Device for Quality Control in Mammography", Seminar on Dosimetry in Diagnostic Radiology, Luxemburg, 19-21 March 1991.

Progress Report

Contract: Bi6-136 **Sector: C22**
Title: Refinement of methods for the assessment of organ doses, and possible reduction of patient exposure.

1 Padovani Unita' Sanitaria Locale N. 7 Udinese

I. Summary of Project and Global Objectives

A. EXPERT SYSTEMS FOR QUALITY ASSURANCE IN MEDICAL RADIOLOGICAL IMAGING

Quality Assurance (QA) programmes assess the performance of radiological equipment by verifying that all parameters believed to influence either the Image Quality (IQ) or Patient Dose (PD) lie inside the tolerance levels. The practical implementation of QA programmes requires specific high-level knowledge which is rarely available. The use of methods using Artificial Intelligence would help to introduce expertise and efficient QA almost anywhere, thus achieving widespread patient dose reduction. The knowledge on the relationships between the equipment performance, IQ and PD includes empirical associations and heuristic methods and seems well suited to an Expert System (ES) devoted to the complex task of finding out causes of, and possibly remedies to, insufficient performance of the radiological imaging system.
The ES will infer situation descriptions and system malfunctions from observables. Values of observables can be obtained directly by monitoring instruments and/or by automatic or interactive image analysis of radiographs of suitable test-objects (to assess performance of film processor a sensitometric strip will be used) and/or simply by interacting with the user at the keyboard.

B. PATIENT DOSIMETRY

The dosimetric laboratory continues to develope and improve the dosimetry system to measure patient doses in diagnostic radiology. Support to international trials of the CEC study groups on the development of quality criteria for diagnostic radiographic images will be provided.

Head of Project 1: Dr. Padovani

II Objectives for the reporting period

Expert System
1. Identification of the problem to avoid tasks too large or unwieldy for the resources available.
2. Definition of key concepts and relations.
3. Choice of appropriate knowledge-acquisition tools.
Patient dosimetry
Measurements of entrance surface doses in the European trial on paediatric radiology.

III Objectives for next period

Expert System
1. Choice of instruments and methods for measuring observables.
2. Knowledge representation.
3. Architecture of the Expert System
4. Implementation of a prototype.
Patient dosimetry
Measurements of patient doses in the second european trial for the validation of the CEC Document on Image Quality Criteria in Diagnostic Radiology.

IV Progress achieved including publications

1. IDENTIFICATION OF THE PROBLEM TO AVOID TASKS TOO LARGE OR UNWIELDY FOR THE RESOURCES AVAILABLE.

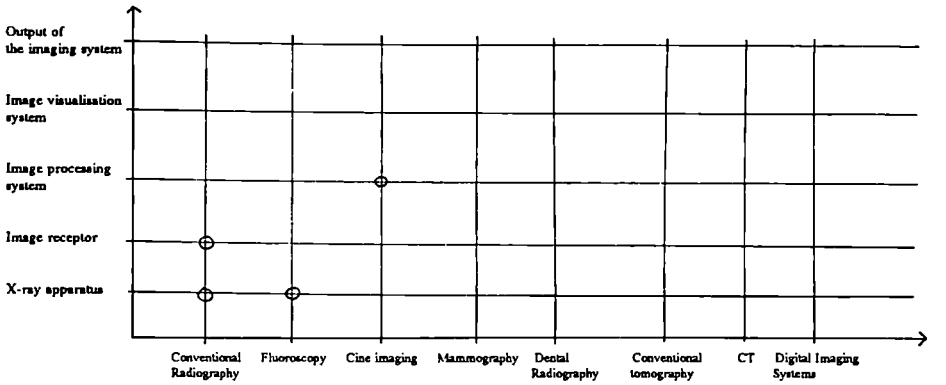
QC deals with two main classes of problems:
- testing of equipment
- troubleshooting equipment problems.

Testing of equipment is carried out under two different headings:
- At acceptance of new or substantially revised equipment to detect defects and ensure that equipment complies with the specifications in the contract of purchase. In this context the baseline level of performance of equipment should be also established for any future comparison.
- Routinely to assist in maintaining the quality of the equipment output, verify the equipment integrity after service or preventive maintenance, evidence, and diagnose, possible causes of, changes in equipment performance before they become radiologically apparent.

Routine tests are carried out also when performance problems are encountered in daily use of equipment. They help to diagnose malfunctions or at least screen hypotheses for troubleshooting. As a first sieve they serve to establish whether the problem is caused by equipment or human factors, thus reducing the time required to correct the problem or the number of useless and costly visits by the service engineer.

QC comprehends a large set of tests, instruments and procedures regarding the whole radiology. This set can be described with reference to a couple of axes (fig. 1). Along the vertical axis the entire process of formation of the radiological image takes place: from the X-ray apparatus to the final output of the imaging system. The horizontal axis is marked by the sectors of radiology which differ at one or more levels in the diagnostic imaging chain.

Figure 1. Environments for Quality Control in medical radiology.



Every intersection of two straight lines passing through a radiological sector and a phase of the imaging process picks out an environment for QC. Hence, the image processing system for conventional radiology selects the photographic QC which includes tests for film storage, darkroom and processing conditions; similarly, QC tests for X-ray tubes, collimators and generators concern the X-ray apparatus for fluoroscopy. This pattern identifies subsets of QC tests which are not mutually exclusive. For example, most of the tests for the X-ray apparatus of conventional radiography also apply to all imaging systems using a conventional X-ray tube (fluoroscopy, dental radiography, conventional tomography ...).

However, each sector of radiology has its own independent set of QC tests, though possibly partly in common with others, thus allowing the factorisation of the search space. On the contrary, along the imaging chain it may be affirmed that QC tests are generally not independent. Any test tool which makes use of film has interdependences with the image processing system (e.g., tests for screen speed). Yet, tests may simultaneously involve more than one phase of the imaging process (tests to check the efficiency of the image intensifier - TV system chain in fluoroscopic equipment). Tests for Image Quality (IQ) imply an evaluation of the whole imaging process and are therefore placed at a different logical level.

2. DEFINITION OF KEY CONCEPTS AND RELATIONS.

IQ and Patient Dose (PD) are critical terms in QA. Producing images of consistently high quality with the lowest possible radiation exposure is the goal of QA. Since the patient body is not completely irradiated during a radiological examination, PD is a comprehensive term to designate all the doses received by patient's organs. Then, the overall risk for patient can be evaluated by means of the risk coefficients specific for each irradiated organ. Agreed methods exist for the evaluation of organ doses in diagnostic radiology (Monte Carlo simulations and thermoluminescent dose measurements). Although IQ represents the ultimate justification of QA, no single objective measurable index of IQ does exist. There are several well established physical parameters which describe an image, including resolution, contrast, threshold contrast, optical density, noise, but there is no consensus on how they correlate with the diagnostic information content of clinical images. However, some of the physical parameters of IQ are usually measured with special test objects.

Other methods are used to evaluate IQ. These methods are based on radiologist impression, visibility of anatomical landmarks (approach adopted in a recent CEC trial) and observer performance (ROC analysis).

IQ and PD are opposing characteristics in a radiological examination. For example, a speed increase of the film-screen system reduces the patient doses at the expense of an image degradation; yet a lower kVp gives more contrasted images, but a greater number of photons loose their energy into the patient body without reaching the image receptor. If PD is a necessary condition to produce radiological images, by no means higher doses are always associated with better IQ: obviously an image located largely on the shoulder of the film characteristic curve loses contrast. Generally, a compromise between IQ and PD is necessary, which takes into account the type of information needed and does not put the patient at risk through misdiagnosis.

Every single QC procedure consists in checking the efficiency of a part of the radiological equipment using a test tool whose result (measurement of one or more observables) must be compared with standards.The characteristics of equipment, both the apparatus to be checked and the test tools, form the basis for any relation between the results of the test and the status of the tested equipment. Other concepts of particular use are those concerning the physical quantities which are observed during test procedure and equipment control.

Events are related to the execution of QC tests, faults of equipment and data record keeping. The different phases of the test procedure define a layout against which the correctness of the execution of the test may be deemed. The results of the test are usually recorded on forms including all the relevant data.

The ES can be conceived as a guide to perform QC tests correctly, evaluate results, take corrective action if necessary .

Tests may be divided into two main categories. The first includes tests which assess the imaging performance of an imaging system through the measure of one or more parameters of the image or evaluate the patient risk through the measure of one or more doses. Test objects for the evaluation of threshold contrast and detail detectability and the measurement of entrance skin dose fall into this group. These tests can be referred to as IQ and PD tests. The second category of tests is concerned with the measurement of a single aspect of equipment performance per se. This aspect certainly affects both IQ and PD, but no attempt is made to correlate the latter two with the former. Tests of the first group tell something about the final result of the radiological examination, but tracing back to the single causes of that result is very difficult. Tests of the second group ascertain changes or drifts in single parameters, but not the extent to which they affect IQ and PD.

3. CHOICE OF APPROPRIATE KNOWLEDGE-ACQUISITION TOOLS.

The type of knowledge and the complexity of the task concerned with QC suggests the use of an ES shell that supports the knowledge representation with frames, a flexible inference engine and LISP facilities. Goldworks II seems a suitable instrument and has the advantage of running efficiently on PC machines.

4. CONCLUSION

As a consequence of the factorisation of the search space an incremental approach to the development of the ES is possible. The first prototype will be devoted to the management of the phantom test in mammography.

Publications

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Contract: Bi6-211 **Sector: C22**

Title: The principles and the practibability of quality control and quality assurance in paediatric radiology

1 Fendel Univ. München Kinderklinik

I. Summary of Project and Global Objectives

The project has the objective to screen and assess problems related to radiation protection in paediatric radiology. Optimization, quality control, and quality assurance of radiological imaging studies of newborns, infants, and children are quite different from those in adults. They are, however, mandatory in terms of radiation protection of the public because paediatric patients represent the most sensitive part of the general population.

The objective of the project is to survey radiographic technique and surface entrance dose for frequent paediatric x-ray examinations in routine practice, and to determine how individual optimization measures can be effective in daily routine and, in addition, to what extent they are practicable with the final goal of establishing standards for quality control and quality assurance in paediatric radiology.

One major part of this study is a survey of equipment, paediatric radiographic technique and phantom measurements within x-ray departments in the Federal Republic of Germany. In a parallel EC-wide study with on-patient thermoluminescent dosimetry (TLD), frequent paediatric x-ray examinations (abdomen, skull, chest using both stationary and mobile equipment, spine and pelvis) for a defined "Euro-baby" of 10 months of age (4 months for pelvis, or premature infants (1000 g) for mobile chest examinations in the incubator) were similarly surveyed.

Head of Project 1: Dr. Fendel

II Objectives for the reporting period

The first part of this study concentrated on those departments headed by a qualified full-time paediatric radiologist. Questionnaire data and phantom measurements from the second part of the phantom study, i.e. in x-ray departments which are not headed by a full-time paediatric radiologist (general radiologists and paediatricians), and from the third part of this study, i.e. from radiologists and physicians in private practices, are completed and are now being analyzed. Results of the EC-wide TLD-study are also available. An expert group of paediatric radiologists who are members of the European Society of Paediatric Radiology (ESPR) has elaborated the document "Quality Criteria for Diagnostic Radiographic Images in Paediatrics" which is specially adapted for children and parallels the similarly named CEC Working Document. The practicability of these paediatric guidelines has been being tested on the survey data and the original x-ray films collected in the first TLD-study.

III Objectives for next period

In the national survey, comparative analysis between radiologists and other physicians not specifically trained in paediatric radiology are planned for the next reporting period. Further statistical analyses of the multitude of data collected in the first TLD-survey are nearly completed. Among these are: the effect of the revision on the image quality criteria on inter-rater agreement and intra-rater variability based on a second film reading. The quality criteria developed by the expert group provide clear and practical guidelines for good radiographic technique of selected x-ray examinations. These have been distributed to paediatric radiologists for review, comments and criticisms. A final version of these quality criteria will be completed by the next reporting period. The results of and the experience with these surveys can serve as a sound basis for similar studies on other age groups (e.g. pre-school and school-aged children), as well as a wider spectrum of x-ray examinations (e.g. computed tomography and fluoroscopy).

IV Progress achieved including publications

Data analysis of the national survey showed that variations in radiographic technique and especially for surface entrance dose in departments and private practices not headed by a paediatric radiologist were considerably greater than those found in departments headed by a paediatric radiologist. Furthermore, it was determined that, in general, patient exposure could have been significantly reduced if the appropriate equipment was selected and an optimized radiographic technique was used. Special knowledge in paediatric radiology is necessary for both.

In the TLD-study, 89 x-ray departments which were headed by an paediatric radiologist were surveyed in 11 EC-countries. Dosimetry was performed by medical physicists and their assistants in the research centres in Neuherberg, Chilton and Udine. A comparative analysis of equipment, radiographic technique, image quality and patient dose has been made. Similar to the national survey, wide variations in technique and dose were also found in this study. Using the criteria for good radiographic technique defined by the ESPR-expert group, it was shown that there was a striking and significant negative correlation between patient dose and the number criteria fulfilled.

Image quality was determined by rating the original x-ray films collected during the survey and was based on the image criteria prepared by this group. Image quality was generally good and was not dependent on dose. The guidelines for good radiographic technique were very practicable and needed only minor revisions.

A second phase of this study is in progress which is designed to confirm the results of the first study, to examine intra-department variations and to clarify many unanswered questions and doubtful replies given on the first questionnaire. Only chest x-rays examinations, with both immobile and mobile equipment will be surveyed, mainly because these are the most frequent examinations in infancy. A total of about 120 departments in the European Community have expressed their willingness to participate in this study.

Publications:

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Progress Report

Contract: Bi6-214 **Sector: C22**
Title: Optimization of protection in medical diagnostic radiology

1 Vano Carruana Universidad Complutense de Madrid

I. Summary of Project and Global Objectives

Extensive data acquisition and achievement of dosimetric evaluations have been carried out at different Diagnostic Radiology Services of the Madrid area, during the first stage of this project (1987-1989) with the aims of analyzing the condition of the Radiation Protection within them and assigning priorities for the gradual implementation of Quality Control and Quality Assurance programmes.

The obtained results, with data from more than 60,000 patients and 3,000 single dosimetric evaluations, can be considered significant in the Community of Madrid (4,800,000 inhabitants). Patient doses measured in about 36% of the evaluated rooms have been found over the EC reference values in some radiological examinations, what has proved the urgency in assuming corrective actions.

The final part of the former project and the first stage of the planned work for the biannual period 1990-1991, have been aimed to the following purposes: 1) development and implementation of a pilot QC programme which permits the detection of anomalies by means of dosimetric and image quality analysis; 2) establishment of a procedure to determine the causes of such anomalies. In each of these stages a computerized treatment has been introduced to help the design and application of an expert system comprising all these goals.

Quality control actions have been first practised in those rooms where anomalous dose values or deficient image quality were found. Such controls included X-ray tubes and HV generators, radiographic and radioscopic image devices and operation procedures.

The foresights in a second step consist of correcting the detected anomalies or proposing for its correction, then carrying out new dose and image quality controls, so that the advantages achieved in each correcting action could be evaluated.

This continuous process of "dose and image quality evaluation/ corrective actions/re-evaluation", requires the implementation of data acquisition and performance procedures simplified enough so as the whole strategy is agreed by the radiology service staff, and with a minimum interference with the operation of the rooms.

The global aims of the project could be then summarized in the application of simplified quality control procedures to detect and correct anomalies in image quality and doses, followed by the effectiveness evaluation of corrective actions and the computerization of all parameters of interest.

Head of Project 1: Prof. Vano Carruana

II Objectives for the reporting period

1. Establishing and putting into practice simplified procedures to evaluate image quality and patient dose levels, getting ready the computerization of the parameters of interest for a future Expert System.

2. Obtaining local levels of patient dose reference values, to compare them with the EC values and use them as sectorial indicators of priority for QC actions.

3. Carrying out quality controls of X-ray equipment and image devices to detect the causes of anomalies.

III Objectives for next period

1. Proposals of corrective actions for anomalous dose and/or image quality findings.

2. Verification of effectiveness of the corrective actions through the re-evaluation of image quality and patient dosimetric controls.

3. Computerization of the results of QC and exploitation of databases for the supervision of levels of patient doses and other parameters of interest which contribute to the development of an Expert System.

IV Progress achieved including publications

A) Simplified procedures: image.

Simplified procedures have been established to evaluate the quality of images. Phantoms TOR-CDR for general Radiology, TOR-MAM for mammography and the nine object set for radioscopy of the University of Leeds are used.

Evaluations of image quality with test objects have been carried out, in some occasions, along with the application of image quality criteria emitted by an EC expert group (CEC Working Document, ref. XII/173/90, second edition, June 1990), with reference to the visualization and reproduction of anatomic details.

To evaluate the image quality obtained through the above mentioned test objects in radiography, it has been taken into account the resolution and low and high contrast sensitivity outcomes. The used criteria have been the following: when resolution was above 6 lp/mm and the number of visible discs indicators of contrast was of 10 or higher the image was qualified as 'excellent'; when resolution was between 5 and 6 lp/mm and the discs indicators of contrast was between 8 and 10, the image was qualified as 'standard', and the image was qualified as 'deficient' when resolution was lower than 5 lp/mm or the visualization of the discs was lower than 8. A deal of over 100 rooms have been controlled.

During the application of the procedure some limitations resulting from the test object practical use in General Radiology have become apparent. Test object requires different exposure conditions from the actual ones used in the radiological examinations, so that a deficient image does not always correspond with quality criteria of EC or vice versa.

In mammography, resolution limits are evaluated, likewise low and high contrast sensitivity thresholds and visualization ability for details simulating micro-calcifications. In this case, the criterion to qualify the images, apart from including a minimum number of test details properly viewed (for example, more than 12.5 lp/mm or less than 10 lp/mm in high contrast), allows for the global score assigned by two observers according to the number of the viewed objects in the test.

The following parameters related to the image quality are stored for the computerization process: identification of the room and equipment (in order to connect them with other data bases); film, screen, cassette types; model and state of processor; state of viewing boxes working in the rooms; expert which

qualify the images; optical density readings in a control point of the test, and resolution and low and high contrast sensitivity. Besides the above parameters, relative contrast and sensitivity for micro-calcification detection are recorded in the case of mammography.

In radioscopy, the parameters of dynamic range, distortion, low contrast sensitivity threshold, and resolution limit are being analyzed. Image scoring is carried out according to these parameters, demanding 4% of contrast threshold in the CsI equipment and 6% in the case of ZnCdS ones, and a resolution limit of 1.2 lp/mm and 1.6 lp/mm in intensifiers of CsI of 23 and 15 cm, respectively, and 0.8 and 1.2 lp/mm in the case of those of ZnCdS.

B) Simplified procedures: doses.

For the analysis of patient doses the simplified procedure involves dose measurements at the entrance for simple examinations or the measurement of dose x area product, apart from the number of images and the fluoroscopy time in 'complex' examinations.

In this last type of examinations, three levels of simplification have been established, to be applied depending upon the completeness of the data achieved in the centres controlled. In all of them, the technical conditions of the examinations are recorded. Dose x area product values from each separate exposure and during fluoroscopy screening periods are recorded along with readings from several TL dosemeters placed on the patient skin, in the first level. In the second level, data capture is similar, but no TL dosemeters are used. In the third level, only the cumulative value of dose x area product during the examination is recorded.

In simple examinations, dosimetric measurements are being carried out in each evaluated room with TL dosemeters or transmission ion chambers in a significant sample of patients. As an alternative, when a given examination type does not show very important shiftings in the operation conditions, these are recorded, to reproduce lately the most frequent and extreme values and to deduce entrance dose values by means of ion chambers.

The computerization is being made by recording the following parameters: keys for the centre, room, examination and radiographer; date; register number in the database (which connects it with other files or bases); age, sex, approximate weight and height and initials of the patient; total number of exposures in the examination; number of useless exposures; number of radiographic films; time of radioscopy; mAs and kVp; dose x area product; breast, gonad and thyroid dose values (if TL readings are available); focus-to-skin and focus-to-film distances; entrance dose if recorded and Monte Carlo factor table which should be used for the evaluation of effective dose. In radioscopy it is also recorded the screening time; mAs and kV in units when automatic brightness control is not used and dose x area product. In order to obtain average weighted values of dose at a local level, the number of examinations per year carried out in each room is also being introduced in the databases.

This computerization allows for the obtention of reference dose values, with a similar criterion to that of the EC expert group, for the dose at the entrance and for the dose x area product in 'complex' examinations, under which there would be 75% of the patients undergoing this type of examinations in the Madrid area. These values which could be considered as a reference to survey the optimization process, together with the analysis of image quality allow the establishment of prioritary QC actions. An approach to the reference levels given by the EC expert group is searched when the values at the domestic level are higher.

The databases are allowing, together with a program of additional actions carried out during the last year with anthropomorphic phantoms, to apply our own calculation method for organ dose and effective dose (for complex examinations) estimates.

C) Results. Image quality.

Image quality in radiography has been evaluated in 57 rooms along 1990, verifying a deficient quality in 13 of them, where it has already been detected the cause of anomalies and it has been proposed the appropriate corrective actions (mostly, adjustments or substitution of processors and changes of spoilt

cassettes).

Image quality in radioscopy has been evaluated in 16 units, having been detected 4 under tolerance standards and 5 in which improvements must be observed when optimizing the working parameters. Equipment under limits are being replaced.

The most frequent causes of the anomalies in image quality in 15 mammography units tested have been, among others, kVp inaccuracies, processor malfunctioning, failures in the automatic exposure control system and in the setting of mobile grids, unsuitable (or inexisting) filtering and unsuitable parameter selection.

D) Results: dose evaluations.

Patient dose controls have been carried out during the last year in 25 rooms. Four of them with anomalous dose values are waiting for corrective actions; in the rest, dose values have come out similar to or lower than the reference values.

The three step QC action cycle above mentioned, planned for the biannual project, has been fulfilled in some cases during 1990 when the dose values turned out to be very high and/or when the corrective actions were simple enough and of low cost. Lack of filtration, deviations and failures in the high voltage generator, use of obsolete image systems, processor malfunctioning and operating procedures not always optimized have been the most frequent causes of anomalous dose findings.

Specific evaluations at six paediatric rooms of two different hospitals have been carried out. Estimates of entrance dose values for different types of examination have been obtained for separate age groups. Image quality has been also evaluated. Corrective actions, such as change of cassette type and screens, adjustments in generators and processors, use of gonadal protectors, and similar ones have been proposed in almost all the rooms.

PUBLICATIONS

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Progress Report

Contract: B17-054 **Sector: C22**
Title: Diagnosis related doses: a comparative investigation in some European hospitals

1. Van Loon Univ. Hospital VUB
2 Thijssen Univ. Nijmegen Acad. Hospital

I. Summary of Project and Global Objectives

The main sources of low dose radiation to the population of Europe are the medical diagnostical examinations in radiology and nuclear medicine. The amount of radiation given before a certain diagnosis is made, depends on factors of medical, technical and/or organizing character.
- In the medical sector we identify the justification of an examination and the protocol leading to a diagnosis. Little work is done on the influence on the dose by these items so far.
- In the technical sector many variables are identified and great effort has been noticed to evaluate the effects of quality assurance and to establish guide-lines for the improvement of image quality and the reduction of patient dose.
- In the organizing sector many extrinsic and logistic factors -e.g. factors that are linked to the differences in the organisation of the health services- can compete with an optimum cost/benefit relation.
No effort has been made to relate the (estimation of) doses and therefore the risk to establish a correct diagnosis in different European Hospitals, to those three factors. One method that can contribute to a better understanding of this relationship is the concept of diagnostic groups (DG's): a DG is the set of examinations that has led to one and the same diagnosis.
Euratom directive 84/488 of Sept 1984 recommends the implementation of measures to lower the dose of ionizing radiation to patients in therapy and diagnosis. Many investigations have been made on mainly technical aspects leading to quality assurance protocols in diagnostic radiology (DR) and evaluations of mean patient doses and risks The ALARA principle as quality assurance tool includes beside these technical aspects also socio-economical factors. The justification of an examination and the protocol leading to a diagnosis show a wide variety and influence the dose to patient, as do factors linked to the differences in organization of health services.
Economic and technical benefit expected are:
- contribution to the elaboration of recommendation or guide-lines for the optimization of diagnostic radiology procedures to lower the exposition of European population to ionizing radiation, and:
- a better use of of national health resources.The object of this program is to develop and test a method for evaluation of a diagnosis related dose. The tool developed in this pilot study may be used for evaluating the diagnosis related doses in European hospitals on a large scale.
This pilot study is devoted to the inventory of existing useful methods of dose assessment in DR; the actual entrance dose in standard condition of different hospitals will be compared by thermoluminescent dosimetry and some ionization chamber measurements. This dosimetry will be based on the work done by the cooperative

group "Dose Assessment and Evaluation of Risk" coordinated by B. F. Wall (NRPB-UK) . A few diagnostic groups (DG) for the pilot study will be selected: the medical teams will evaluate the existing work done in the field, define relevant groups of diagnosis and test that in the participating hospitals. Once the procedure is established, the teams will analyse a number of relevant patient files; the inventory will be made of the different radiological examinations performed before the diagnosis is definite; the corresponding dose per exam will be calculated. The physical/technical details and the medical parameters together will yield the received dose per DG for each participating hospital. Finally the applicability and validity of the total procedure will be tested by analysing the different data sets per DG.
The first question to be answered will be: is it possible to collect the relevant medical and technical data with this procedure for a sufficient number of patients?; the second is: which other elements are detectable within diagnostic procedures that are related to the doses per diagnosis, such as organization of health services and other reasons for the use of a certain protocol.

The Global Objectives can be summarized as follows.
- Inventory of diagnoses that are frequently encountered and require examinations involving a medium amount of ionizing radiation.
- Selection of the most suitable ones regarding the availability of the information of the number and the type of examinations, clarity of the entry and exit points, of the gold standard (unambiguous "proof" of the correctness of the diagnosis), criteria of exclusion. This selection will be done after a test on a limited number of patient files.
- Establishment of patient and examination data collection sheets and collection of data in the different hospitals. The ultimate goal would be to collect data in several different countries with different health service organisations medical infrastructure and traditions. This pilot study will only "compare" same diagnosis in hospitals in two countries, Belgium and the Netherlands.
- Agreement on the dosimetric procedure to establish a dose to organ or energy imparted to the patient comparable between the two countries. Also, this procedure will be linked to previous dosimetric studies done in the EC, the NPRB protocol will be analysed on applicability in this project, in cooperation with the NRPB, and TLD dosimetry of entrance dose will be compared to NRPB dosimetry by mailing.
- Collection of dosimetric data for the examinations currently used in the selected diagnoses. University hospitals were choosen, since for most examinations standard procedures are used, so retrospective analysis will give a good approximation of the received dose. Important parameters for later evaluation will be collected: kVp, geometry, sensitometry, size, density, etc. ..

Head of Project 1: Prof. Van Loon

II Objectives reporting period

- Search for diagnoses that could enter the study
- Elaboration of a model protocol for inclusion in the study, and data collection form for patient data and for the different examinations
- Evaluation of the TLD dosimetry: possibility of local read-out of TLD's, comparison with read-out in Nijmegen and linking to the NRPB calibration.
- Set-up of the hardware in Radiology department AZ-VUB for determination of the necessary parameters of the X-ray beams (classical X-ray) : kVp, geometry, sensitometry, phantoms.

III Objectives for next period

- Further investigation of suitable diagnostic groups
- Further evaluation of the TLD-dosimetry and comparison with NRPB
- Collection of patient data for "Lumbar Hernia Discalis" and for "Renal Cell Carcinoma"
- Inventory of the different radiological examinations performed before the diagnosis
- Assessment of the dose per exam for the relevant examinations
- Evaluation of the procedure

IV Progress achieved

Several meetings have been held for discussion of possible diagnoses. Brussels suggested: radiological diagnosis of bone metastases of the spine (Bordet Cancer Institute), acute pyelonephritis in children (Reine Fabiola Children's Hospital), Lumbar Hernia Discalis (AZ-VUB); draft proposals from Nijmegen included Renal Cell Carcinoma,
A consensus was reached on the "Lumbar Hernia Discalis" as a diagnosis to be included in the study. The general outline was followed to have a clear protocol for inclusion. This led to following procedure:
DIAGNOSIS: detection by imaging procedures of a hernia discalis resulting in surgical treatment;
GOLDEN STANDARD: hernia discalis was proved by surgery (operation protocol)
EXIT POINT: surgical treatment of the hernia
ENTRY POINT: first radiological examination following the complaint of LBP or sciatalgy;
CRITERIA OF EXCLUSION: only accepted patients are those where we can account for all their radiological examinations, even with some in other hospitals; excluded are patients with a previous surgery for hernia discalis.
EXAMINATIONS FOR DOSIMETRIC STUDY: standard radiography, conventional tomography, CT, MRI, myelography, discography and other pre-op examinations; including those in other centers;
DATA COLLECTION: data sheet was elaborated with a chronological summation of all the radiological examinations and their technical data.
A first search was done in the patient files of the AZ-VUB hospital, and a sufficient number (over 30 patients already) can be included, and sufficient technical details were available.
This protocol was transmitted to the colleagues in Nijmegen for feasibility study in their hospital(s).
TLD dosimetry at radiation levels encountered in diagnostic radiology was tested: complications arose with the TLD equipment (although a very performing modern system), but in close cooperation with our colleagues of Nijmegen a reproducibility of ±2% was achieved. Agreement was achieved with the NRPB team on a mailing of a number of TLD's to NRPB to cross check our dosimetry.

The University provided funds for Quality Assurance equipment, and a transmission chamber (Diamentor®) and a Digi-X® are currently available for determination of energy imparted to the patient and for peak kV values, total filtration or HVL,...
Discussions were started on the procedure to be followed and the phantoms that will be used in CT and diagnostic radiology examinations, and on the way to link film density and image quality to entrance dose.

Head of Project 2: Dr. Thijssen

II Objectives reporting period

- defining the limits for this pilot study to maximize the value of the result
- find radiological examinations that are suitable within these limits
- define entry and exit points for individual patients to be included in the study
- create the possibility to measure the dose and parameters

III Objectives next period

- production of data collection from for patient data
- collection of patient data on two selected diagnostic groups
- calibration of the TLD measurements to the NRPB- system
- evaluating the time-effects on our TLD-materials for the exchange of TLD chips by mail
- selection of the method and exams to calculate the entrance dose and radiation risk per exposure
- set-up and test of a measuring protocol for the examinations
- evaluation of the procedure

IV Progress achieved

After several meetings two diagnostic groups were selected to start the pilot study. The main problem was to limit the examinations to a diagnosis and to exclude examinations of which the dose can not be estimated, e.g. exposures that are given before the patient entered the hospital
On the first diagnostic group -hernia discalis- half of the aimed number of patients is already selected. The possibility of the proposed data form is evaluated.
The dosimetric part has started with the selection of a colleague-physicist to perform the calibrations and measurements. To be able to line up with earlier data the NRPB was visited.
The TLD-measuring system of the University was calibrated for new TLD chips in cooperation with the Brussels team. A high reproducibility is obtained.

Contract: Bi7-057 **Sector: C22**
Title: Patient dose from radiopharmaceuticals

1	Mattsson	Univ. Lund
2	Smith.	MRC Clinical Research Centre
3	Henrichs	GSF Neuherberg

I. Summary of Project and Global Objectives

The general goal of the project is to improve the accuracy of the current data on absorbed dose from radiopharmaceuticals to patients and to produce dose data for new radiopharmaceuticals. There is a specific interest in data for children of various ages.
This will be done by:

1. Improving the biokinetic data for selected radiopharmaceuticals by repeated uptake and retention measurements on patients and healthy volunteers. Special attention will be given to:

a) new radiopharmaceuticals recently taken into clinical use and
b) biokinetics and dosimetry for children and new-born.

2. Improving the physical basis for the dose calculations by using detailed voxel-phantoms based on CT/MR data for Monte Carlo calculations of new S-values, especially for children.

The results will be used to quantify organ doses as well as effective dose equivalents or effective doses. The results will enable comparison of different radionuclides for labelling and of different pharmaceuticals in the view of balancing diagnostic benefit and radiation dose and to determine the appropriate activity to be administered in order to avoid unnecessary radiation exposure of the patient.

Head of Project 1: Dr. Mattsson

II Objectives for the reporting period

Biodistribution and retention will be studied in patients undergoing investigations with new radiopharmaceuticals. For these studies gamma-cameras as well as whole body counters will be used together with measurements on samples of blood, urine, etc. Biokinetic data will be collected for newborn and children up to 18 years of age who are subjected to nuclear medicine investigations, whenever such opportunities arise. Experimental measurements will be carried out on the excretion through breast milk, of radiopharmaceuticals for which there still is a lack of data.

III Objectives for next period

During the remaining time of the project we will continue
- to collect biokinetic data for new substances and for substances for which there is a lack of data
- to collect biokinetic data for children and newborn
- to make models and dose estimates
- to report and publish the results

IV Progress achieved including publications

Since the beginning of the project on Sept 1st, 1990, the work has been concentrated on experimental studies of the biokinetics of radiolabelled HM-PAO, MIBG and MIBI and of the excretion of radionuclides in breast milk.

Tc-99m HM-PAO in children

At the Göteborg East Hospital, the biokinetics of Tc-99m HM-PAO has been studied in eight children aged between 7 weeks and 18 years. In addition to the routine SPECT study of the brain, the children were scanned with a gamma-camera at 1, 7 and 24 hours post injection. Urine was collected during a 24 hour period. The activities in the brain, lungs, liver, kidneys and intestines were estimated using conjugate counting and biological half-times were calculated.
There is a strong age-dependence on the uptake in the brain, with high values (20%) for the very young children, four times higher than those reported for adults (5%). The uptake in the other organs showed no similar age-dependence.
The absorbed doses to various organs were calculated with the standard MIRD formalism using published S-values for children of various ages. The organs or tissues receiving the highest absorbed doses are the gall-bladder wall, bladder wall, kidneys, liver and upper large intestine wall.
The effective dose (equivalent) per activity unit for a new-born (0.11 μSv/MBq) is more than one order of magnitude higher than the value for an adult (0.008 μSv/MBq).

In a separate study, it was found that if the same activity per unit body-weight is given to patients of various ages, this gives equal image quality. With this dosage method patients of different ages will receive about the same effective dose equivalent from an examination with Tc-99m HM-PAO independently of age.

I-131 MIBG in children

MIBG is used for the diagnosis and staging of neuroblastomas in children. MIBG labelled with I-131 can also be used for radiotherapy. At Göteborg East Hospital and Sahlgren Hospital, the biokinetics and dosimetry of I-131 MIBG in two patients, a boy aged 3.5 years (one diagnostic study and one treatment) and a girl aged 7 years (two diagnostic studies), have been studied up to now. The children were scanned at 3, 24, 48, 72 and 96 hours p.i. The activity in the liver, the tumours and in the whole body was estimated. Blood samples were taken at 5, 15, 30. 60 and 120 and 240 minutes and at 24, 48, 72 and 96 hours p.i. The clearance from blood was very rapid.
Bone marrow aspiration was done at 24 hours p.i. and the activity in the bone marrow was measured. The study is ongoing.

Tc-99m MIBI in adults

At Malmö General Hospital, ten adult patients who were undergoing myocardial perfusion imaging with Tc-99m MIBI (methoxy isobutyl isonitrile), were followed with respect to the biodistribution and retention of Tc-99m. Gamma-camera whole body scans were performed at 15 minutes, 6 hours, and 24 hours post injection. Blood samples were taken on the same occasions. Urine was collected up to 24 hours p.i. The activities in different organs and tissues were determined using the geometric mean of posterior and anterior counts corrected for attenuation in the body. The absorbed dose to different organs and tissues were determined using the MIRD formalism and the effective dose was estimated.
The substance is to a large extent eliminated through the hepato-biliary system and excreted via the gall bladder through the gastro-intestinal tract and through the kidneys. Consequently, the upper large-intestine (50 uGy/MBq)), lower large-intestine (22 µGy/MBq), kidneys (29 µGy/MBq) and gallbladder (27 µGy/MBq), receive the highest absorbed doses. The effective dose was estimated to 0.01 mSv/MBq, somewhat higher at rest and somewhat lower at stress. Tc-99m MIBI does not redistribute in the myocardium and for a full investigation, at rest and during stress, two injections have to be given. This gives a total effective dose of 13 mSv for a complete investigation.

Excretion of Tc-99m labelled radiopharmaceuticals in breast milk

At Malmö General Hospital, with the assistance of several other south Swedish hospitals, new data have been collected concerning the excretion of Tc-99m labelled radionuclides in breast milk.
The following substances and number of patients have been evaluated within this project:

Radiopharmaceutical	Number of patients	Extrapol excreted fraction per ml at time of injection	T 1/2	Excreted fraction of inj avtivity #)
Tc-99m pertechnetate	7	10^{-3}	2.7 h	7%
MAA	11	10^{-4}	4.8 h	7%
DTPA	2	10^{-6}	4.4 h	0.04%
MDP	3	10^{-6}	4.5 h	0.04%

#) Assuming breast feeding every 4th hour, starting 4 hours after injection, the child is given 140 ml at each occation.

The activity concentration in breast milk is relatively independent of the total milk volume. The difference in $T_{1/2}$ between pertechnetate and Tc-MAA primarily reflects the rate of Tc loss from MAA. The low excretion in breast milk for Tc-99m DTPA and MDP is due to the fact that these substances are excreted through the kidneys and bound to the skeleton respectively. Tc is also firmly bound to these substances.

Publications:
Leide S, Diemer H, Ahlgren L and Mattsson S: In vivo distribution and dosimetry of Tc-99m MIBI in man.
To be published in Proc 5th International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, Tennessee, U.S.A., May 7-10, 1991.

Vestergren E, Jacobsson L, Mattsson S, Bjure J, Sixt R Uvebrant P
Biokinetics and dosimetry of Tc-99m-HM-PAO in children.
To be published in Proc 5th International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, Tennessee, U.S.A., May 7-10, 1991.

Head of Project 2: Dr. Smith.

II Objectives for the reporting period

To carry out internal dosimetry studies on patients undergoing clinical investigations and on volunteers to whom radiopharmaceuticals are administered specifically for dosimetry. In addition, every effort will be made to obtain biodistribution and biokinetic data for newborn and children whenever such opportunities arise. These studies will be supported, when appropriate, by experiments with phantoms.

III Objectives for next period

During the remaining time of the project we will continue
- to finish the study of the biokinetics and dosimetry of Tc-99m P53
- to extend the Tc-99m S12 (antisclerotic plaque antibody) study.
- to make models and dose estimates
- to report and publish the results.

IV Progress achieved including publications

Tc-99m P 53 in adults.
Dosimetric studies of a recently developed myocardial perfusion imaging agent, Tc-99m-P53, a lipophilic technetium phosphine cation, has been carried out.
Following pre-clinical studies in animals (not part of the CEC contract), Tc-99m P53 was investigated at Clinical Research Centre at Northwick Park Hospital, Harrow, and at the Royal Infirmary at Aberdeen. While image anlysis and sample measurement were performed independently at the two centres, subsequent analysis of all aquired data and the dosimetric evaluation were performed at the Clinical Research Centre.
Six normal healthy male volunteers (22-35 years old) were recruited at each centre and each subject was investigated on two occasions, first at rest and not less than 7 days later, after exercising to 85% of peak heart rate. The measurements include quantitative whole body imaging with anterior and posterior views on 8 occasions between 5 minutes and 48 hours post-injection; monitoring of whole-blood and plasma clearance up to 24 h, and total urinary and faecal clearance up to 48 h. Regions of interest were used to determine whole-body counts in anterior and posterior views and in various organs, which showed significant activity content, at least in the early post-injection period. In particular, the organs were heart, liver, lungs, kidneys, salivary glands, gall-bladder, urinary bladder and gastrointestinal tract.
Whole-blood activity fell rapidly to less than 0.2% of administered activity per litre within 30 minutes. Whole-body retention fell rapidly during the first hour and was only

about 20% after 48 h. After exercise, whole-body retention was consistently about 10% higher than that at rest, largely as a result of enhanced uptake in leg muscles. Excreted activity was roughly equally shared between the urinary and faecal routes and approximately 80% of the administered activity was accounted for in total 48 h excreta. Residence times were determined for the organs listed above and for the "remaining body". Dose estimates were done according to MIRD (MIRDOSE 2 software). At rest the highest dose was calculated to the gall-bladder (49 µGy/MBq), followed by the GI-tract (17-39 µGy/MBq), urinary bladder (19µGy/MBq) and salivary glands (12 µGy/MBq), with all other organs receiving less than 10 µGy/MBq. After exercise, doses to these organs were reduced to about 70% of those at rest, but total body doses were similar in both studies. The effective dose was 8.9 µSv/MBq at rest and 7.1 µGy/MBq after exercise, assuming a bladder voiding period of 3.5 hours.

Studies similar to those described above have been initiated to estimate the dosimetry of Tc-99m S12, an antibody against arthrosclerotic plaque. At the date of this report, two studies on patients undergoing investigation of peripheral vascular disease using Tc-99m S12 have been performed but are yet not fully evaluated.

Publication:
Smith T, Lahiri A, Gemmell H G, Davidson J, Smith F W, Pickett R D and Higley B
Dosimetry of Tc-99m P53. A new myocardial perfusion imaging agent. To be published in Proc 5th International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, Tennessee, U.S.A., May 7-10, 1991.

Head of Project 3: Dr. Henrichs

II Objectives for the reporting period

By means of the voxel phantoms developed by dr Drexler et al., specific absorbed fractions and specific effective energies , should be calculated. There are voxel phantoms, derived from CT-pictures of one 7 years and one 4 weeks old child available. The calculations will enable us to compare the results of this method to that using mathematical anthropomorphic phantoms and to decide whether the improvements, which may be expected especially for children, are sufficiently substantial to continue with the development of other voxel-phantoms (other ages) for internal dosimetry.

III Objectives for next period

During the remaining time of the project we will continue
- to improve calculational procedures
- to quantitatively compare the results (dose coefficients) obtained for important radiopharmaceuticals with those based on earlier anthropomorphic phantoms.
- to publish and report the results.

IV Progress achieved including publications

Up to now the work has been concentrated on the development of tools to simulate the transport of radiation within the body. The method chosen uses Monte-Carlo techniques. The necessary geometric information concerning the positions, sizes and forms of the important tissues and organs is taken from a computer-tomography investigation of a 7-weeks old baby. For the simulation of the radiation transport, an existing computer code was used which was developed for the dosimetry of external irradiations, i.e. for diagnostic radiology, by Drexler and Zankl, GSF. The program was modified to allow the use of arbitrary internal sources in any tissue of interest.
The code was developed and tested on a parallel-computer, which is especially well suited for Monte-Carlo simulations. The computational power of such a computer guarantees the possibility to reduce statistical uncertainties as far as necessary (at present below 10%)
The results of the first runs are expected at the end of this spring.

Progress Report

Contract: Bi6-128 **Sector: C24**

Title: Methodology for evaluating the radiological consequences of radioactive materials released in accidents including uncertainty analysis and economic impact.

1 Kessler KfK Karlsruhe

I. Summary of Project and Global Objectives

The overall objective of the project is to develop improved and more complete models, data sets and systems for use in probabilistic accident consequence assessment (ACA). This work is being undertaken primarily in close cooperation between the Kernforschungszentrum Karlsruhe (KfK) in FRG and the National Radiological Protection Board (NRPB) in the UK.

An important objective of the project is to maintain and further develop the ACA program package COSYMA (Code System from MARIA) for use within the European Community. The full COSYMA system, intended for detailed studies and research on a large computer, has been distributed through the Commission of the European Communities to interested parties within the EC and other countries. However, its support and maintenance (including modelling and data improvements and up-dates), and continual adaptation to the needs of users continue to be a significant part of the project, much of this work being undertaken at KfK.

A second and more simple COSYMA system is now being developed, intended to allow non-expert users to undertake ACA studies. This system will enable more routine accident consequence assessments to be performed on a small computer, such as a PC. A feature of this system will be an interactive interface. Much of the work on the simplified COSYMA system is being undertaken at NRPB. It is possible that in the future alternative simplified systems could be developed which would contain different degrees of flexibility but which would be consistent in basic assumptions and data.

Specific modelling aspects of the work which is largely being undertaken at KfK includes the refinement of models for assessing the off-site economic costs of accidents, for calculating deterministic and stochastic health effects and the corresponding loss of life expectancies.

The project also includes code distribution and giving advice to users during the implementation, test and assessment phases. In particular, during the NEA/CEC Intercomparison Exercise on Probabilistic Accident Consequence Assessment Codes, this will become an important aspect, when results from COSYMA obtained by different users have to be analysed and interpreted.

NRPB topics under this contract are described in an attached Progress Report.

Head of Project 1: Prof.Dr. Kessler

II Objectives for the reporting period

After the completion of the program package COSYMA and the presentation of its main features and ingredients during a CEC seminar in Athens in May 1990 /1/, the international distribution of the code, the preparation of its documentation and the feedback with future users were the main aims of the reporting period, in particular with respect to the NEA/CEC Benchmark exercise beginning in 1991. To that purpose the released version had to be completed by inclusion of options and modifications which emerged from discussions with potential users and the analysis of example runs.

III Objectives for next period

Besides code maintenance and up-dating, the participation at the NEA/CEC Benchmark exercise and the analysis and interpretation of results obtained with COSYMA running at different institutions will require a significant amount of effort. This work includes the preparation of data sets for dose and health risk calculations based on NRPB data. Work on preparing a simple version of COSYMA for use on a PC will be performed in close cooperation with NRPB.

IV Progress achieved including publications

1. The program package COSYMA

As a result of a joint venture between KfK and NRPB, the program package COSYMA has been developed within the MARIA programme. During a seminar held in Athens in May 1990, its structure, models, data sets and endpoints have been presented and discussed. To prepare the distribution of COSYMA to interested parties in the EC and other countries, a one week training course has been organized with support of CEC at KfK from 17 to 21 September 1990. To that purpose, a variety of documents were prepared including an overview report /2/, the user guide /3 /, input and output examples, and detailed modelling information. The 39 participants came from 11 European and 7 other countries.

As a result of the discussions during the meetings mentioned above and the example runs performed with COSYMA, several modifications were necessary, such as missing options, recognized modelling weakpoints and lack of data. Special aspects were the inclusion of hereditary effects and the quantification of loss of life expectancy in a simplified manner, the completion of the ingestion pathway with different options for calculating collective doses and for introduction of food-bans, the preparation of the land-sea matrix, and a more flexible coding of countermeasures and economic modelling. These improvements are contained in the Version 90/1 of COSYMA, which has been released in February 1991. The corresponding documents - in particular the user guide - have been up-dated to take account for the modified input/output options. In the meantime, 16 institutions in the EC and other countries got copies of the program package for implementation on their computers together with revised documentation.

2. Expert judgement

The probability distributions and correlations placed on model parameters in the extensive uncertainty and sensitivity studies with UFOMOD /4/ were derived from internal discussions among those involved in model development and from literature reviews. With the aim to develop and standardize knowledge acquisition and elicitation techniques for uncertainty analyses, the CEC is supporting an expert judgement study focused on the dispersion-deposition module of COSYMA/NE. The principal contractor is the Department of Mathematics and Informatics at the Delft University of Technology in The Netherlands. NRPB and KfK are providing technical and logistic cooperation. Within Germany, 10 experts from 6 institutions could be identified, who agreed to be interviewed about uncertainty distributions of variables relevant for atmospheric dispersion and deposition processes.

3. NEA/CEC Benchmark study

An international code comparison exercise sponsored by CEC and OECD/NEA was initiated during the 1st Meeting of the ad-hoc group on probabilistic accident consequence assessment codes, Paris, 16-17 January 1991. The exercise is coordinated and managed by a small project management group comprising representatives from CEC, NEA, KfK, NRPB, SRD and USNRC, whereby a large part of the planning for the exercise has been carried out by SRD.

During the 'pre-launching'-period, a basic document has been prepared, containing the main objectives, the task specifications and the endpoints of the Benchmark calculations. Based on a questionnaire distributed to potential participants, an additional technical document has been prepared by the project management group with data and instructions needed when doing the code comparison calculations. In particular, it contains detailed descriptions of gridded data, meteorological and economic data, source terms, and result sheets for each consequence to be calculated. Before the calculations start, an informal meeting will take place in April 1991, during which any residual problems can be discussed and clarified, which may arise from the final specifications and from implementation of the various data bases provided by KfK, NRPB and SRD.

4. Other Activities

To generate a simplified version of COSYMA for smaller computers and less experienced users, computing times and storage requirements have to be reduced, and model simplifications have to be considered leading to a smaller list of options and modelling details neglecting those which are unimportant in standard applications. Investigations are under way in close cooperation with NRPB to determine the specifications of simplified versions for special purposes without significant altering the model predictions in the corresponding area of application.

In conjunction with the extension of the economic model to consider site-specific characteristics, the evacuation/relocation cost calculations will be based on the number of employees in different economic sectors rather than using total GDP values on a per capita basis. The respective statistical data for Germany acquired during the last National Census in 1989 are presently processed for further use within the COSYMA grid systems.

References

/1/ Proceedings of the "Seminar on methods and codes for assessing the off-site consequences of nuclear accidents", Athens, 7 to 11 May 1990, Vol. 1 and 2, Commission of the European Communities, Report EUR 13013, 1991

/2/ COSYMA: A new program package for accident consequence assessment. Joint report by Kernforschungszentrum Karlsruhe GmbH and National Radiological Protection Board. Commission of the European Communities, Report EUR-13028 (1991)

/3/ I. Hasemann, J.A. Jones
COSYMA: Program description and user guide
Commission of the European Communities, Report EUR-13045 (1991), Report KfK-4331 B (1991)

/4/ F. Fischer, J. Ehrhardt, I. Hasemann
Uncertainty and sensitivity analyses of the complete program system UFO-MOD and of selected submodels
Kernforschungszentrum Karlsruhe GmbH, Report KfK-4627 (1990)

Contract: Bi6-127 **Sector: C24**

Title: Methodology for evaluating the radiological consequences of radioactive effluent released in accidents.

1 Cooper NRPB

I. Summary of Project and Global Objectives

The overall objective of the project is to develop improved and more complete models, datasets and systems for use in probabilistic accident consequence assessment (ACA). This work is being undertaken primarily in close cooperation between the Kernforschunsgszentrum Karlsruhe (KfK) in FRG and the National Radiological Protection Board (NRPB) in the UK.

An important objective of the project is to maintain and further develop the ACA program package COSYMA (Code System from MARIA) for use within the European Community. The full COSYMA system, intended for detailed studies and research on a large computer, has been distributed through the Commission of the European Communities to interested parties within the EC and other countries. However, its support and maintenance (including modelling and data improvements and up-dates), and continual adaption to the needs of users continue to be a significant part of the project, much of this work being undertaken at KfK.

A second and more simple COSYMA system is now being developed, intended to allow non-expert users to undertake ACA studies. This system will enable more routine accident consequence assessments to be performed on a small computer, such as a PC. A feature of this system will be an interactive interface. Much of the work on the simplified COSYMA system is being undertaken at NRPB.

Specific modelling aspects of the work which are largely being undertaken at NRPB include the development of models for the off-site economic costs of accidents, for the calculation of external γ dose from deposited material, (particularly in urban areas) and the formulation of advice so that an appropriate choice among the available models for atmospheric dispersion can be made. The project also includes an assessment of the uncertainty in the results of ACA codes, and work to describe the ranges of parameter values using expert judgement.

KfK topics under this contract are described in an attached Progress Report.

Head of Project 1: Dr. Cooper

II. Objectives for the reporting period

The COSYMA system is an important tool for research and application, and as such requires continuous up-dating, testing and improvement. The code and its databases were to be maintained and improved throughout the period of the project by KfK and NRPB. The increasing availability of small computers, and the need of people without experience in ACA code development to carry out routine analyses, necessitate the development of a reduced and simplified version of the code. Work to develop this simplified system was to be carried out.

Continuing work was to be carried out on the analysis of the uncertainty in the predictions of ACA codes. This included the use of expert judgement to determine the ranges of values for the input parameters.

Work was to continue on the development of the COSYMA programs by including the results of detailed models for deposited γ doses in urban areas in COSYMA. Work on the validation of the EXPURT model was also to be carried out. Work was to be carried out to allow advice on the choice of atmospheric dispersion models for different applications of ACA codes to be given.

III. Objectives for the next period

The work on preparing a simple version of COSYMA for use on a PC will continue, so that the first version of the system will be available by the end of the reporting period.

The work to give advice on the choice of atmospheric dispersion model for use in ACA codes will be finalised, so that clear advice on this topic can be given. Work will continue on the development of models for deposited γ doses in urban areas. This will concentrate on 2 areas, namely ways in which ACA codes can use the detailed results which can be obtained from the EXPURT code, and the improvement of the EXPURT model to include the dose from material on trees. Work to compare the predictions of different models for the economic costs of accidents will continue.

IV. Progress achieved including publications

The Simplified Version of COSYMA

A number of runs of the NE and NL sub-systems of COSYMA have been carried out to examine the feasibility of running the system on a small computer, and to see where the complex models contained in the full system could be simplified. A series of probabilistic runs of the NE sub-system with a range of simplifying assumptions have been carried out for 2 source terms for a PWR at a UK site. Source term A represented a very large degraded core accident at a PWR, while source term B was a smaller accident in which about 10% of the volatile material was released.

Most of the simplifications considered here did not affect the calculations of the numbers of people who would need to be evacuated, but did affect the dose which those people would receive, and hence the predicted numbers of early effects. In some cases, the simplifications had different impacts on the different source terms.

The model included in COSYMA for the actions of the population during the sheltering and evacuation period is very complex. The results of the sensitivity studies considered here showed that these parts of the model could be simplified without altering the predicted numbers of early deaths by more than about 50%, and generally by only about 10%.

Both source terms show a sensitivity to the number of phases assumed for the release and therefore the ability to consider a release as a series of phases should be kept in the simplified version of COSYMA.

Choice of Atmospheric Dispersion Model

Comparisons between the results of calculations in which wind direction changes during travel were and were not considered have been carried out as part of the process of determining what should be in the simplified version of COSYMA. The results are described above.

Uncertainty Analyses

This progress report summarises the work done in an analysis of the uncertainty in the predictions of the consequences of hypothetical accidental releases of radioactive material to atmosphere using the MARC-2A computer program. The analysis follows on from earlier work in which the uncertainty in the predictions of some of the individual modules of the MARC program was analysed. However in this analysis the uncertainty in the predictions of the whole of the program was considered.

The study considered the uncertainty in the parameter values describing the transfer of material through the environment from the release point to man, the calculation of doses from the concentrations of material in air, in foods and deposited on the ground, the health effects arising from those doses and some aspects of the economic costs of the accident.

A total of 98 uncertain input parameters was considered and 150 runs of the MARC program were carried out for each of five analyses considered. Each of these runs of MARC included an assessment of the consequences of the release in about 100 sequences of atmospheric conditions. The output of the MARC-2A program is in the form of complementary cumulative distribution functions for each of a number of endpoints of the accidental release. These include the numbers of health effects in the exposed population, the impact of countermeasures in terms of the numbers of people evacuated or relocated, the amount of agricultural produce which could not be consumed due to food restrictions, and some indicators of the economic cost of the accident. The quantities considered in the analysis were the expectation value and some of the percentiles of the ccdfs for the different endpoints. In addition, the amount of CPU time required by the programs was also analysed. The uncertainty in each of these endpoints was considered, and the input parameters whose uncertainty makes major contributions to the overall uncertainty were identified for each. However, only the results for early death and fatal cancer are described in this progress report.

The study investigated the uncertainty in the predicted consequences of two hypothetical accidental releases from a Pressurised Water Reactor (PWR) in the UK. The releases considered were those designated UK1 and CB2. UK1 represents a very large degraded core accident, and has been found to dominate the predicted risks of early death from all accident sequences considered in risk assessments for the reactor design. CB2 represents smaller but more likely accidents. Both source terms make major contributions to the overall risks of late health effects from the reactor.

The most important parameter uncertainties contributing to uncertainty in the number of early deaths were identified using PRCCs and PCCs. This analysis included several uncertain parameters related to calculating numbers of health effects from skin irradiation. One of these has been identified as the

most important parameter uncertainty for early deaths. A second one is also highly ranked. Given the preliminary nature of this analysis, it seems reasonable to identify the whole topic of prediction of early health effects from skin irradiation as an important source of uncertainty rather than trying to identify specific parts of the calculation of skin effects as being the ones with the most important uncertainties. Similar remarks apply to the model for calculating deposited γ doses in urban areas, and it is probably reasonable to identify the calculation of deposited γ doses in urban areas as being an important source of uncertainty in the MARC model.

The upper envelope of the ccdfs for fatal cancers for UK1 from each run of MARC is roughly twice that corresponding to the ccdf for the best estimate parameter values, while the lower limit is about an order of magnitude below the best estimate value. Similar ranges are observed for the smaller CB2 source term. The most important parameter uncertainties contributing to the overall uncertainty in the expectation value of fatal cancers for UK1 are the risk coefficient at high dose rates and the DREF are the two highest ranked parameters. A number of parameters have reasonably high values of the indicators. The ordering of these lower ranked parameters derived from the different indicators is rather different, with parameters ranked highly by one indicator not necessarily having a high ranking according to other indicators. Similar results were obtained for the CB2 source term.

Expert Judgement

The ranges adopted for the parameter values in the MARC uncertainty analysis were obtained without using formal techniques of expert judgement. This topic is being covered by a group at the University of Delft in a separate CEC contract. NRPB, together with KfK, is assisting in this work in a number of ways; in the study the uncertainty in the atmospheric dispersion part of consequence assessments is being examined. We have had a number of discussions with, and given advice to, the researchers at the University of Delft.

Deposited γ Doses

A model was developed at NRPB several years ago for calculating the external γ dose from material deposited in urban areas; the model includes the effects of transfer of the deposited material between different surfaces in the urban area. The model was developed using data available prior to the Chernobyl reactor accident which are mainly based on studies on caesium. Several studies and measurement programmes have been carried out since Chernobyl, particularly in Scandinavia and Germany, and data from these studies are now being used to test EXPURT predictions. Areas which are being tested include the modelling of processes such as the partitioning of material onto various surfaces during wet and dry deposition and the weathering of material off buildings and roads. Data on the behaviour of nuclides other than caesium, particularly iodine and ruthenium, on building surfaces are also available. These are being used to examine the uncertainty of the EXPURT predictions when used for nuclides other than caesium.

MECA/COCO-1 Economic Model Intercomparison

Under previous stages of the MARIA project, two models for predicting the economic consequences of accidents have been developed. The COCO-1 model was developed jointly at NRPB and KfK and has been incorporated into the COSYMA code. The MECA model has been developed at the University of Madrid and is appropriate for use in conjunction with the MACCS ACA code developed in the US.

In this phase of the study, a draft report comparing the two models was produced, prepared by Dr Christopher Heady of the University of Bath in the UK. This report has indicated the relative strengths of the two models and has also revealed several omissions and areas of weakness. Some modelling aspects are common to both codes, and in these areas similar results may be expected. However, there are a few areas where different approaches have been taken; some differences in modelling results may be expected as a result of these different approaches.

It is anticipated that a comparison of results from the two models will be undertaken as part of the CEC/NEA ACA code intercomparison exercise in 1991 and 1992.

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J A Jones
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Contract: Bi6-125 **Sector: C24**
Title: Methodology of probabilistic uncertainty analysis of computational assessments

1 Hofer GRS

I. Summary of Project and Global Objectives

The work being carried out within the 1990 - 1991 period of the Programme consists of three parts:

1) A so-called Driver programme is being developed for the package. Its tasks are to

   - guide the user through the steps of a probabilistic uncertainty and sensitivity analysis
   - support selection of a suitable combination of options from those offered by the programmes in the package
   - perform the data transfer between the programmes as well as, where possible, between the programmes and the assessment model
   - support design extensions for parameters where the quantitative expressions of the state of knowledge (or where the expressions of state of knowledge dependence) cannot be readily handled by MEDUSA.

   The aim is a largely continous flow of the analysis requiring as little user interference as possible.

2) After completion of the Driver programme the package is available for use on mainframe computers. It would greatly promote the performance of uncertainty and sensitivity analyses and encourage the use of the package within the community if a Personal Computer version were available supplemented by sample analyses to illustrate its application. This PC version of the complete package, including the Driver programme, will be produced in the second part of the project.

3) The value of an uncertainty and sensitivity analysis depends largely on the quality of the subjective probability distributions needed for propagation of the state of knowledge through the model. A major source for the required quantitative expressions of the state of knowledge (as well as state of knowledge dependence) is expert judgement which needs to be elicited. In the third part of this project guidance will be provided for this important aspect of the analysis to support the user of the package.

   The guidance will specifically cover the subjects

   - Compilation of uncertainties
   - Typefication of uncertainties
   - Elicitation of expert judgements to obtain quantitative expressions of the state of knowledge
   - State of knowledge dependence
   - Processing of expert judgements.

Head of Project 1: Hofer

II Objectives for the reporting period

- Development of Version 1 of the Driver programme to the GRS programme package for uncertainty and sensitivity analysis

III Objectives for next period

- Completion of the Driver
- Preparation of the PC-Version of the package
- Provision of guidance for preparation of the analysis input

IV Progress achieved including publications

1) Methodology

A draft of Version 1 of the 'Driver' program and its panel structure was completed. Additionally the programmes DIVIS and EQUUS were extended for their application under the control of the 'Driver' program. The extensions are:

- DIVIS
  - inclusion of mixture distributions
  - conditional distributions to model state of knowledge dependence
- EQUUS
  - graphical presentation of uncertainty statements to scalar model results;

Version 1 of the 'Driver' program was documented in form of a report and the respective software developments were initiated. Specifically these developments deal with the:

- preparation of panels and skeletons
- preparation of the CLIST to organize the user dialog;

2) Results

The draft of Version 1 of the 'Driver' program guides the user through the steps of a probabilistic uncertainty and sensitivity analysis and prompts the user for the necessary input at the respective stages. The first four steps deal with the preparation of the analysis input. Here the user is supported in the specification of suitable types of distributions and in the inclusion of suitable measures or functional expressions to account for the identified state of knowledge dependences. The Driver establishes the necessary connections to the programmes DIVIS and MEDUSA and provides for design extensions if necessary. The parameter compilation and all the quantitative expressions of the user's state of knowledge are finally transferred to MEDUSA. Together with the citations of supporting references,

this input to the analysis is printed in a form suitable for the accompanying documentation. Subsequently the panels for selection of the respective MEDUSA options follow. Presently, the interface to the computer model is prepared to enable automatic performance of the required model runs under control of the Driver in step 5. The draft of the sequence of panels guiding through the subsequent steps 6 to 8 and prompting for the input needed for selection of the respective EQUUS, SAMOS and TUSSIS options is also completed.

3) Discussion

While the draft of the continous analysis flow through all eight steps under the control of the Driver in the interactive (foreground) mode is established there is still some work to be done to promote a successful application of the package. The Driver panels will need to be backed up by an efficient HELP function which is to support the user in the selection of the most suitable combination of the options offered by the programmes in the package. This HELP function still needs to be developed.
The programme package (not yet including the Driver) was presented at the 'CEC-Seminar on Methods and Codes for Assessing the Off-Site Consequences of Nuclear Accidents'. Contributions to the 'Training course on the use of the probabilistic accident consequence code COSYMA' consisted of an application-oriented presentation of the programme package for uncertainty and sensitivity analysis.

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Contract: Bi6-227 **Sector: C24**
Title: Optimization of off-site recovery actions following nuclear reactor accidents.

1 Alonso Universidad Politécnica de Madrid

I. Summary of Project and Global Objectives

(1) The main objective is the development of a model that combines long-term dose predictions, based on measured surface contamination, with the resulting economic costs of the different countermeasures against chronic exposure, following a large accidental release of radionuclides. The model will facilitate the cost-effectiveness analyses of different alternative actions and criteria, comparing the effectiveness, in terms of dose reduction, against the resulting economic costs and social disturbances.

For dose calculations, the model will consider both external and internal exposure pathways. Direct exposure from gamma emitters deposited onto various locations and surfaces are assessed using a dynamic linear compartment model to calculate the retention and migration of radionuclides. Three different environmental areas (urban, rural and other) will be considered, with different contributors to the external dose and to the internal dose by inhalation of resuspended material. Different suitable countermeasures (decontamination and interdiction mainly) will be evaluated for each area.

Indirect exposure resulting from the ingestion of contaminated foodstuffs and water are estimated using a dynamic model to simulate the transport of fallout radionuclides through food chains to man. The impact of countermeasures, like food disposal or temporary interdiction of agricultural areas for food production, will be also evaluated.

The model will use, for the assessment of the economic impact of countermeasures, the previously developed economic model MECA and its associated social-economic data base, that includes detailed distributions of population, livestock and agriculture for all the municipalities of Spain.

Uncertain parameters affecting countermeasures and dose calculation will be treated probabilistically.

(2) A second objective is an intercomparison of the model MECA (Model for Economic Consequence Assessment, developed in the previous period of the contract) against the model COCO-1, developed by NRPB and KfK, and presently included in the European ACA code COSYMA. The intercomparison consists on three phases: (1) theoretical analysis and discussion of the different concepts and assumptions used by each model; (2) comparison of results for deterministic scenarios; and (3) comparison of results for probabilistic scenarios using MECA with the U.S. MACCS code and COCO-1 with COSYMA.

Head of Project 1: Prof. Alonso

II Objectives for the reporting period (January, 1990 - May,1991)

(1) Development of a computer model for evaluating the external exposure from radiactive material deposited in the urban environment (URBAPAT).
(2) Design of a model for evaluating the internal (ingestion of contaminated food) and external exposure resulting from radioactive material deposited in rural environment (AGROPAT).
(3) Coupling of the previously developed model for economic consequence assessment, MECA, to the version 1.5 of the U.S. code MACCS.
(4) Theoretical comparison of MECA and COCO-1.

III Objectives for next period

(1) Completion of the model for the indirect exposure through the agricultural pathways (AGROPAT).
(2) Demonstrative analysis of the new model, including uncertainty-sensitivity assessment.
(3) Final report.
(4) Completion of the intercomparison of MECA against COCO-1.

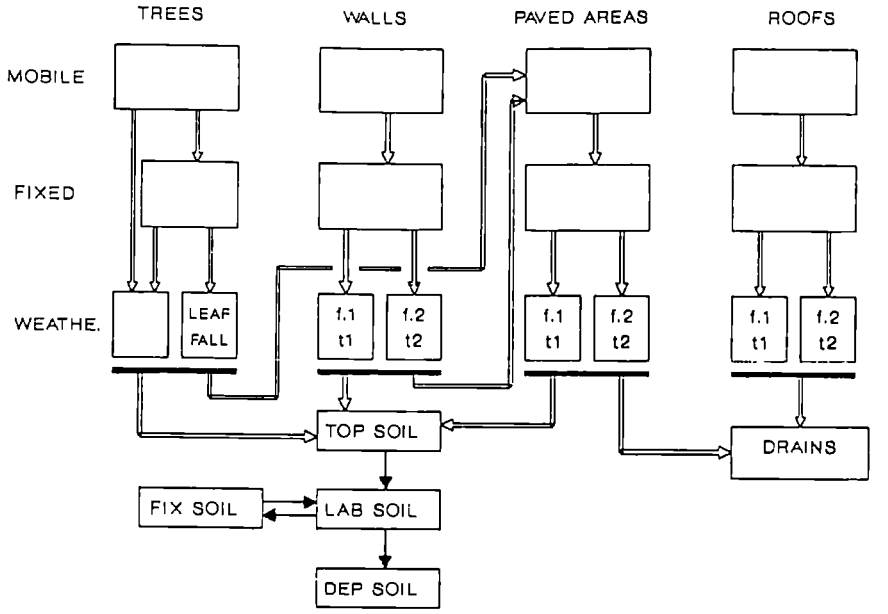
IV Progress achieved including publications

The first objective has been achieved with the development of the URBAPAT code. URBAPAT is a computer code that calculates the time evolution of the activity deposited on several urban surfaces and the resulting external doses, making use of the relationships between Kerma-rate and surface contamination as published by Meckback, Jacob and Paretzke[1]. Internal doses by inhalation of resuspended material are also being modelled. The model can consider the decontamination or dose reduction achievable on the individual surfaces using appropriate methods. Their associated costs will be estimated using the MECA model for decontamination cost itself plus the cost for the disposal of the resulting radioactive wastes. If a temporary or permanent interdiction of the area is selected as alternative for dose reduction, their economic impact will be evaluated with the MECA models.

The mathematical treatment of the model is based on the linear compartment model theory employing two alternative methods of resolution: an analytical method and a fifth order Runge-Kutta method. The model comprises 21 compartments plus one added to simulate the activity removed by decontamination. An schematic diagram of the URBAPAT model is shown in the Figure. There are five main surfaces in the model: trees, walls, paved areas, roofs and permeable surfaces (including grassland, parkland, soil and gardens), with four different states at which radiactive matter may be found on the particular surface.

URBAPAT is very flexible in order to perform uncertainty/sensitivity analysis of the different parameters.

(1) Meckback R., Jacob P., Paretzke H. G., "Gamma exposures due to radionuclides deposited in urban environment" Radiation Protection Dosimetry, 25; N° 3 pp. 167-179 (1988)



The second objective is still in progress, with a conceptual design of AGROPAT already completed. The programming of the model will start in the next period.

With regard to the third and fourth objectives, a coupling of the model MECA to the MACCS code, version 1.5, has been made and tested for various cases without significant problems. Also, as a result of the theoretical comparison of MECA with COCO-1, performed by Dr. Heady of Bath University (United Kingdom)[2], some of the models in MECA have been modified, originating the version 2 of the model, MECA2. A final revision of the draft MECA reports (CTN-58/89 and CTN-80/89) is being performed.

The theoretical intercomparison has been a valuable way to a better knowledge of both models and their modelling differences. The comparison of results for similar problems will be the way to verify their functioning coupled to general probabilistic accident consequence assessment codes, as a part of the quality assurance process. A comparison of results for deterministic and probabilistic scenarios using MECA with the U.S. code MACCS and COCO-1 with COSYMA is to be performed in the framework of the NEA/OCDE - CEC "Intercomparison exercise on Probabilistic Accident Consequence Assessment Models".

(2) Heady, C., "Intercomparison of CEC Economic Consequence Models". School of Social Sciences. University of Bath (February, 1991)

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1.- Alonso A., Gallego E. and Martín J.E., "The Modelling of Off-Site Economic Consequences of Nuclear Accidents". In Proc. CEC Seminar on Methods and Codes for Assessing the Off-Site Consequences of Nuclear Accidents. Athens (Greece), May, 7th-11th, 1990. Report EUR 13013.

2.- Alonso A.,Gallego E. and Martín J.E., Off-Site Economic Consequences of Nuclear Reactor Accidents (Final Report, Draft). Internal Report CTN-80/89. Revision 1. (Madrid, June 1990).

3.- Martín J.E. and Gallego E., Distribution of the Agricultural and Livestock Census of Spain in the European Grid. Internal Report CTN-82/89. (Madrid, November 1990).

Progress Report

Contract: Bi7-010 Sector: C24

Title: Deposition of radionuclides and their subsequent relocation in the environment following an accidental release to the atmosphere.

1	Underwood	SRD AEA
2	Roed	Risø National Laboratory
3	Paretzke	GSF Neuherberg
4	Nixon	SRD AEA

I. Summary of Project and Global Objectives

The objective of the project is to improve, as necessary, the models and parameterizations used in estimating (a) the intensity and spatial distribution of deposited activity and (b) the total health/economic impact of such deposits in assessments of the consequences of accidental releases of radioactivity. To this end, the aim is to attain a better understanding of:

- the influence of various weather conditions on deposition, particularly weather conditions which can lead to high deposition fluxes such as fog, snow or intense rain;

- the resuspension of deposited ^{137}Cs activity;

- the weathering of deposits in urban and rural environments and its impact on long-term external exposure;

- the ultimate fate and dosimetric impact of radionuclides carried by urban run-off water;

- the impact that a change in the method of representing the atmosphere's dispersion capabilities would have on the end-points of consequence assessment.

Head of Project 1: Dr. Underwood

II Objectives for the reporting period

To investigate the factors influencing the incorporation of radionuclides into fog, in particular to address the question of the competition between the released aerosol and the natural aerosol for the available fog water.

To identify the dominant mechanisms determining the patterns of retention and redistribution of radionuclides throughout the urban drainage system, in particular to carry out a review of pertinent information.

III Objectives for next period

To assess the feasibility of utilizing routinely-recorded meteorological data to provide information on the presence of fog and an indication of its intensity and type. To consider the design of algorithms for including foggy conditions in consequence-assessment codes.

To quantify the radiological impact of run-off into the urban drainage system in terms of its contribution to the endpoints of consequence assessment.

IV Progress achieved including publications

A) THE INCORPORATION OF RADIONUCLIDES INTO FOG

Methodology

The chief task was to calculate how the condensed water in a fog would be partitioned between the naturally-occurring aerosol (on which fog droplets would normally form) and the additional released aerosol, and how it is shared amongst particles of different size.

Attention was focused on the situation in which foggy conditions are encountered some time after the initial release, either as a result of a change in meteorological conditions leading to radiation fog or as a result of the plume encountering orographically-induced fog at some distance downwind of the release point.

The released aerosol was taken to have a log-normal size distribution in its dry state, and be characterized by its total mass concentration (m_t), the mass-median aerodynamic diameter (D_{50}), the geometric standard deviation of the number distribution (σ), the fraction (by volume) of soluble material (ε) and the dry particle density (ρ_d). Ranges of these parameters to be considered are defined, bearing in mind broad physical constraints and past analyses.

The enhancement in deposition produced by the released particles taking up water is expressed in terms of the 'enhancement factor', defined as the ratio of the 'mean'

deposition velocity after water uptake to that before. The weighting factor used in calculating this 'mean' is the released (dry) mass concentration in each size sub-range, which is assumed to be proportional to the amounts of radionuclides of interest present.

A detailed determination of how the condensed water is shared amongst all the particles present requires solution of a set of coupled differential equations representing the growth of particles of differing initial size and the consequent feed-back of the loss of water on the saturation ratio of the air. In this work, however, two approximate approaches were developed which avoid explicit numerical integration of the differential equations but which nevertheless provide powerful insights into the dependence of the enhancement factor on the characteristics of the released aerosol.

Results and Conclusions

The enhancement factor approaches an asymptote at low mass concentration, with a value of order 100 when the initial D_{50} is below 2μm (provided the released aerosol contains a minimum fraction of soluble material). This is a large factor capable of having a major impact on some of the parameters used to quantify the consequences of accidental releases of airborne radionuclides. As the mass concentration of the released aerosol increases, the enhancement factor falls because (a) the available water has to be shared amongst more particles and (b) the supersaturation achieved for the same cooling is diminished, which leads to a smaller portion of the size distribution being activated.

At high mass concentrations, where the saturation ratio does not exceed unity during the transient, enhancement factors significantly above unity can still be found, so that activation is not a prerequisite for appreciably enhanced deposition.

Bearing in mind the dilution factors (i.e. the concentration per unit release rate) at various distances downwind, the enhancement factor in orographic fog is likely to be close to its asymptotic value in most practical situations. In radiation fog - for which the dilution factors are less and which could readily be encountered close to the release point - significant reductions in the enhancement factor are possible.

Usually the enhancement factor decreases with increasing D_{50}, other parameters being held constant. However, the value only drops to a small fraction of 100 near the upper limit of the range of D_{50} considered (5μm), where growth is limited by the time available for water uptake.

The enhancement factor usually shows only a modest dependence on σ, suggesting that it may often be possible to ignore the spread of sizes in the released aerosol (i.e. use a single representative size), when considering the impact of fog on deposition.

Similarly, the dependence of ε is weak. Although the dependence of the enhancement factor on ρ_d is stronger, it is not likely that a wide range of particle specific gravity needs to be considered.

Thus it appears that the key parameters required to specify the deposition velocity appropriate in fog are the total mass concentration and the mass-median aerodynamic diameter. Approximate estimates of the other parameters will usually suffice.

Discussion

The work indicates that it should be possible to make an adequately reliable estimate of the impact of fog in probabilistic consequence assessment despite the fact that detailed information on the released aerosol characteristics for various accident scenarios may be lacking.

B) RADIONUCLIDE TRANSPORT IN URBAN DRAINAGE SYSTEMS

Methodology

A review has been carried out with the aim of gathering information necessary for development of an approach to quantifiying the urban drainge pathway.

The review covers the following aspects.

1. Urban Drainage Systems

Here, the pertinent features of the various types of urban drainge systems common in Europe are described, including a discussion of flow rating and the provisions made for overflows. The stages of sewage treatment are described, as are the methods for sewage-sludge treatment and disposal.

2. Radionuclide Behaviour in the System

Consideration is given to which are the key radionuclides of interest. The processses of removal during transport in sewage pipes, removal from the liquid phase during sewage treatment, concentration in sewage sludge and dilution on discharge are considered. In particular, pertinent experimental studies (small scale) and field data are reviewed, and consideration given to whether the experience of heavy-metal contamination yields any pertinent information.

3. Radiological and Economic Implications

Here, doses to the public, worker doses and economic costs are discussed. Within the context of doses to the public, contributions from terrestrial pathways and aquatic pathways are considered. Under the heading of economic costs, the costs of countermeasures (such as having to make alternative arrangements for disposal of contaminated sewage sludge) are discussed, as is the possible disruption to the sewage treatment process.

Conclusions

Although there are a variety of urban drainage systems in operation, some generalizations are nevertheless still possible. For example, combined systems (in which storm water and foul discharge are carried in the same set of pipes) are still common in Europe indicating that sewage treatment is a factor that has to enter into an assessment of the ultimate effect of radioactivity in the drains.

Generally, information on the absolute amounts of water entering the system will be required to assess the public-health impact of contaminated run-off water entering the drains. For example, this parameter can affect the extent to which flow is diverted from its normal route due to overflow provisions, thereby enabling a more direct path to the discharging watercourse.

Empirical information on the partitioning of radionuclides between the solid and liquid phases in the sewage system is sparse, although it may be possible to fill in some of the 'gaps' using data obtained for other types of pollutant.

Little is known on the effect of chemical treatment of sewage - carried out in some countries - on radionuclide behaviour.

Discussion

The review has revealed what are the key features of the urban drainge system pertinent to an assessment of the impact of radionuclides in run-off water entering the drains, and highlighted the important parameters needed for quantification of the consequences.

PUBLICATIONS

B Y Underwood (1991) The Incorporation of Aerosol Particles into Fog Droplets. 22947/R1. Topical (contract) report to the CEC.

J MacKenzie (1991) A Review of Radionuclide Transport in Urban Drainage Systems. 22947/R2. Topical (contract) report to the CEC.

Head of Project 2: Dr. Roed

II Objectives for the reporting period

- is to set up two raincollectors in different heights in order to find the influence of height on resuspended matter that is collected by rain.
- to follow the influence of natural decontamination and weathering processes in urban area by measuring the levels in contaminated areas in Sweden as well as in Denmark.
- in order to be able to model the natural decontamination and weathering processes to find the depth-profile of caesium in asphalt.

III Objectives for next period

- is to measure the amount of resuspended material in rainwater collected at two different heights in order to find the influence of resuspension on height.
- to follow the influence of natural decontamination and weathering processes in an urban area by measuring the contamination on different urban surfaces as a function of time.
- to find the depth profile of caesium of lawn in order to be able to find kerma on different locations in the urban area.

IV Progress achieved including publications

In August 1990, a measurement campaign was conducted in Gävle and many of previously examined surfaces was again assessed for levels of ^{134}Cs and ^{137}Cs. The results are shown in Table 1.

Surface type	Location	Cs-137 ($kBq \cdot m^{-2}$)	Cs-134 ($kBq \cdot m^{-2}$)
Concrete paved area 1.	Industrial Area	3.21 ± 7%	0.49 ± 10%
Concrete paved area 2.	"	6.61 ± 6%	1.15 ± 8%
Asphalt surface. Crossroads.	"	0.50 ± 15%	-
Concrete paved area 1.	Town centre	3.15 ± 7%	0.52 ± 10%
Concrete paved area 2.	"	4.26 ± 7%	0.69 ± 9%
5.8 m wide road	"	0.44 ± 17%	-
Grassed area	"	66.9 ± 5%	9.85 ± 8%
Grassed area	Harkskär, 10 km N-E of Gävle	117 ± 5%	17.6 ± 7%

Table 1. Radiocaesium levels in the Gävle area in August 1990.

From a comparison with measurements in 1988, it can be seen that there has been little or no decrease in the ^{137}Cs levels on grassed surfaces. The decrease in the ^{134}Cs levels was due to the relatively short half-life (2.05 a) but after allowing for radioactive decay the decrease was the same as for ^{137}Cs.

On the paved surfaces, the levels had decreased by 53-70% in the two years between the two series of measurements.
A model TACTUC for describing the time dependence of the contamination, is under development and the contamination levels on different urban structures are simulated. The following three figures show the contamination level on different urban structures, relative to that on a smooth, cut lawn, where there was no penetration.

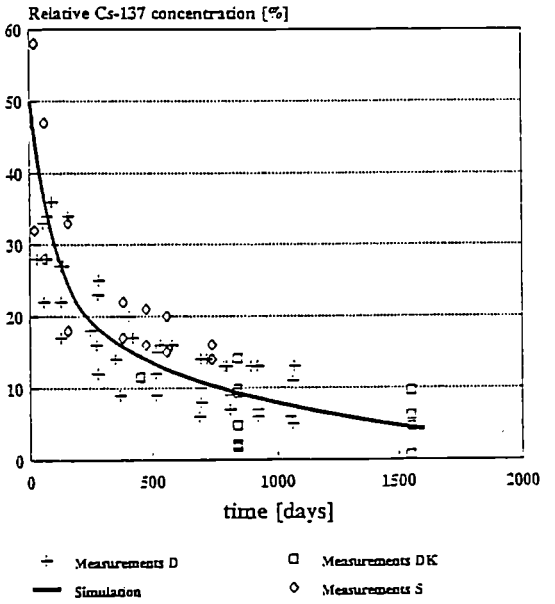


Figure 1. Simulation on Pavings.

The simulations are compared to field measurements made in Sweden, Danish measurements in Gävle, Sweden and measurements in Germany.

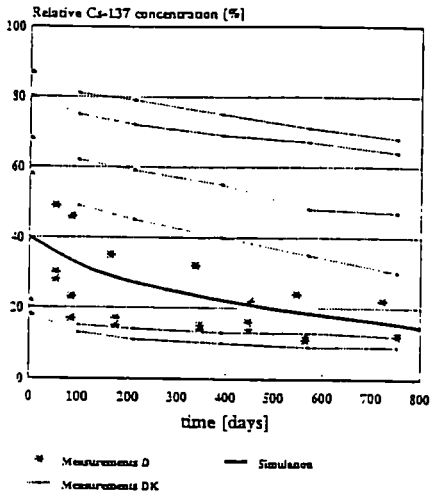


Figure 2. Simulation on Roofs

Here, the model simulations have been compared with measurements of wet deposition on tile roofs in Bavaria, and with results of Chernobyl wet deposition measurements at Risø for a very wide range of different roof materials. The model reflects the typical flow on roof pavings.

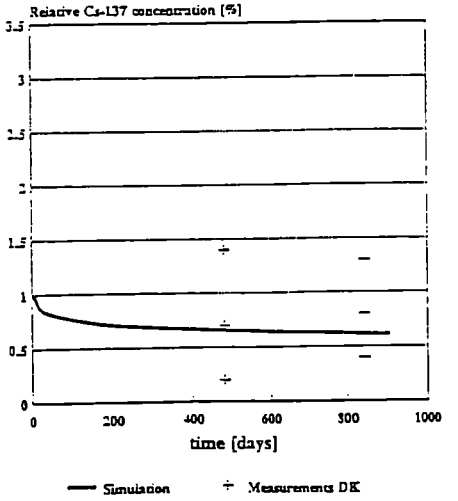


Figure 3. Simulation on Walls.

The model simulations for contamination on walls have been compared to the results of the measurements in Gävle in Sweden. Very little experimental information was available in the literature. However, the deposition on walls is likely to be very small compared with that on other surface types, and the migration processes therefore of lesser importance.

The depth-profile of Caesium in asphalt roads has been measured at three different locations, see fig. 4.

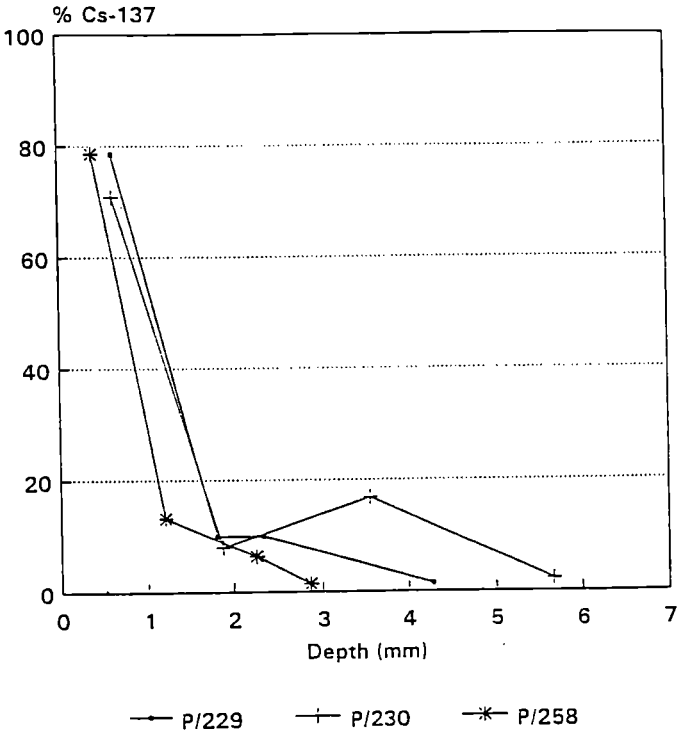


Fig. 4. Depth-profile of Caesium in asphalt.

It can be seen that 80-90% of the total Caesium is still in the uppermost 2mm of the asphalt layer.

The rain collectors for measuring the resuspended material in rain has been erected at two different heights and the measuring of the collected rainwater is in progress. The first measurement did not show any significant difference in the collection of resuspended material at the two heights, yet further measurements has to be done in order to give a valid conclusion.

Head of Project 3: Dr. Jacob

II Objectives for the reporting period

A) Application of a tracer method to the measurement of washout of particles by rain and snow. A modified La Mer generator will be used to produce a monodisperse aerosol (in the size range 0.3-5 μm) tagged with a tracer. Deposition parameters for each specific particle size will be determined from the measured tracer concentration in the precipitation.

In the second year - and beyond if appropriate - SRD will consider the design of algorithms for including foggy conditions in consequence-assessment codes. It would also continue the model uncertainty/sensitivity analysis commenced in the first year.

B) In-situ γ-ray spectrometry measurements at about 50 locations in urban, suburban and rural environments in Bavaria which have been continuously investigated since the reactor accident at Chernobyl. The spectra will be evaluated with respect to the reduction of the γ-dose due to weathering.

C) Measurement of the resuspension of Cs in urban environments and subsequent deposition on construction surfaces, vegetation etc. in Goiania (in cooberation with the Inst. Radioprotecao e Dosimetreia, Rio de Janeiro), where an accident with a medical Cs-source led to highly localized contamination in a city of 1.3 million inhabitants. The procedure will be to take size-differentiated air samples in weather episodes and to make measurements on environmental and construciton surfaces (roofs, walls etc.).

III Objectives for next period

A) Direct measurement of the wet deposition of atmospheric aerosol. Here, the trace element content of the atmospheric aerosol will be used to quantify aerosol deposition parameters. Trace elements on aerosols transported over long range may be scavenged predominantly by processes in clouds, ie by rain-out, whereas wash-out determines the deposition of trace elements with dominant local sources. The use of a set of trace elements which encompass both these deposition routes will allow wet deposition to be studied in detail under various meteorological conditions. The use of generated aerosol and trace elements will provide a complete picture of wet deposition: wash-out both on its own and together with rain-out can be studied. The methods will be applied to precipitation in the forms of rain and of snow.

B) Continuation of recording and evaluating γ-spectra for the 50 sites mentioned above. The time behaviour of the reduction of the γ-dose rate in the first five years after deposition will be approximated by analytical functions.

C) Development of a resuspension model which will enable an estimate to be made of the redistribution of initial contamination and thus the usefulness of decontamination measures in urban environments after major radioactive contaminations.

IV Progress achieved including publications

A) The tracer method was established and first measurements were made. These experiments showed in the considered size range (0.6-4 µm) only a weak dependence of the washout-process on the particle size, while a significant difference was oberserved depending on the precipitation type (rain/snow) or the precipitation intensity. The direct measurement of the wet deposition of atmospheric aerosol also was started. The focus of this investigation was the determination of the scavenging efficiency of snow. It was found that the scavenging efficiency of snow is up to one order of magnitude higher than that of rain and that the dependence on particle size is similar to that of rain: small aerosol particles (< 0.5 µm) and large aerosol particles (< 1.5 µm) are scavenged most effective.

B) The evaluation of the photon spectra recorded at 50 locations in Southern Bavaria showed that the site-specific differences of the attenuation of the γ-radiation from deposited cesium increases with time after deposition.

C) In and around the IRD-laboratory house in Goiania, Brazil, the resuspension of deposited Cs-137 was measured. To this purpose the specific activity of surface soil and street dust as well as the airborne Cs-activity was measured. The aerosol collection was done by EPA-type airsamplers (ca. 30 m^3/h) at different heights and locations, by large (0.8 x 0.8 m^2) water surfaces and by sticky papers. Typical time integration periods were 14 days. Preliminary results on air concentrations indicate a rather local source of resuspension and negligible differences in air concentrations between 1 m and 2 m height above ground. Apparently over 90 percent of airborne activity is attached to aerosols larger than the cut-point of the EPA-air sampler. The street dust results show after 3 years a rather minute dispersion of the initial urban contamination. This is in contrast to the Sovjet reports on similar measurements in the towns and villages of the Chernobyl region and requires clarification.

Publications

Tschiersch, J., B. Hietel, P. Schramel and F. Trautner, Wet deposition of aerosol by rain and snow, in: AEROSOLS, Science, Industry, Health and Environment (S. Masuda and K. Takahashi, Eds.), Vol. 2, pp. 1033-1036, Pergamon Press, Oxford, 1990.

Tschiersch, J., B. Hietel, P. Schramel and F. Trautner, Saharan dust at Jungfraujoch, J. Aerosol Sci., 21, Suppl. 1, S357-S360, 1990.

Frank, G., J. Tschiersch and H. Behrens, Wet deposition of tracermarked aerosol, J. Aerosol Sci, 21, Suppl. 1, S213-S216, 1990.

Head of Project 4: Dr. Nixon

II Objectives for the reporting period

SRD would commence the assessment of the impact of changing the representation of the dispersive capability of the atmosphere. The vehicle for carrying out this investigation would be an uncertainty/sensitivity analysis of the atmospheric dispersion module of the CONDOR code (a state-of-the-art probabilistic consequence assessment code). In contrast to the more usual parametric uncertainty analyses, this study would examine the impact of changes in some aspects of the modelling implemented in the atmospheric dispersion module. The study will emphasise the impact on standard consequence endpoints, eg health effects, rather than limiting the analysis to the variation in dispersion characteristics, eg concentration levels.

III Objectives for next period

Since the project has not yet commenced (see below) the complete assessment will be carried out during the next reporting period.

IV Progress achieved including publications

Timescale restrictions for other work within the department at SRD, have made it more convenient to postpone the proposed work for the first year of the contract. Project 4 will now be completed in the second/final year. Since this is a minor part of the whole contract, this rescheduling will not restrict the amount of effort available for the project, but merely moves the completion towards the end of the two year contract.

Progress Report

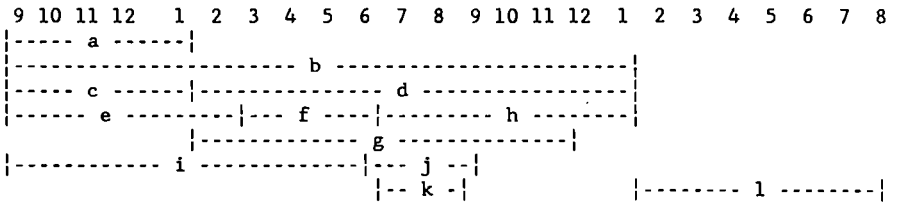
Contract: Bi7-012 **Sector: C24**
Title: RADE-AID, the development of a Radiological Accident DEcision AIDing system

1	Wagenaar	TNO
2	Ehrhardt	KfK Karlsruhe
3	Morrey	NRPB

1 Summary of Project and Global Objectives

The current RADE-AID contract, the development of a Radiological Accident DEcision AIDing system is a continuation and a extension of the work on the system during the period 1988-1989. Within this period a prototype computer program was developed to assist in decision on countermeasures following radiological emergencies. The program helps a decision-maker structure the problem and investigate the consequences of different countermeasures. In order to demonstrate the potential of this decision tool, some illustrative applications were developed.

During the current contract the computer system will be further developed with particular reference to the user-interface, the decision logic and a database of model predictions supplied for use with the system.
Consultation with decision-makers to prove the practical value of the system is also an important subject within the contract. Two separate computer programs are developed: DATUM and DATARADE. DATUM is a multi-attribute analysis tool for general applicability. DATARADE is the version of DATUM for radiological applications; it contains the database mentioned above.



a. List user-interface improvements (TNO)
b. Implement user-interface improvements (TNO)
c. List input elicitation improvements (TNO)
d. Implement input elicitation improvements (TNO)
e. Design database (NRPB)
f. Implement limited UK version of database (NRPB)
g. Provision of German & Netherlands data for database (KfK, TNO)
h. Implement full database (NRPB)
i. Establish contacts with decision-makers (TNO, NRPB, KfK)
j. Detailed discussions with decision-makers (TNO, NRPB, KfK)
k. Preparation of progress report (TNO, NRPB, KfK)
l. Preparation of final report, user guides etc. (TNO, NRPB, KfK)

Head of Project I: Dr. Wagenaar

II Objectives for the reporting period

The objectives for the reporting period (September 1990-May 1991) are (as shown in section I):
- constitution of a list with improvements on the user-interface;
- implementation of the improvements on the user-interface (partial);
- constitution of a list with improvements on the elicitation of input data;
- implementation of improvements on the elicitation of input data (partial);
- provision of Netherlands data for the database;
- establishment of contacts with decision-makers.

III Objectives for next period

The objectives for the next period (June 1991-August 1992) are (as shown in section I):
- implementation of the improvements on the user-interface (partial);
- implementation of improvements on the elicitation of input data (partial);
- provision of Netherlands data for the database;
- establishment of contacts with decision-makers;
- detailed discussion with decision-makers.

IV Progress achieved including publications

Lists with improvements on the user-interface and the elicitation of input data have been constituted and been approved by KfK, NRPB and TNO.
The implementation of both the improvements on the user-interface and the improvements on the elicitation of input data is an on-going activity. Several improvements are already operational; an example is the option to print a summary of the current problem. In discussion with NRPB activities to define the interface between the decision analysis part of the program and the database have been started. The design of the interface has been made; implementation is scheduled for the months to come.

Provision of the Netherlands data for the database and the detailed discussions with the decision-makers will be realized in contacts with authorities. Preliminary interviews have been held; further progress is scheduled for the rest of 1991. Contact with decision-makers has not been restricted to authorities responsible for radiological protection. Exercises with the decision-aiding tool have also been performed in the field of selection of methods and techniques for cleaning of (chemically) contaminated areas.

An abstract for a paper entitled "Using a multi-attribute decision tool to aid decisions on countermeasure strategies" has been submitted to the "International seminar on Intervention Levels and Countermeasures for Nuclear Accidents" (7-11 October 1991, Cadarache, France).

Head of Project 2: Dr. Ehrhardt

II Objectives for the reporting period

Work at KfK is more directed towards investigations about the applicability of the RADE-AID methodology in decision support systems for nuclear emergencies (see Contract BI7-045). In addition, contributions of a more advisory character will be provided with some calculational support in case studies for different source terms or countermeasure strategies.

III Objectives for next period

The usefulness of an integrated approach combining both knowledge based (expert) system methods and multi-attribute decision analysis techniques as evaluating part of a decision support system will be investigated. Ad hoc assistance will be provided in modelling problems or calculation tasks.

IV Progress achieved including publications

As part of a computerized real-time decision-aiding system for assisting the emergency management in the case of a nuclear accident, an expert system shell has been developed by the Deutsches Forschungszentrum für Künstliche Intelligenz, Kaiserslautern, FRG, under contract with KfK.

To develop the structure and content of the rules to be implemented into the system, several meetings with German experts involved in decision-making have been organized by KfK. Based on case studies with different release characteristics, countermeasure strategies, environmental situations, and the corresponding spectrum of consequences precalculated with the program package COSYMA, various questions and problems were discussed for pre-release and release conditions, such as

- principle rules of emergency response,
- relevance of different consequence types and quantities in the decision process (doses and/or health effects, economic impact),
- the availability of technical equipment and manpower,
- the size of areas with sheltering and evacuation and the timing of these actions including the behaviour of the population,
- the role of existing recommendations and emergency plans.

As an essence of these meetings, a fundamental rule structure could be identified; however, the fact that people involved in decision-making have not been confronted till now with the whole spectrum of consequences, they have not yet developed preferences, judgements and weights for balancing the various consequence types. Therefore, with the experience gained during the RADE-AID development, example sets of preference functions and weighting factors have

been generated without input from the decision-maker side and integrated in the form of separate routines into the rulestructure of the expert system. Thus, a combination of a rule-based system with multi-attribute value theory resulted, which allows to explore the interaction between both methods and their potential application in computerized real-time decision support systems. The existing expert system has been integrated into the comprehensive decision support system presently under development at KfK with support of the Federal Ministry of Environment and the Commission of the European Community, DG XII, under contract No. BI7-045.

Head of Project 3: Mrs Morrey

II Objectives for the reporting period

As indicated in Section I, two versions of the decision-aiding software are being developed, namely DATUM and DATARADE. NRPB's responsibilities with regard to DATUM were to initiate contacts with UK decision-makers, in order to explore ways in which the software could be tailored more closely to their needs, in terms of the presentation of information, functionality provided and ease of use (activity i on the barchart). With regard to the development of DATARADE, NRPB were responsible for designing a radiological database to be integrated with the decision software, which would enable the consequences of adopting different emergency response strategies to be evaluated (activity e). The full database will contain information on the consequences of adopting a range of relocation and food interdiction strategies. During the reporting period, NRPB were responsible for developing a limited version of this database (activity f). NRPB were also responsible for requesting data from TNO and KfK which would be required to provide a database appropriate for the Netherlands and Germany (first part of activity g).

III Objectives for next period

During the next reporting period, NRPB will pursue their contacts with UK decision-makers, and provide TNO with feedback on any desired changes to the software (activity j). NRPB will also develop the full radiological database for both food interdiction and relocation strategies for integration with DATARADE (activity h).

IV Progress achieved

Initial contacts have been made with three organisations within the UK who have responsibility for decision-making in a radiological protection context, namely the Nuclear Installations Inspectorate (NII), the Ministry of Agriculture, Fisheries and Food (MAFF), and the Scottish Office (SO). Demonstrations of the DATUM software have been given to NII and MAFF. It is now intended that NRPB will collaborate with SO and NII to explore the potential application of DATUM within these organisations. Some feedback has already been provided to TNO concerning useful extensions to the existing decision-aiding software.

The radiological database for the DATARADE software has been designed, together with the necessary interface to the decision-aiding software. Existing accident consequence models have been adapted to predict the long term radiological, economic and social consequences of notional UK accidents occurring in different weather conditions. A limited version of the database has been developed, based on these results for a range of relocation strategies. TNO and KfK have been advised of the data required to expand this relocation database for application to The Netherlands and Germany. Currently the use of data interpolation procedures are being explored in order to limit the eventual size of the database.

An abstract for a paper entitled 'Modelling the Consequences of Relocation and Decontamination Strategies for Input to Multi-attribute Decision Tools' has been submitted for the CEC International Seminar on Intervention Levels and Countermeasures for Nuclear Accidents (October 1991).

Contract: Bi7-015 **Sector: C24**
Title: Indoor deposition and relationship between indoor and outdoor air concentration

1 Roed — Risø National Laboratory
2 Goddard — ICSTM

I. Summary of Project and Global Objectives

In order to assess the effect of staying indoors during an air pollution episode, including that caused by a nuclear accident, it is essential to find as well the reduction in inhalation dose as the deposition on indoor surfaces.

Full-scale investigations of house filtration and indoor deposition have so far used polluted air from natural air pollution episodes, including the Chernobyl case. A technique using labelled monodisperse silica particles, however, is believed to enable full scale measurements of indoor deposition to be made with particular particles sizes in the range of interest for accidental releases and so give improved underlaying and sound data for modelling purposes.

Protective measures that may be taken by the householder are relevant to seeking optimal strategy in protecting the individual. In relation to aerosol, one decontamination method, that will be investigated, is the use of vacuum cleaner as a tool for cleaning indoor air.

The global objectives for the study are.

- to examine - and improve if necessary - the techniques for estimating the reduction of inhalation dose by staying indoors and the deposition on indoor surfaces.
- to examine the influence of the surface type, for instance the importance of furniture on the deposition process.
- to consider the range of measures that may be taken to alleviate indoor exposure and particular to examine a vacuum cleaner as a tool for reduction of the indoor air pollution.
- to thereby improve models for generic and risk assessment purposes in radiation protection.

Head of Project 1: Dr. Roed

II Objectives for the reporting period

The objective of this reporting period is to implement in collaboration with Imperial College the use of monodisperse silicon particles labelled with dysprosium for indoor deposition measurements in full scale.

- to perform large scale experiment in a real house using this method combined with a cromatographic method for measuring the air exchange rate using SF_6 as tracer, and by combining the two measurement to find the indoor deposition in the house for two different particle sizes

III Objectives for next period

To conduct 1-2 more full-scale experiments using monodisperse aerosol in collaboration with Imperial College.

- in a test house using cosmogenic ^{7}Be-particulate as tracer to find indoor/outdoor air concentration and by varying the air exchange-rate to find the filtering factor and the deposition constant.
- to investigate vacuum cleaners as a tool for reduction of indoor pollution and to test their performance under real condition in a building.

IV Progress achieved including publications

Two full scale tests have been performed using Dysprosium labelled silicon particles. In both cases the test house was Risø-huse no. 27, where the living-room was used.

The air exchange rate, λ_r was measured using SF_6 as a tracer gas and monitoring its decay by chromatography. 5μm dysprosium labelled silica particles were dispersed from a Palas RBG 1000 powder disperser. To insure a uniform distribution of the aerosol within the room, a fan was used. Ten filter samples were taken in the room at ten minutes intervals.

The filter samples were activated in Risø's research reactor. The activity of the filters was then analysed by gamma spectroscopy using Ge-Li detector and a multi-channel analyser.

The filter papers used, were Whatman 42: 15cm$^{\circ}$. The size was determined by the pumps ability to draw air through the filter.

In order to avoid cross-contamination the generator and source were cleaned during runs using different particle size. This procedure did not include complete dismounting of the brush unit in the generator.

This was found subsequently to be desirable. The new procedure removed most doubt concerning cross-contamination though it still may be possible for some particles to lodge in remote areas of the generator.

The sodium content of the filter paper however, produced unacceptably large background of ^{24}Na, after irradiation.

In order to lower this background for the second experiment we used double punch pumps with a very rigid characteristics and was then able to use smaller filter papers. From an investigation of the background after irradiation of the different types of filter-paper, we found that Whatman 542 was the best.

In the second experiment we measured the deposition of particles labelled 1μm and 5μm from the manufacturer. As shown in the progress report from Imperial College, these particles were not found to have the size given by the manufacturer. Measurement was done in the living room both with and without furniture in order to find the influence on deposition of the furniture.

The relative concentrations of the particulates in the air as a function of time are given in figures 1 - 4.

In table 1 are given the deposition velocities to the walls and floors in the room.

As can be seen from table 1, the deposition velocity is lower for the smaller particles than for the larger particles, the difference, however, seems to be smaller than expected, this is because the particles labelled 1μm were found actually to be closer to 2μm and those labelled 5μm were closer to 4μm.

Fig. 1

1 micron Dy particles.
Unfurnished

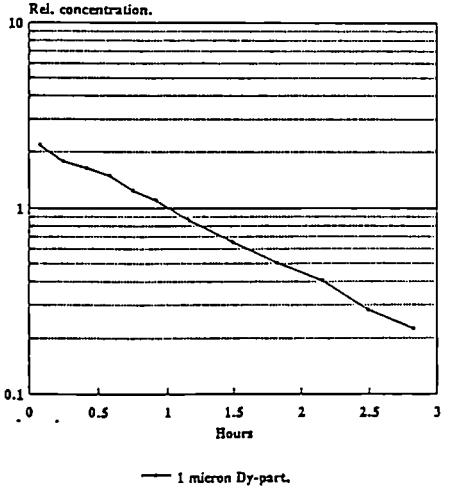


Fig. 2

5 micron Dy particles.
Unfurnished

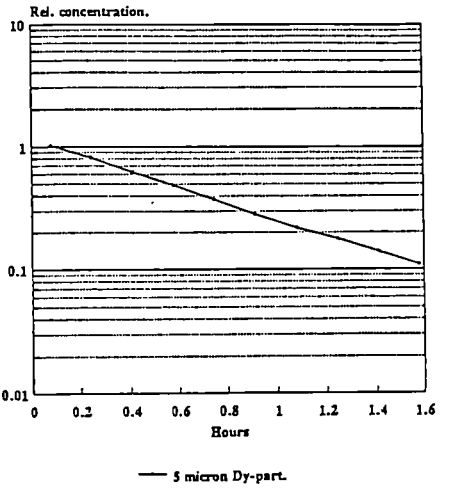


Fig. 3

1 micron Dy particles.
Furnished

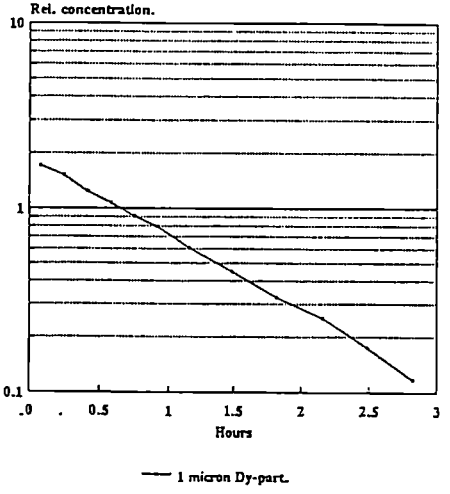


Fig. 4

5 micron Dy particles.
Furnished

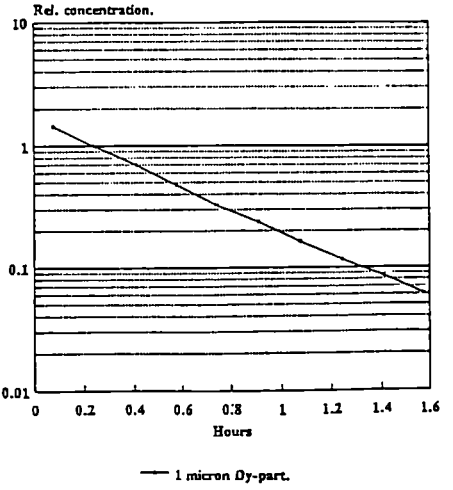


Table 1. Deposition constants and deposition velocities

	Particle size	Deposition constant	Mean deposition velocity to internal surfaces
Unfurnished	1 μm	0.84 h^{-1}	0.015 $cm \cdot s^{-1}$
-	5 μm	1.33 h^{-1}	0.024 $cm \cdot s^{-1}$
Furnished	1 μm	0.95 h^{-1}	0.017 $cm \cdot s^{-1}$
-	5 μm	2.10 h^{-1}	0.038 $cm \cdot s^{-1}$

Further it can be seen from table 1 that furnishing the room increases the mean deposition velocity, this is in good agreement with earlier findings.

The relatively high deposition-velocities measured are probably due to high turbulence in the room caused by the experimentators during the measurements.

It can be concluded that the method developed for finding deposition characteristics (deposition constant deposition velocity) has worked very well in the experiment performed.

Head of Project 2: Prof. Goddard

II Objectives for the reporting period

Samples of monodisperse silica particles in the size range 1-10μm would be obtained and the size characteristics would be checked using the Imperial College APS analyser.
The stable tracer labelling technique would be confirmed using the Imperial College Reactor Centre facilities.
The assistant would travel to Risø to participate in indoor deposition measurements with labelled monodisperse aerosols, using air filter techniques. Necessary equipment from Imperial College would be brought to Risø.
Models would be used to assist experimental design.

III Objectives for next period

Imperial College would participate in additional full-scale house experiments using monodisperse aerosol in the size range 1-10μm. This again will involve a period spent at Risø and the transport and provision of the appropriate College equipment.
Data and models would be developed, in collaboration with Risø, that can take account of the differences in behaviour between particles in the 1-10μm range, and to disseminate the results of the model studies.

IV Progress achieved including publications

Studies were carried out at the Imperial College Reactor Centre to confirm the particle labelling technique and its detection limits.

Transporting the appropriate equipment, the assistant travelled to Risø to participate in indoor deposition experiments using labelled monodisperse aerosols. An efficient experimental procedure was quickly established after minor modifications. Experiments were carried out in furnished and unfurnished conditions using two aerosol particle sizes, which APS analysis at Imperial College had shown to be 2μm and 4μm, respectively.

Co-operation was established with the developers of a simple compartment model at Imperial College, with a view to assisting experimental design and understanding experimentally-observed aerosol deposition behaviour.

PARTICLE LABELLING

A technique has been developed at the Imperial College Reactor Centre at Silwood Park which allows silica particles to be labelled with a neutron-activatable tracer, such as dysprosium or caesium. The silica particles used are supplied by Phase Separations Ltd and are available in various monodisperse size distributions. The particles are suitable to tracer adsorption due to their large surface area and the presence of a large number of 8nm pores on their surfaces.

The labelling procedure is as follows: the silica particles are shaken for 24 hours in an aqueous solution of the tracer metal salt (of typical concentration 10 mg/ml), filtered and rinsed throughly with distilled water (to remove excess metal solution from the particles' surface). The typical uptake of metal ions resulting from this procedure is 5mg per gram of silica, in the case of dysprosium.

Attempts to increase the level of labelling of the silica particles, by increasing the metal salt solution concentration and the solution agitation time, have yielded no significant improvement. However, using the observation that the pore size of porous glass increases upon heating, it has been found that heating the labelled silica to 500° C overnight, followed by re-labelling, increases the uptake of metal ions by the particles by a factor of two.

The bonding of the metal ions to the silica has not been investigated. However, it is thought to be attributed, to some extent, to the replacement of sodium by the metal ion label, since the native sodium content of the particles (typically 5mg/g) is reduced upon labelling.

PARTICLE SIZE MEASUREMENT

The linearity of the decay curves obtained from the experiments indicates that the particles used were satisfactorily mono-disperse. In order to characterise the particles fully, a size analysis was carried out, using the TSI Aerodynamic Particle Sizer (APS 33) at the Imperial College Reactor Centre. The APS is capable of particle size measurements in the 0.5-30 μm range. The instrument has a size-discriminating capability of 0.18 μm.

Using the APS, it was found that the "1μm particles had an aerodynamic diameter (A.D.) close to 2μm and the A.D. of the "5μm" particles was in fact 4μm. These values may explain the surprisingly small difference between the deposition velocities calculated for the two particle sizes used in the experiments.

Contract: Bi7-017 **Sector: C24**

Title: Validation- training- and uncertainty-study experiments for real-time atmospheric dispersion models.

1 Mikkelsen — Risø National Laboratory
2 Werner — Deutsche Forsch.anst.Luft Raumfahrt

I. Summary of Project and Global Objectives

The objective of this study is to quantify and assess uncertainties likely to arise during nuclear accidents involving a near-site atmospheric dispersion scenarios.

The work task is to perform and compile full scale aerosol-plume dispersion experiments over various types of terrain, - for a variety of different atmospheric conditions, including non-idealized but nevertheless realistic dispersion events occurring during transitional (time-changing) meteorology.

The final contribution will consist of sets of "Reference and Validation Data", applicable as case studies for real-time dispersion models, and will include experimental data suitable for training and evaluation of real-time uncertainty handling and on-line emergency training relevant for nuclear accidental releases.

Head of Project 1: Dr. Mikkelsen

II Objectives for the reporting period

To conduct extensive full-scale dispersion experiments over flat and complex terrain appropriate for real-time dispersion model evaluation and training.

To build a comprehensive experimental data base consisting of reference experiments based on detailed LIDAR measurements of the meandering and time-changing plume dispersion.

To improve our plume tracking capabilities by improving the mini-LIDAR systems for remote sensing of aerosol plumes (in conjunction with our German partners at DLR).

III Objectives for next period

Performing diffusion experiments based on releases from elevated sources.
Continuing studies of dispersion based of short time releases (puffs).
Studies of building effects on near-site dispersion by LIDAR.
Data processing, quality assurance and corrections.
Construction of the "uncertainty-knowledge and training data base"
based on previous (flat terrain) and new (complex terrain) diffusion experiments.
Construction and testing of a new and improved mini-LIDAR system suitable for two-dimensional plume scanning and for plume measurements at longer ranges.

IV Progress achieved including publications

IV.1 Introduction

With technological support from our German cooperators at DLR, we have to date participated in two full scale aerosol-plume dispersion experiments, during two different experimental campaigns. They are:

I. Atmospheric dispersion experiments over complex terrain in a Spanish valley site (Guardo power plant, Oct., Nov. Dec 1990, Palencia, Spain)

II. Elevated puff diffusion experiment over flat terrain (Ravlunda field test site, March 1991, Sweden).

During both experimental campaigns, our mini-LIDAR system was a central instrument for fast and high-resolution plume tracking measurements of the dispersion sceneries:

Real-time sequential data of plume dispersion, in the form of "movies" of instantaneous concentration profiles, were recorded for the compilation of a realistic diffusion data base. Subsequently, we establish plume-profile statistics of important statistical quantities such as mean- and mean-square concentration profiles of the horizontal and vertical plume spread, in addition to the entire concentration probability function (pdf) at a variety of locations during each experiment.

Extensive meteorological mean- and turbulence measurements were simultaneously taken during each experiment in order to provide extensive input-data for the dispersion models to be evaluated and used for simulations.

IV.2 Atmospheric dispersion experiments over complex terrain in a Spanish valley site (Guardo power plant, Oct., Nov. Dec 1990, Palencia, Spain)

An intensive field experimental campaign, sponsored by the spanish companies of electricity, and organized by Hidroelectrica Española, Madrid (under project PIE-134.036) were here conducted in the fall of 1990 with our participation in order to quantify atmospheric dispersion within a deep and steep valley located amid rough, mountainous terrain located in the southern foothills of Picos de Europa. The Spanish program was launched in the summer 1990 with the intention to validate existing plume models and to provide an scientific basis for future model developments.

In the course of these "Guardo"- experiments, the atmospheric transport and diffusion processes were studied over a 40 km by 40 km domain in order to:

1) Evaluate smoke and continuous SO_2 and SF_6 releases from a 185 meter tall power plant chimney
2) Study dispersion from a ground level source located on the floor of a valley surrounded by complex terrain.

As a result of the five week experimental campaign in Spain, a total of fourteen tracer-gas

experiments, of approximately 2 hours duration each, were conducted by the spanish investigators.

Coincidentally, we performed some 18 evaluation and training experiments using artificially generated smoke, and measured the detailed dispersion with the lidar-system from various sites, including measurements taken over the valley floor. In addition we provided detailed meteorological mean flow and turbulence measurements based on a 25-meter high meteorological tower equipped with cups, wind- vanes, thermometers and a sonic anemometer. Also, we contributed measurements of the boundary layer profiles taken from a tethered balloon. Other experimental groups also provided mean-flow measurements from a network of 10-meter towers distributed over the 40 km x 40 km terrain, and also radio-sonde and SODAR-measurements were taken, ref. Ibarra (1991).

While being aware that flow and dispersion in complex terrain can be "site specific", special attention was nevertheless given to diurnal flow reversal and local effects influencing the dispersion characteristics.

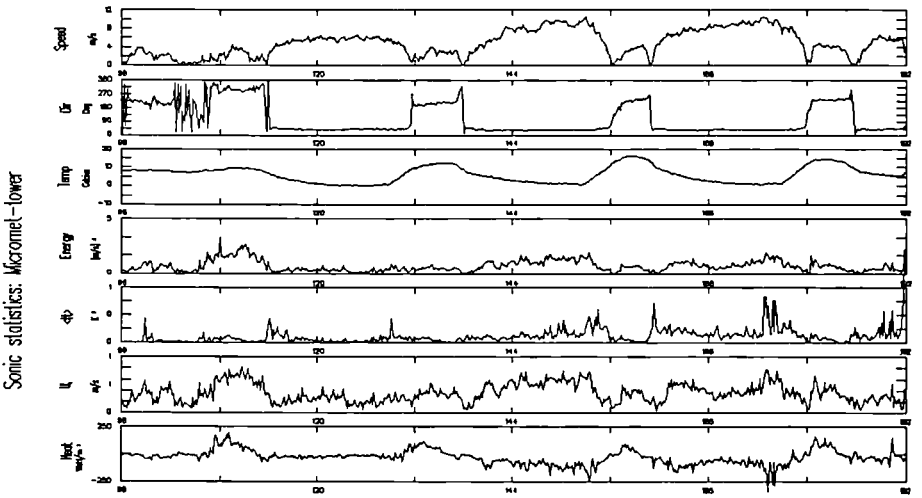


Fig. 1 Four days of repetitive diurnal flow reversal in the valley.

The figure shows the flow- and turbulence characteristics from a four day period (Nov. 5. through Nov. 8) measured by a 25 meter tall met-tower located on the 300 meter wide valley-floor in the upper end of a North-South oriented valley, which is surrounded by high topography on both sides.

The three first traces show the 10-min mean wind speed, the mean wind direction, and the temperature. All do they exhibit a strong diurnal variation with strong (8-10 m/s) nocturnal drainage flow running down the valley (from north) and lasting till almost noon, then followed by a 5-6 hr period of much lighter up-valley (southerly) breeze. In the evening about 1800, the wind again turns back to nocturnal drainage, persisting with strong local winds for the next approx. 18 hours. The remaining four traces shows the corresponding measurements of turbulent kinetic energy (turbulence), the temperature variance, the sheer stress (drag), and a strong but local heatflux mainly caused by advection.

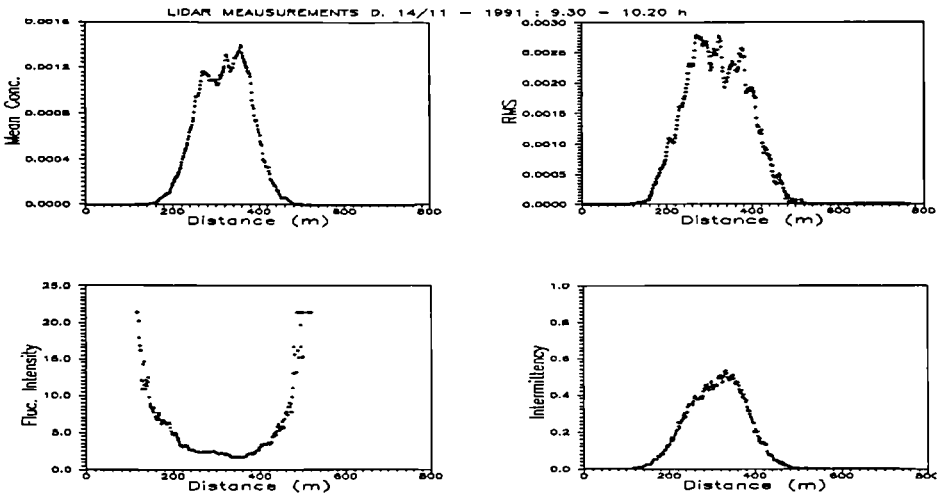


Fig. 2 Examples of plume statistics from the Guardo power plant plume.

The figure gives examples of statistics obtained with the lidar system from the power plants chimney plume: Sequential snapshots of the instantaneous crosswind plume dispersion were taken at 3 sec intervals in the period from 9:30 to 10:20. The plume statistics presented is consequently based on approx. 1000 instantaneous plume profiles and shows, as function of the distance from the lidar system:

a) The mean concentration profile across the plume,

b) The fluctuation profile (Root Mean Square),

c) The fluctuation intensity profile, and

d) The measured intermittency function.

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Head of Project 2: Dr Werner

II Objectives for the reporting period

- improvement of evaluation methods for lidar signals with strong influence of multiple scattering, especially designed for the DLR microlidar

- improvement of the hardware performance of the DLR microlidar (new amplifier design, four simultaneous detector channels)

- test of DLR microlidar with all four channels operating simultaneously and under flight conditions

III Objectives for next period

- to participate in full-scale aerosol diffusion experiments with various types of lidar hardware available at DLR

- to perform concentration profile measurements and to obtain raw data for subsequent calibration and statistical analysis

- further improvement of evaluation methods for lidar signals including effects of multiple scattering

IV Progress achieved including publications

One application of backscatter lidar systems is the observation of the dispersion of aerosol plumes close to the earth surface. Figure 1 shows the measuring geometry used in such situations.

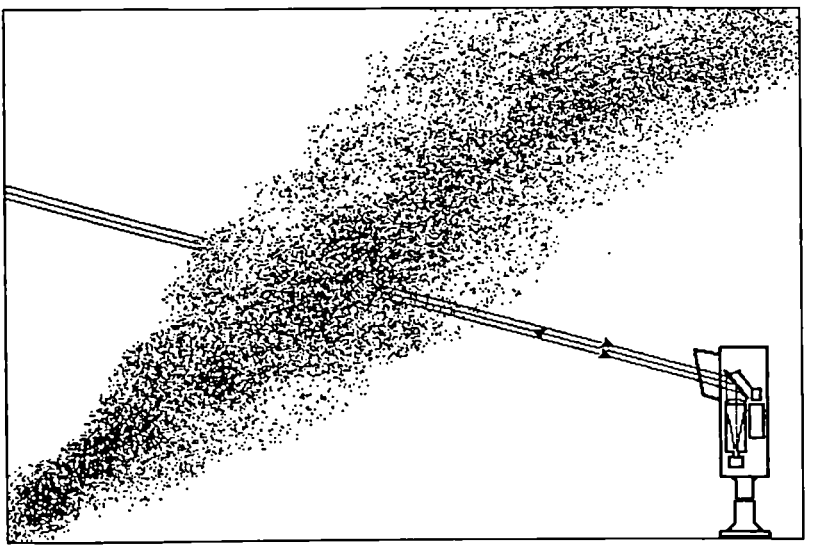

Figure 1. Scheme of lidar measurement of plume dispersion

Estimation of the ground level concentration field of an inert pollutant downwind of a point source is often achieved by the Gaussian plume model. The following equation is used for the concentration χ at a distance x downwind from the source:

$$\chi(x, y, z, H) = \frac{Q}{2\pi\sigma_y\sigma_z U} \exp\left[-\frac{1}{2}\left(\frac{y}{\sigma_y}\right)^2\right] \cdot \left[\exp\left[-\frac{1}{2}\frac{(z-H)^2}{\sigma_z^2}\right] + \exp\left[-\frac{1}{2}\frac{(z+H)^2}{\sigma_z^2}\right]\right]$$

where σ_x and σ_y are the vertical and crosswind standard deviations of the pollutant distribution, U is the wind speed, Q the strength, and H the effective source height. Ground level reflection is assumed.

The lidar measures the signatures $S(R) = \beta_s \tau^2$, where β_s is the volume Mie backscatter coefficient and τ^2 is the extinction loss. If we neglect the extinction loss τ^2, S(R) is in first approximation proportional to β_s. Improvement can be achieved by using the Klett method (Klett (1985)).

But this is only correct if we have a lidar signal consisting only of single scattering. However, in dense aerosols there is always a more or less important contribution of multiply scattered radiation to the signal; in this case the single scattering lidar erquation is no longer valid, also the Klett algorithm can not be used. Therefore the influence of multiple scattering on lidar signals has to be studied carefully to achieve a correction method by which the single scattering signal can be extracted from the total lidar signal including higher orders of scattering. This task can be made much more easier if lidar hardware is used which is designed especially for the measurement of multiply scattered radiation. Therefore the first period of this project has been devoted to

- theoretical studies of multiple scattering to extract the single scattering information from lidar signals both for monostatic (DLR microlidar) and bistatic (modified cloud ceilometer) configurations
- hardware improvement and test of the DLR microlidar especially designed for multiple scattering studies

A. Theoretical Studies

In the last few years a stochastic model has been developed for the theoretical prediction of lidar signals including multiple scattering and for the evaluation of actually measured lidar signals (Krichbaumer and Oppel (1988); Oppel et al. (1989); Oppel (1989); Krichbaumer (1989)). This model has been expanded to calculations of signals from slabs of dense aerosols, as the slab geometry is especially appropriate for cloud and plume measurements (Krichbaumer and Oppel (1990); Oppel and Krichbaumer (1990)). Using the equations provided by this stochastic model, the single scattering contribution to the signal can be calculated from the lidar signal containing multiple scattering, and the evaluation methods based on the single scattering lidar equation can be used once more.

Moreover it seems possible to use the multiply scattered signal as a whole to develope new evaluation methods. First ideas have already been reported here but could not have been tested up to now; they need special hardware configurations as they are provided by a multiple-field-of-view lidar like the DLR microlidar; these evaluation methods use relations between the signals detected in the different fields of view of the lidar. Cf. Oppel and Krichbaumer (1990).

B. Experimental Studies

Parallel to the theoretical work on the influence of multiple scattering a lidar system was constructed which is capable to detect multiple scattering and depolarization simultaneously. It uses two different fields of view and for each field of view two orthogonal directions of linear polarization, as depolarization and multiple scattering are closely related to each other. (On the mechanism of depolarization by multiple scattering cf. the Appendix in Krichbaumer (1989).) In May 1991 flight test measurements with the microlidar were performed, for the first time using all four channels. Although the data have not yet been evaluated, the correct performance seems established; moreover new results with respect to certain types of clouds are to be expected.

To explain some of the possibilities given by the microlidar, Figure 2 shows two examples of lifted fog, measured using only three of the four channels. The signals of the three channels are plotted versus range.

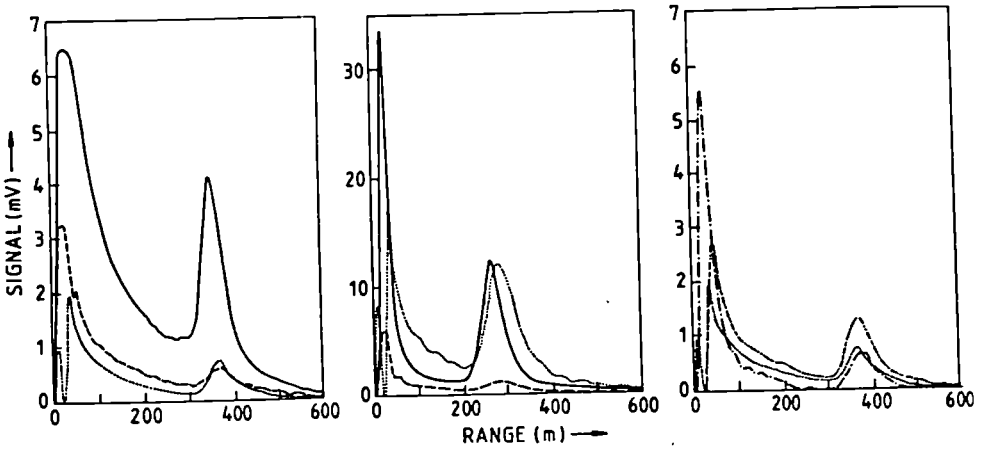

Figure 2. Lidar signals of lifted fog (left: water droplets; middle: ice particles; right: aperture influence of depolarization channel)

Solid curve: FOV 10 mrad - parallel lidar return

Dashed curve: FOV 30 mrad, fieldstop 10 mrad - parallel polarization, multiple scattering

Dotted curve: FOV 10 mrad, perpendicular polarization

Dashed - single dotted curve: field stop 10 mrad, perpendicular polarization

Dashed-double dotted curve: 30 mrad, perpendicular polarization

Each signal is the averaged value over 10 single lidar measurements in 10 sec intervals, averaged also over 8 range gates corresponding to 0.75 m each. The following effects can be seen from Figure 2:

a. Pulse lengthening because of multiple scattering: the multiply scattered signal reaches its maximum at a few meters behind the linearly depolarized signal

b. Different depolarization for water droplets and mixed phase droplets (left and middle)

c. The depolarization for water droplets is similar to the multiple scattering for similar fields of view (right).

More recent results on cloud and plume dispersion experiments can be found in Werner et al. (1990a) and Werner et al. (1990b).

For the next period, we expect a more quantitative treatment of the four-channel-signals; furthermore experimental campaigns together with other systems (both lidar and radar) promise to give new results.

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Contract: Bi7-045 **Sector: C24**

Title: Development of a comprehensive decision-aiding system for the off-site emergency management.

1	Ehrhardt	KfK Karlsruhe
2	Müller	GSF Neuherberg
3	Robeau	CEA - FAR
4	Caracciolo	ENEA
5	Thykier-Nielsen	Risø National Laboratory
6	ApSimon	ICSTM
7	Bartzis	NCSR "Demokritos"
8	Persson	Inst. Meteorological and Hydrolog.

I. Summary of Project and Global Objectives

After a nuclear accident, quick and well-founded decisions are needed in order to mitigate the potential impact on the exposed population. A prerequisite for such decisions is access to good quality information in a timely manner. Computer based systems can satisfy these needs and provide information after collection and processing of data in a manageable and effective form.

With a view to minimizing the duplication of effort and optimizing the use of limited resources, research and development work being carried out in separate institutes will be coordinated and concentrated within a major programme aiming at a comprehensive decision support system for use in Europe. Main purpose of this research programme is to structuralize and to develop the hardware and software framework and the essential ingredients of a real-time system providing support in the case of a real emergency and serving as a powerful tool in training of decision makers, in performing emergency exercises, and as a means for gaining experience with existing emergency plans and recommendations. The system must be able to make predictions from the vicinity of the release to far distant areas and to enable an easy transfer of information and data unperturbed by national boundaries. It has to provide great operational flexibility to cope with differing amounts and quality of measured meteorological and radiological data, site and source term characteristics, environmental conditions, national regulations, emergency plans, responsibility structures, and needs of the users.

The main aims of the system are to convert all relevant data and information with the help of models and procedures into easy interpretable pictures of the actual and future radiological situation. It has to offer the possibility to simulate different countermeasures in order to assess in advance their respective merits and disadvantages in terms of dose or health effects saved and the associated social and economic costs. This would enable countermeasures to be ranked in terms of their effectiveness and practicability and aid in the selection of an optimum approach.

Head of Project 1: Dr. Ehrhardt

II Objectives for the reporting period

Development of the framework for a prototype version of a comprehensive decision support system. To that purpose, the hard- and software structures of the real-time system RESY for aiding decisions about emergency actions in the early phase of an accident (evacuation, sheltering) is being extended to allow for implementation of software products provided by the other contractors. An expert system for evaluating and ranking of alternative actions will be provided as part of the system.

III Objectives for next period

Extension of the hardware and the software of the framework for the prototype version. Coordination of the software developed by the other contractors with respect to the modular structure and interface requirements of the decision support system. Implementation of data and programs, and coding of the graphical software for presenting results.

IV Progress achieved including publications

1. Overall structure of a comprehensive decision-aiding system

In a discussion document prepared with support of CEC, DG XI (Contract No. 89-ET-019), the structure of a comprehensive decision support system for nuclear emergencies in Europe has been set out by KfK. During several occassions, in particular at the contractors meeting in Neuherberg on July 5 and 6, 1990, it has been extensively discussed and finally broadly agreed with some changes of details in the text describing its main features. The system comprises three subsystems which are controlled by an operation system OSY (Fig. 1):

(1) analysing subsystem ASY:
screening of the continuously updated estimates of the present and future environmental distributions of activity concentrations, derived doses/ dose-rates and health effects together with monitoring/measurement data and the uncertainties in the absence of countermeasures;

(2) countermeasure subsystem CSY:
estimation of individual/collective doses, health effects and economic costs of alternative courses of actions together with the technical and personnal aid required and the associated uncertainties;

(3) evaluating subsystem ESY:
judgement of alternative courses of actions under the aspects of practicability in the actual situation, acceptance by and behaviour of the population, and socio-political implications.

The system can be run in two different modes: automatically and interactively. Both modes are allowed in parallel or in isolation. In the automatic mode, the system will provide the user at all times after the start of the system - as far as possi-

ble - with information from each of the three subsystems. In the interactive mode, the user will communicate directly with the system by menu driven input. He can address the various subsystems and options, and use different ways of graphically presenting the results.

The control of the interactive and automatic mode, the linkage of the three subsystems ASY, CSY and ESY, the transfer of data from external sources to the single subsystems, and the exchange of data between them, the data and memory management and the preparation and processing of data for graphical presentation is performed by the operation system OSY. Its careful design and coding is one of the most important tasks when developing the system, because it ultimately determines the flexibility of the system and the communication with the user, independent of the degree of sophistication achieved in the three subsystems.

Experience in this area has already been gained at KfK during the development of the German computerized system for aiding the emergency management, called RESY, supported by the Ministry of Environment. Main purpose of this system is to help in decision making about early emergency actions in the vicinity of the site, such as sheltering and evacuation. The software structure of its operation system OSY is being extended to allow for implementation of software products provided by the other contractors. The system software is being developed as a transportable package to run with a standards based UNIX operating system, X-window user interface package with STARBASE/GKS graphics and SQL/ALLBASE data base manager. The hardware configuration consists of a HP 9000/835 S host computer, connected with 3 workstations and a special graphical station for presenting results and site characteristics on maps. Advice is being given to those contractors who intend to provide software products for integration in the prototype version which is intended for demonstrating the main functions and the principal input/output options of a comprehensive decision support system at the end of 1992. In particular, the dose assessment program EURALERT developed under contract No. BI-6-0255-D is being restructured by GSF for integration in this prototype version.

2. Coordination

The development of emergency response systems, which are able to support decision makers in the event of a nuclear accident, is a relatively new area of R&D work. Till now no comprehensive decision support system exists and only a small number of systems are already in operation, which - in general - can only respond to a limited number of questions posed by a decision maker. To a great extent, this is due to the fact, that by far not all methodological problems are solved and all information and data available to built a complete system.

During the discussions about the structure and the functional specifications of a commonly agreed system, several areas were identified where additional research/investigation is necessary to complement the existing contracts in order to assure that all aspects of the system are being addressed and receiving appropriate attention. Those which were considered to have high priority are briefly described:

(1) Development of criteria for selecting meteorological and atmospheric dispersion models from a hierarchy of models according to the actual conditions.

(2) The use of model predictions and monitoring data in the optimisation of emergency response (data assimilation).

(3) The further development of atmospheric dispersion models, and their associated meteorological inputs (e.g. forecast, windfields), in order to provide probabilistic as well as deterministic estimates of relevant quantities.

Several meetings on these topics were organized by CEC, DG XI and DG XII, with input from KfK in the form of problem descriptions and proposals emerging from the needs of the various subsystems and their software realisation. Besides the existing close cooperation with the contractors and the associated institutions (JRC-ISPRA, S.C.K./C.E.N. Mol), new contacts have been established with the University of Leeds, UK, the Federal Office of Public Health, National Emergency Operations Centre, CH, and the National Radiological Protection Board (NRPB), UK, already indirectly involved as contractor of the RADE-AID project. An ongoing effort is the administrative coordination of the contract and the corresponding communication with the contractors.

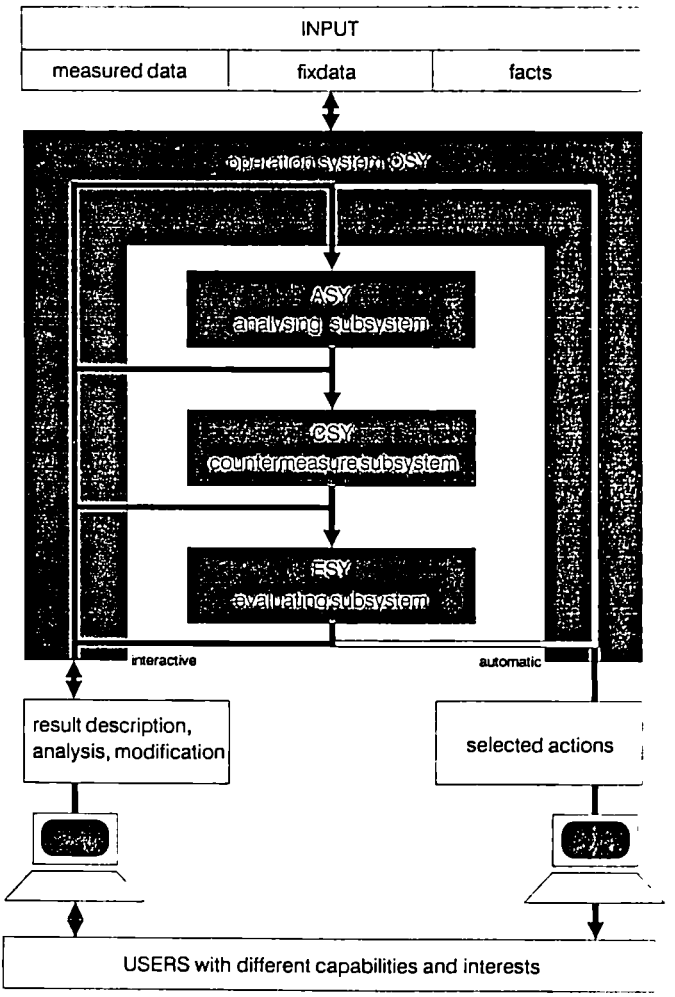


Fig. 1 Overall structure of the decision support system

Head of Project 2: Dr. Müller

II Objectives for the reporting period

- Design and development of a modified version "EURALERT-91" of the computer code "EURALERT" for the calculation of the transport of radionuclides through the foodchain and of doses due to all relevant exposure pathways. The modifications comply with the requirements and the structure of the proposed decision support system as far as they are fixed at present in agreement with the other contractors.
- Extension of the foodchain model in EURALERT to products not yet taken into account, which are of some importance in other parts of Europe.

III Objectives for the next period

- Adaption of the models and data of "EURALERT-91" according to the detailed technical structure of the operation system provided by KfK and the interfaces to be agreed upon.
- Test calculations with regard to the identification of bugs and the optimization of the code.
- Preparation for the installation of the modules in the decision support system.
- Extension of the data base of the code to some more radionuclides including fission products of minor importance, corrosion products, actinides and noble gases.
- Provision of default parameters for different regions in Europe.

IV Progress achieved including publications

In the framework of the research programme "Radiological aspects of nuclear accident scenarios" the real-time emergency dose prediction system EURALERT has been developed during 1987-1990. This computer code has been designed to allow its adaption to the different living habits, climatic and agricultural conditions in the different regions of the European Community.

In the current research programme, the EURALERT program system is used as a basis for the foodchain transport module, the dose module, and for parts of the countermeasure modules in the proposed decision support system. According to the requirements of this system, a refined version "EURALERT-91" of the EURALERT computer code has been designed and is under development.

The main features of EURALERT-91 are:

- EURALERT-91 consists of a frame program and a number of modules.
- During the development of EURALERT-91 the frame program has to do the tasks which will be performed lateron by the operation system OSY, esp. input and output operations and controlling the sequence of the modules. In the established decision support system the frame program is no longer necessary.
- The modules of EURALERT-91 are outlined to fit into the decision support system without changes or at most minor adaptions. For this purpose, all input and output operations have been banished from the modules, and the modules are coded as subroutines without parameter list.
- Input and output data are submitted to and from the modules via COMMON-blocks located in include-files together with the definition of these data. Progress in the capabilities of workstations regarding available RAM make it possible, at least for the greater part of the interfaces, to avoid data transfer from one module to another via external files but to keep the data inside the memory. This implies, however, an effective use of data space and limitations on the dimensions of the calculated data.
- For the purpose of an easy adaptability the declaration of dimensions is performed by PARAMETER-statements and submitted to the modules via include-files.

According to these items the computer code of EURALERT has been disintegrated, modified, and is being recompiled as EURALERT-91. At present, in EURALERT-91 the exposure pathways inhalation, external exposure from ground and from cloud are established and from the ingestion pathway the calculation of normalized activities in primary products is implemented.

Beside these program developments the foodchain model of EURALERT has been refined by developing a model for predicting activity concentrations in rice after deposition of radionuclides. The inclusion of rice into the list of foodstuffs improves the applicability of the program for regions in Southern Europe.

Head of Project 3: Dr. Robeau

II Objectives for the reporting period

The objectives of this contract for the reporting period is to bring and expand the existing knowledge in order to obtain prediction capabilities concerning airborn radioactive pollutant dispersion and deposition under any terrain complexity and atmospheric conditions. During this period we have drawn, expanded and improved a complete set of calculation tools in the form of a system code permitting to do forecasting or real time dispersion computations, to be used in dose assessments in case of radioactive release in the air.

III Objectives for next period

During next period, objectives are to study the specific aspect of dispersion such as transport and deposition in the presence of complex topography, surface water, rain, especially when such conditions simultaneously exist. The primary aim of this task is on one hand to expand and improve the performance of the proposed system code and on the other hand to contribute towards better understanding of these complex phenomena.

IV Progress achieved including publications

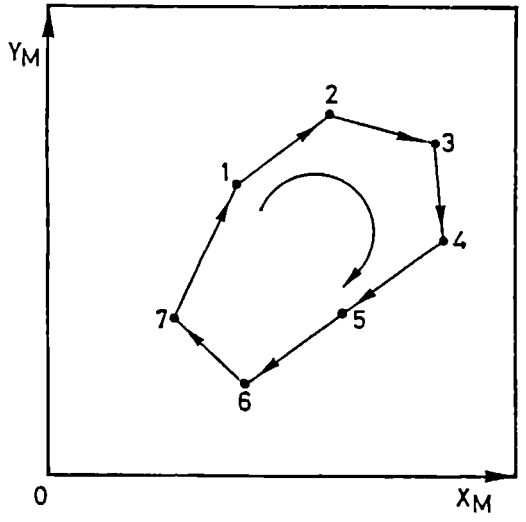
The achieved work concerns a code system development based on the MC 31 and ADREA codes, including descriptive data homogenization ; the first code system preliminary version has been generated by the existing version, ADREA and MC 31 codes. The interface and data homogenization work will cover topics such as ADREA and MC 31 interface introducing a common three-dimensional representation of the orography of sites. A common support programme development to perform automatic and optimum discretization of the complex domain under considerations has been developped.

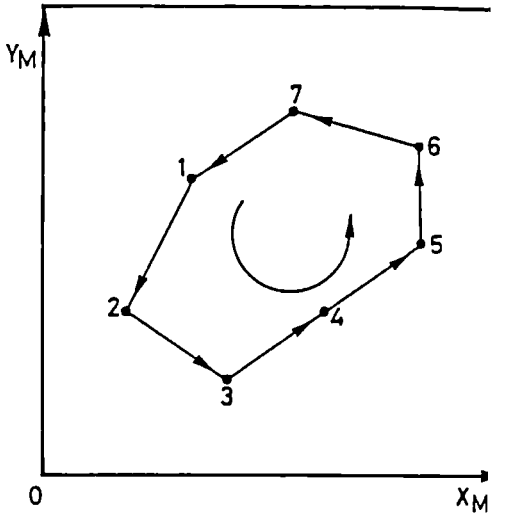
Principles of the design of the 3D-Relief

It is to define a relief on a finite part of a three dimensional space. This space is sliced up by parallel planes (x = 0, y = 0, z), (x, y = 0, z = 0) and (x = 0, y, z = 0). Thus, the 3D-Space is made up by a set of rectangular parallelepipedic boxes. Inside this space, a relief is defined. This relief is a set of obstacles : mountains, buildings, lakes, etc...

The surface of each obstacle is defined on a set points coming from orographic plotting.
After, this surface will be automaticaly built up by three-dimensional triangular meshes. Rectangular axes (O ; x, y, z) are associated to the 3D-Space, and the dimensions of this space are : $[O, X_M] \times [O, Y_M] \times [O, Z_M]$. Inside this space, it is needed to draw obstacles on the planes Z = 0 ; all these obstacles constitute the relief.

The base of an obstacle is defined on a polygon. This polygon is defined on a set of points. The points must be introduced following a logical order, (clockwise or inverse, but no out of sequence).





The bases of the obstacles must recover the domain [O, X_M] x [O, Y_M] totaly. Consequently, the points composing the polygon must be located in the area determined by the four points [O, O], [X_M, O], [O, Y_M], [X_M, Y_M]. The relief of an obstacle is defined on a set of points located inside the polygon. Each point is located by its 3 coordinates (X_i, Y_i, Z_i).
In the Z-axis direction, the maximum spot heigh must be smaller than Z_n. Inside an obstacle or the equivalent polygon, two points defined on identical coordinates cannot have differents altitudes.

The data necessary to the definition of the relief have two objectives :

- Describe the surface of the relief, i.e. ,
the obstacle number,
the coordinates (x, y, z) of the points defining the surfaces of the obstacles ;
the coordinates of the points defining the bases of obstacles.

- Define a 3D-Grid permitting to slice up the space on 3D-Rectangular boxes.

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Head of Project 4: Dr. Caracciolo

II Objectives for the reporting period

To define the input/output and the flow diagram of a meteorological preprocessor for atmospheric dispersion models as a component of the Analysing Subsystem. To define the most suitable methods for deriving the meteorological parameters based on the state of the art of the boundary layer parameterization and the requirement of felxibility of the preprocessor with respect to the available data. To find out the available routines which can be included into the preprocessor.

III Objectives for next period

To write the technical specifications of the software package for the preprocessor. To implement or develop routines for deriving meteorological parameters. To develop the software package. To test the software package.

IV Progress achieved including publications

The object of the ENEA-DISP contribution to the CEC contract " Development of a comprehensive decision support system..." is the development of a software package for the assessment of meteorological parameters needed by real time atmospheric dispersion models.

During the first phase of the contract, the general structure and characterics of the meteorological preprocessor have been defined. The first requirement was to design the preprocessor as a independent module that can easily fit into the general architecture of a comprehensive decision support system, in particular into the Analysing Subsystem (ASY) of the CEC project. For this purpose, the preprocessor is independent on the atmospheric dispersion model(s) included into the system,

and is flexible with respect to the meteorological data that can be available on site (which can range from a single wind measurement and cloud cover observation to vertical profiles of wind and temperature).

As far as the hardware environment is concerned the best solution should be represented by either high performance Personal Computer or or Workstation due to their large diffusion and the cost-effectiveness ratio. For similar reasons, system software is going to be either DOS or UNIX.
All functions of the module (praparation of input data, selection of the parameterization method, analysis of the results) are driven by a user-friendly menu.

The meteorological preprocessor estimates boundary layer parameters generally used by atmospheric dispersion models such as mixing height, friction velocity, Monin-Obukhov length, etc. In order to identify the most suitable and updated methods for deriving these parameters, a review of recent works available in the literature was carried out. In addition, a scientific collaboration with Risoe National Laboratory, which has a consolidated experience in the real time assessment of atmospheric dispersion parameters, was established. After informal agreements occurred during technical meetings carried out in Luxembourg and in Paris, Dr. Desiato of ENEA-DISP met Dr. Mikkelsen and Dr. Thikier-Nielsen at Risoe in April 1991.
During the meeting an agreement on the structure of the preprocessor was reached and technical informations and suggestions regarding the methods for estimating the meteorological parameters were exchanged.

At present, the flow diagram and most of the routines of the preprocessor have been defined. Some of the routines are available in a different software environment and will be modified and adapted to be included into the preprocessor. Others will be developed and tested in the next period.

Head of Project 5: Dr. Søren Thykier-Nielsen

II Objectives for the reporting period

Improvement of the modelling of plume rise, building wake, dry deposition and gamma-doses in RIMPUFF.
Interfacing with other modules in the emergency preparence system.
Model evaluation for complex terrain.
Creation of a flexible user-interface for the RIMPUFF/LINCOM system.
Flow modelling for unstable and stable meteorological situations.

III Objectives for next period

Implementation and testing of the new models for plume rise, building wake, dry deposition and gamma-doses in RIMPUFF.
Optimization of the computer codes for RIMPUFF and LINCOM.
Interfacing with regional scale models in the emergency preparence system.
Development and testing of the user-interface for the program system.
Development of an interactive graphic package for presentation of windfields, doses and other informations pertinent to the evaluation of a dispersion scenario.
Interfacing with pre-processors for the RIMPUFF/LINCOM system.
Model evaluation.

IV Progress achieved including publications

IV.1 Introduction

During the reporting period work has concentrated on topics within the areas of model development and evaluation. Substantial progress has been made although none of the "sub-tasks" will be finished before the end of 1991. Emphasis has been placed on identifying and improving the areas where the RIMPUFF / LINCOM model complex needs improvements in the context of the real-time emergency response system.

IV.2 Model Development

New and updated models for plume rise, building wake and penetration of the inversion lid are being introduced in RIMPUFF. These sub-models are based on the methods used in the OML-model (*OML=Operational Air Pollution Model*) developed by the danish environmental pollution laboratory. The work is done in cooperation with IMK, Karlsruhe.

A better model for calculating the gammadoses from puffs is being introduced in RIMPUFF. The model is based on the methods described in Slade.

A system for coupling RIMPUFF with the flowmodel LINCOM has been implemented on a PC. LINCOM is under improvement by introducing temperature forcing, so it becomes able to deal with both unstable and stable situations. In addition to the neutral a method for calculating a mass consistent flow field based on data from a combination of the flowfields from several wind station will be operational mid 1991.

Modelling of chemical reactions involving the materials released to the atmosphere will be included in RIMPUFF. Work has started in april 91 and a preliminary model will be available in august 91.

A closer cooperation has been established between Risø, the Swedish Meteorological and Hydrological Institute (SMHI) and the danish meteorological institute (DMI). Risø and SMHI are coupling the Risø mesoscale puff (RIMPUFF) to the SMHI regional scale dispersion model RAM. Methods for connecting the HIRLAM flow model to RIMPUFF are being investigated by DMI and Risø. The aim of this work is to establish an integrated system for calculating the dispersion of toxic material over long (1000 km) and medium range. HIRLAM and RAM are used for calculating the regional scale dispersion, while RIMPUFF, using the windfield data from HIRLAM, should calculate the dispersion on local/medium scale (100 km).

IV.3 Preprocessing of input data

Preprocessing of meteorological data for dispersion models is studied in cooperation with ENEA (*Project no. 7*). A simple pre-processing system for use with RIMPUFF have already been established in connection with the development of a model for the dispersion of virus

released to the atmosphere.

IV.4 Model evaluation

RIMPUFF/LINCOM are being evaluated using data from two complex terrain experiments: *SIESTA* and *Guardo*.

An experimental evaluation of flow field and dispersion modelling has been performed, using data from the complex terrain SIESTA experiment (SF6 International Experiment in STagnant Air). Two alternative flow-field calculation methods were evaluated as "drivers" for the dispersion model RIMPUFF: One is the diagnostic mean-flow model LINCOM based on (linearized) Navier-Stokes equations, the second is based on simple interpolation method using tower data (objective wind analysis).

SIESTA was performed by several European groups in November 1985. Measurements were taken of advection, turbulence and dispersion during neutral and convective weak-wind situations over complex terrain of the Jura ridge and the hilly prealpine region. Dispersion characteristics were determined by use of SF6-tracer gas released from the met-tower of the Gösgen nuclear power station at 6 meters height.

Three of the SIESTA experiments were simulated, using RIMPUFF/LINCOM. In one of the experiments, wind direction and turbulence condition changed strongly with time. The comparison between measured and calculated values were quantified by the determination of Chi square, relative Chi square, correlation coefficient and mean error factor. In an orographically influenced dispersion scenario, like SIESTA, potential of improvements by use of high-resolution mean flow model LINCOM was found.

The *Guardo* experiment, which was performed in northern Spain in november 1990 is now being evaluated. As the two experimental sites where located in mountainous terrain, RIMPUFF run on the output from the fast high resolution mean flow-model LINCOM (see fig. 1.). Further evaluation is in progress.

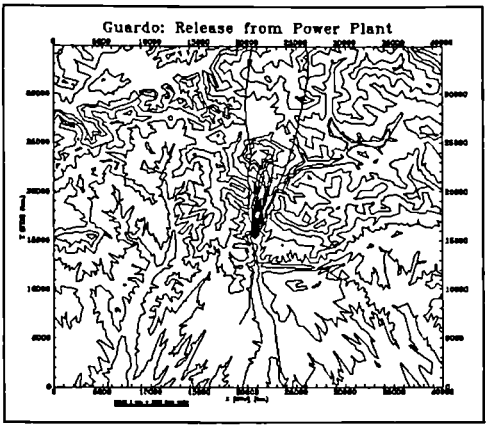

Figure 1 Release from Guardo Power Plant

IV.5 References

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Head of Project 6: Dr ApSimon

II Objectives for the reporting period

Initial development has been undertaken of a model to simulate atmospheric dispersion of an accidental release out to continental scales for use in the event of a nuclear accident. In conjunction with data from a weather forecasting model this is designed to provide predictions of the levels of contamination in air and deposited on the ground. A Monte-Carlo approach has been chosen, representing a release as an assembly of particles advected according to the mean 3-dimensional wind-fields, but with random displacements to represent smaller scale eddies and turbulence. An initial version of the code is operational, and has been made available through ENEA in Italy, as a component of a joint collaborative package produced with our co-contractors in Italy, france and germany.

III Objectives for next period

It is now intended to concentrate on some specific factors which have been identified as particularly important for the potential consequences of a major accident. These include the transport of radionuclides within frontal systems where precipitation is likely to occur; and also parameterisation of convective clouds which can also yield concentrated wet deposition, as well as venting to higher levels in the atmosphere. Further effort will be put into improving paramaterisation of the boundary layer.

Attention will also be focussed on the practical applications of model simulations for the longer range effects in an accidental situation. This includes rapid response and computer efficiency (in which ancillary studies on the use of parallel processing techniques will contribute), and the combined interpretation of model results and radiological measurements to revise assessments of contamination during an evolving emergnecy scenario. This is highly dependent on a thorough understanding of the uncertainties involved in the assessments.

IV Progress achieved including publications

Experience gained in applying our relatively simple Lagrangian puff model, MESOS, to interpret the radiological measurements in the immediate aftermath of the Chernobyl accident, convinced us of the advantages of the Lagrangian approach ,in which the histories of different components of the release are traced independently as they move across the map area and can be cross-correlated with observed evolution of the areas exposed (by contrast an Eulerian approach integrates the advection-diffusion equation for all the material together moving through a fixed spatial grid). However it was apparent that MESOS, which largely ignored variations of wind with height, and vertical motion, was inadequate to represent the full 3-dimensional nature of the transport across Europe. Cloud processes and precipitation systems were also identified as requiring improved treatment.

Accordingly it was decided to develop the 3-diemensional Monte-Carlo particle model 3DRAW (3-Dimensional RAndom Walk) which represents a release as an assembly of particles advected with the mean wind, and with random perturbations to represent sub-grid scale variations in wind. Each partcile represents variable amounts of selected nuclides, these quantities being depeleted during transport according to the probability of decay or deposition. Deposition is calculated due to both dry and wet processes, the latter with provision to distinguish between convective and frontal precipitation where meteorological data from forcasting models enables this (the height of origin of material scavenged can be very different in the two cases).

The model calulates time-integrated atmospheric concentrations in ground-level grid cells over specified periods of time (eg 3 or 6 hourly intervals) and cumulative dry and wet deposition over the same grid, spanning the map area. This is in contrast to other particle models which tend to produce a sequence of instantaneous snap-shot distributions of atmospheric concentrations: it reflects a novel feature of the model in that contributions to exposure are calculated along the whole trajectory of each particle, rather than using just positions at certain times (thus reducing the number of particles which it is necessary to track and reproducing more closely the observed quantities). This statistical approach can also be interpreted to give a more probabilistic indication of exposure at a location of interest.

The model has been constructed in such a way that it can be readily adaptable to meteorological data from different national or European forecasting models; for example it can take winds specified either for straight or staggered grids. However it has been applied so far with data from the UK Meteorological Office fine-mesh model. It is written in standard FORTRAN, and has been implemented on a VAX computer at ENEA in Italy in its present preliminary form, where it is available together with other modules produced by co-contractors as computer tools for emergency response purposes.A detailed description may be found in the EUR report listed below.

However this is a preliminary version and significant improvements are envisaged in the next phase of the programme as indicated above.Initial studies of transport through frontal sytems have already begun. Recognising the advantages of better spatial and temporal resolution in the meteorological data to avoid interpolation difficulties, archived data has been obtained from the UK Meteorological Office mesoscale model for selected frontal systems

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Head of Project 7: Dr. Bartzis

II Objectives for the reporting period

Draw, expand and improve a complete set of calculation tools to obtain prediction capability concerning airborne radioactive pollutant dispersion and deposition under any terrain complexity and atmospheric conditions, in close collaboration with the CEA (France). The particular objectives were: development of a preliminary version of a system code by interfacing the ADREA-I ("Demokritos"-Greece) and MC31 (CEA-France) codes, development of a code performing automatic and optimum discretization of the complex terrain considered, review on modelling the precipitation, wet scavenging and deposition.

III Objectives for next period

Ground-dependent dry deposition modelling; determination of rain water runoff stream channels network and identification of pollutant accumulation areas on the ground surface; implementation of new mathematical formulations for orographically induced precipitation, wet scavenging and deposition; case study calculations over selected site(s) will be carried out, to test the code system capabilities.

IV Progress achieved including publications

The MC31 and ADREA-I codes have been merged, the former performing pollutant dispersion calculations in the wind field produced by the latter. The topography of the complex terrain they both take into account, is simulated by the DELTA (Discretization by ELements of Triangles Approach) code /1/, jointly developed by "Demokritos" and the CEA.

The current version (1.0) of the DELTA code may accept user-supplied data, or may directly use digitized charts built according to French (CEA) or Greek ("Demokritos") specifications.

The modellization of the topography in the DELTA code is obtained using adjacent triangular surfaces in number and size depending only on the accuracy required. Each triangle simulating the ground surface may have its own characteristics: orientation, area, albedo, soil type, roughness, deposition velocity etc. Each user-defined cartesian calculational cell can include many ground surface elements and corresponding one-eighth-type surface and volume porosities (percentage of volume and fluid-fluid or fluid-solid contact surfaces occupied by fluid) which are important items in hydrodynamic calculations, are analytically computed.

For subsequent air/ground energy exchange calculations, the DELTA code determines each triangular surface element to be either sunny or shaded, given the geographical location of the area under treatment and the timing of the event studied.

More details concerning the DELTA code may be found in the description of progress achieved in Project No 2 of the current contract.

A review study has been carried out concerning the mathematical modelling, in Eulerian formulation, of clouds, precipitation and wet deposition. First the modelling of clouds and precipitation in mesoscale system was surveyed and next the modelling of pollutant transport into a precipitating atmospheric system was reviewed /2/.

Regarding precipitation modelling the following remarks can be mentioned:

The presence of water substance constituents, like water vapour and suspended or falling water particles, is taken into account indirectly by formulating the equation of state in terms of, somehow artificial variables, such as the virtual temperature or the liquid water potential temperature. Such an approach is based on constant physical properties, including latent heat can introduce substantial errors in the real temperature in the upper troposphere. In most models separate transport equations are used for cloud water, rain water and water vapour. The advantage of the latter approach is the ability to treat explicitly certain microphysical transformation processes. However, explicit modelling is required for the above mentioned microphysical processes difficult to be directly verified in real conditions.

Based on the review remarks and the fact that the code should be also focused to low CPU times, the following features have been adopted to "wet" conservation equations for the present model /3/:

A single prognostic equation for the whole of water substance will be utilized. The advantage of the present approach is the model simplicity and the potentiality for lower computer time by avoiding the addition of more differential equations for solution. In addition no explicit modelling is required for microphysical processes difficult to be directly verified.

The energy transport is expressed in terms of internal energy instead of liquid potential temperature.

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Head of project 8: Christer Persson

II Objectives for the reported period

Our part of this programme have, during the reported period, been devoted to:

* adaption of an Eulerian meso-γ scale dispersion model to the European scale
* development of the model description of the emission phase of an accidental release
* development of the model description of the initial 24 hours plume dispersion
* include non real time precipitation data
* adaption to different sets of meteorological input data
* recoding to improve the portability of the model code
* some model applications on the European scale
* preparations for real time applications.

III Objectives for the next period

For the next period our aim is to:

* further develop the model description of the initial 24 hours dispersion of the plume by implementing a plume-splitting technique where the vertical wind shear is better described
* include precipitation data, both near real time analyses of synoptic observations and forecasts from HIRLAM (joint Nordic-Dutch-Irish high resolution weather foracast model) and from ECMWF (European Centre for Medium-range Weather Forecasts)
* improve the analyses of some essential dispersion parameters, as the boundary layer height and the eddy diffusivity, based on output from HIRLAM or ECMWF
* some model evaluations on data from the Chernobyl accident
* further preparations for real time applications based on both HIRLAM and ECMWF data
* have a first version of the model code transmitted to the coordinating group at Karlsruhe.

We suggest that the last point takes place during the autumn 1991, which is some months later than given by earlier plans. However, it will then be possible for the present EC-project to take advantage of results from a cooperative study which we intend to have together with CRAY Research Ltd during the summer 1991. Our dispersion model will be used on a CRAY computer for scientific purposes and the model code will be adjusted by CRAY personnel. The code can then be transmitted to Karlsruhe as soon as the CRAY staff is ready with the adjustment.

IV Progress achieved including publications

The dispersion model is based on an Eulerian meso-γ scale model which has been adapted to the European scale. The emphasis in the further modelling work has been put on the description of the inital phase of the accidental release and on the description of the first 24-hour dispersion of the plume. A correct description of the initial phase of the release and the first hours transport and dispersion is a very important part of emergency response systems for large scale transport. Improvements have been done of the description of the initial spread of the released cloud. Presently a Gaussian puff model is used. We have improved the technique of handling the puffs accurately and having them introduced into the grid point model in a proper way.

The model code has been recoded giving a more general structure and, what is more important, a large emphasis has been put on increasing the portability of the code. We have restricted the coding, as far as possible, to FORTRAN 77. The Eulerian gridpoint model was intentionally developed for a regular grid with equal grid spacing. When adapting to the European scale various map projections have to be considered. The system was therefore recoded to be independent of projection. However, the map projections used have to be specified and at present the following projections are accounted for:

- regular equally spaced grid
- latitude-longitude grid
- rotated latitude-longitude grid.

Other projections are easily implemented in the system, and the system therefore has a great feasibility to use analysed or forecast meteorological input fields from various data bases. However, the interface to such data has to be carefully specified.

The system has been used with either HIRLAM or ECMWF meteorological input data in some different test calculations. Examples of results from these applications are briefly presented below. HIRLAM data have, so far, only been applied to non real time simulations while ECMWF data also have been used for forecasts in real time applications. Model calculations, based on ECMWF meteorological forecasts, were performed just before the Persian Gulf war started. Our intension was to give simplyfied forecasts, up to five days ahead, concerning the dispersion of soot/SO_2 and radioactive material from hypothetical emissions in the area. The oil fires were assumed to consume 10 million barrels of oil per 24 hours. When the calculations were performed, still no oil fires had started. Examples of forecasts, transmitted before the war started, are given in Fig 1-2.

A model application, focusing on the importance of accurate precipitation information, has been performed using some data from the Chernobyl case. The dispersion model has been applied using wind fields from HIRLAM while precipitation fields were generated by careful subjective analyses which then were digitized. The analyses for Sweden were based on a network of 800 daily precipitation stations and 140 synoptic weather stations. A comparison between model calculated, Fig 3, and observed, Fig 4, deposition of ^{137}Cs shows very good agreement in this case. The agreement is obviously to a large extent depending on the accuracy in space and time of the precipitation fields used in the model. The importance of precipitation for the deposition pattern can be seen from a comparison of the detailed analysis of precipitation amounts, Fig 5, with observed ^{137}Cs deposition, Fig 4. However, the detailed precipitation analyses, which have been used in this model

application, are much more accurate than could be accomplished by objective analyses or from forecasts available in real time.

Therefore, the uncertainty of precipitation analyses and forecasts has to be addressed. We suggest that some work, during the next period, will be devoted to tentatively investigate the possibilities of using a statistical approach of estimating the uncertainty due to precipitation.

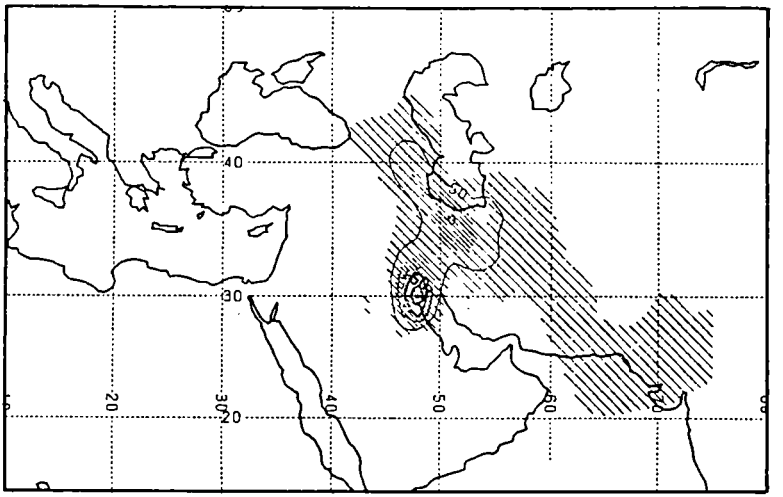


Figure 1. Assumed continuous burning over Kuwait. Assumed start of emission: 17 January 00 UTC. Concentration map refer to time: 21 January 12 UTC. Predicted concentrations of SO_2/soot ($\mu g/m^3$) are given.

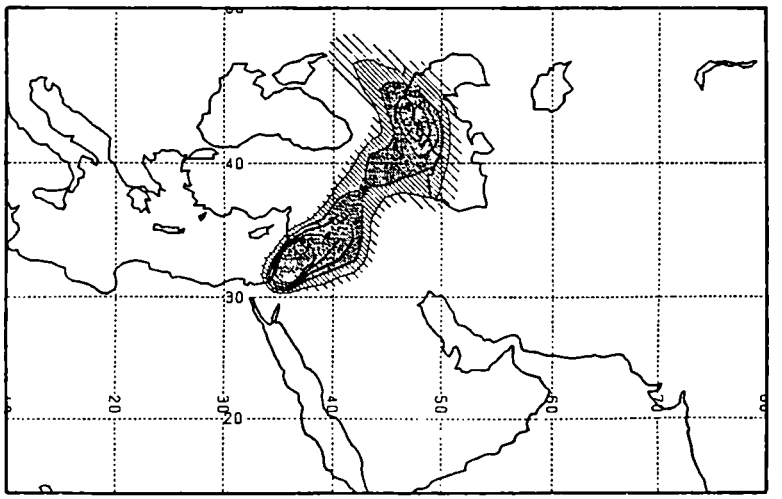


Figure 2. Assumed short time emission over Tel Aviv. Assumed emission time: 17 January 00 UTC. Concentration map refer to mean value for: 17-21 January. Predicted concentrations given in relative units.

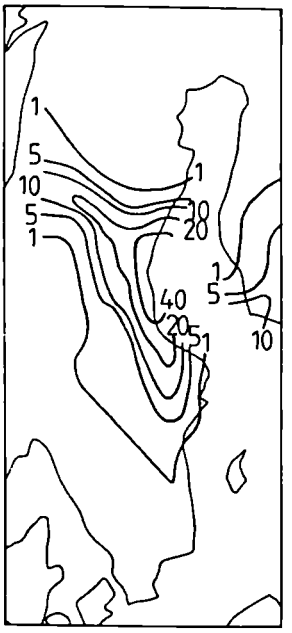


Figure 3. Calculated wet deposition of ^{137}Cs (kBq/m^2) from Chernobyl, 26-29 April 1986.

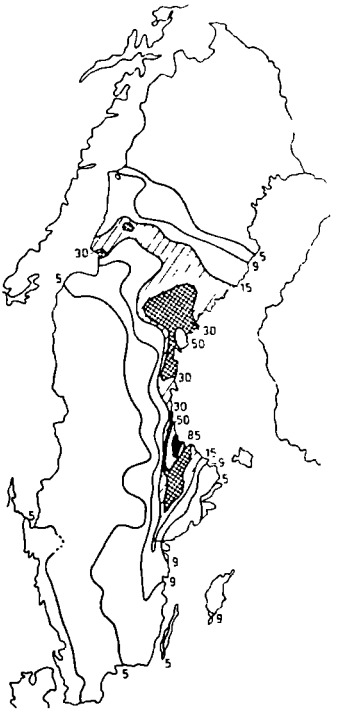


Figure 4. Measured deposition of ^{137}Cs (kBq/m^2) over Sweden. Measurements made from aircraft by the Swedish Geological Company during 1-23 May 1986.

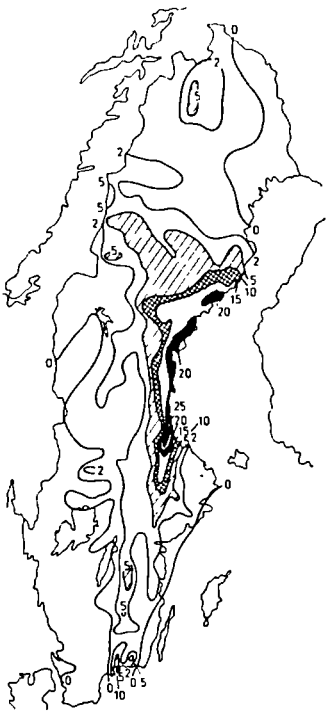


Figure 5. Recorded precipitation (mm) over Sweden for the period April 28 06 UTC to April 30 06 UTC.

Proceedings:

Persson C. and Robertson L. 1990.An operational Eulerian dispersion model applied to different scales. NATO/CCMS Meeting, Vancouver Canada.

Persson C. and Robertson L. 1990. An operational Eulerian dispersion model applied to different scales - Applications possible on a subgrid level in the EMEP grid. EMEP Workshop, Potsdam, Germany.

Robertson L. and Persson C. 1991. On the Application of Four Dimensional Data Assimilation of Air Pollution Data Using the Adjoint Technique. EUROTRAC Workshop, Wiesbaden, Germany.

Contract: Bi6-106 **Sector: C24**

Title: Real-time uncertainty handling and development of a computer based training system for the management of off-site nuclear emergencies.

1 Govaerts CEN - SCK

I. Summary of Project and Global Objectives

In case of an accidental release of radioactive materials to the atmosphere, it is a major task to make as soon as possible a realistic assessment of the environmental impact of the releases, in order to optimize interventions for the protection of the public. Past accidents have learned that the availability of data on source terms and transfer parameters might be rather weak. Reliable environmental monitoring data might be considerably delayed.

This project aims to develop a methodology to maximize the use of available information at each moment. The model predictions are compared with the early environmental monitoring results, in order to revise model input data and transfer parameters. This method aims a smooth transition between the early impact assessment, based exclusively on models and the final impact assessment based exclusively on environmental monitoring.

Head of Project 1: Dr. Govaerts

II Objectives for the reporting period

1. To discuss the incorporation of a "environmental survey monitoring data feedback" - module in the "Comprehensive decision support system for Nuclear Emergencies in Europe", developed under contract : BI7-0045-6.
2. To work out the previously developed methodology, based on a numerical optimization process for a realistic environmental monitoring system.
3. To discuss alternative methods to handle the differences between predicted and observed environmental data, in order to decide on the approach to be used in the European decision support system under development. such an approach should avoid the difficulties associated to the numerical method, developed up to now by the contract.

III Objectives for next period

1. Preparation of a demonstration unit on PC, using the results discussed by IV.2.
2. Further analysis of the alternative approaches discussed by IV.3.

IV Progress achieved including publications

1. Incorporation of a "feedback" - module in the "Comprehensive Decision Support System for Nuclear Emergencies in Europe"

The incorporation of a module to correct predictions, based on a real-time dose assessment by considering some environmental data is discussed with the coordinator and the partners of the CEC-Radiation Protection Programme, contract BI7-0045-C during working sessions, organized at Neuherberg, Brussels and Karlsruhe. The main conclusions are :

- To develop an off-line module, with an input-output compatibility with the main system. The module can be inserted in the system under the control of the user. The module has to be considered as a tool for the user to explain differences between predictions and observations. It remains up to a users'decision to correct the predictions in the way proposed by the feedback module, and to associate weighing factors to the reliability of individual monitoring results.
- Regarding the complexity of the exercice a feedback exercice has to be based on a bigaussian atmospheric dispersion model. Eventually it can be organized to confirm or correct in a first instance the trajectory of the center of mass of the releases, followed by a correction of source term, height of release and dispersion around the trajectory.
- Parameters optimized by the application of an optimization loop to a simple model, can then be introduced in a new assessment applying a more complex dispersion model.

2. Demonstration of the numerical optimization technique

The previously developed numerical optimization technique has been demonstrated on the measuring data collected by a set of gamma exposure rate monitors. The response of a gamma exposure rate meter can be predicted by :

$$R(x,y) = f\ (S,\ H,\ \text{stability},\ \text{windspeed},\ \text{wind direction})$$

where
- S is the source term, to be considered as a sum of release terms associated with consecutive gamma energy windows
- H is the effective height of release.

A given response constraints the possible values of the arguments in a parameter space, defined by this physical relationship. The response of all monitors constraint those values to the intersections of the specific subspaces. In the ideal case this intersection will be a point giving the exact value for each argument, as soon as the number of monitors equals the number of arguments. In real cases this intersection will never be a single point.
A numerical optimization technique allows to choose a best value for each argument.
The most sensitive parameters will be the source term, the effective height of release and the wind direction.

As an example the analysis of the response of gamma-ray detectors has been undertaken for a set of 3 receptors on an arc at 500 m distance from the source.
Taking an error on the true wind direction a set of 3 curves can be drawn in the Source-Height space (fig. 1 - solid line). The feed-back procedure will minimize the intersection area. The exact solution is given on the intersection of the 3 dashed lines on the figure.

3. Discussion of alternative methodes

3.1. Fuzzy Logic

An increasing number of practical optimization systems use the so-called fuzzy logic instead of numerical optimization techniques. Fuzzy logic is appropriate to translate a qualitative judgement into a degree of membership to a given group. A logic has been developed to derive a degree of "possibility" or "necessity" to conclusions, based on the combination of several fuzzy informations (Fuzzy Sets, Zadeh 1965).

Each environmental observation can in this way be appreciated and qualified as e.g. in very good agreement, good agreement, bad agreement, very bad agreement with the predictions, using an agreement membership function. An application of the fuzzy logic might allow to define boundaries to the possible values of the model input data or parameters. This approach has been preliminary discussed with an expert in those mathematical techniques. It is not yet decided whether this option deserves further efforts for this application.

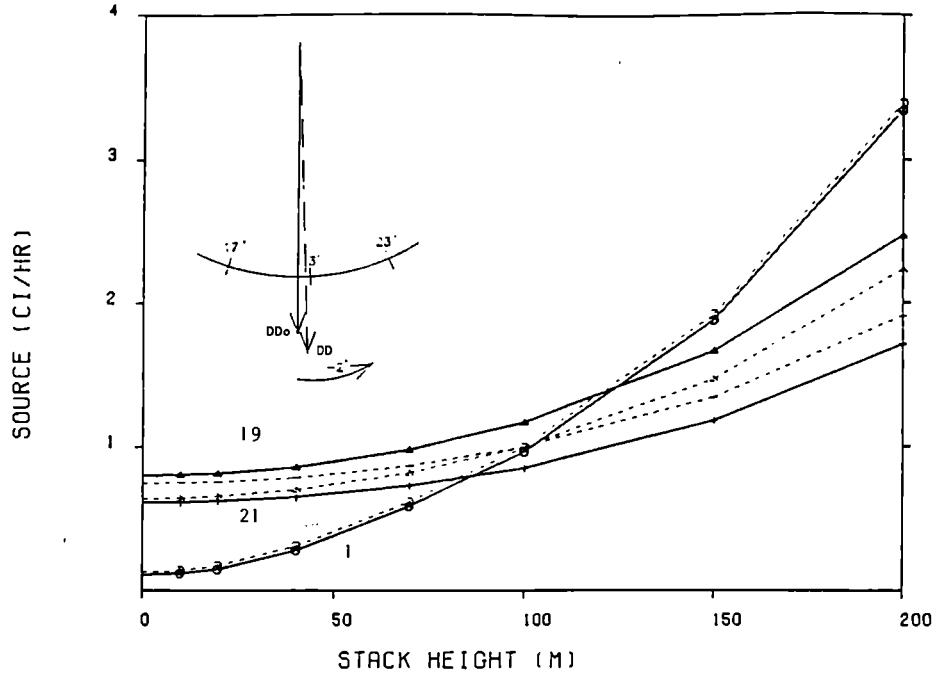
3.2. Analysis of spatial distribution of P/O ratios

A variation of a key-parameter of the prediction model will introduce a specific spatial distribution of the predicted to observed ratios. In a normal situation with a good fit between predictions and observations the P/O ratios are distributed symmetrically around 1, without any correlation between the P/O ratio and the geographical position. An artificial decrease of the source term, e.g. will decrease all the P/O ratios. A shift in wind direction will decrease

the P/O values under the real wind direction and increase the ratios under the assumed wind direction. Analogous graphical transformations of the P/O distributions can be associated with variations of other parameters.
It is proposed to analyse typical P/O distributions and to develop an expert system to propose variations to the input parameters, transforming the P/O ratio distribution to an acceptable distribution centered around 1, without a spatial correlation.

4. Publications

P. Govaerts, A. Sohier
Optimisation of real time dose assessment models, including the interface with environmental survey.
- Seminar on methods and codes for assessing the off-site consequences of nuclear accidents, Athens (Greece), May 1990.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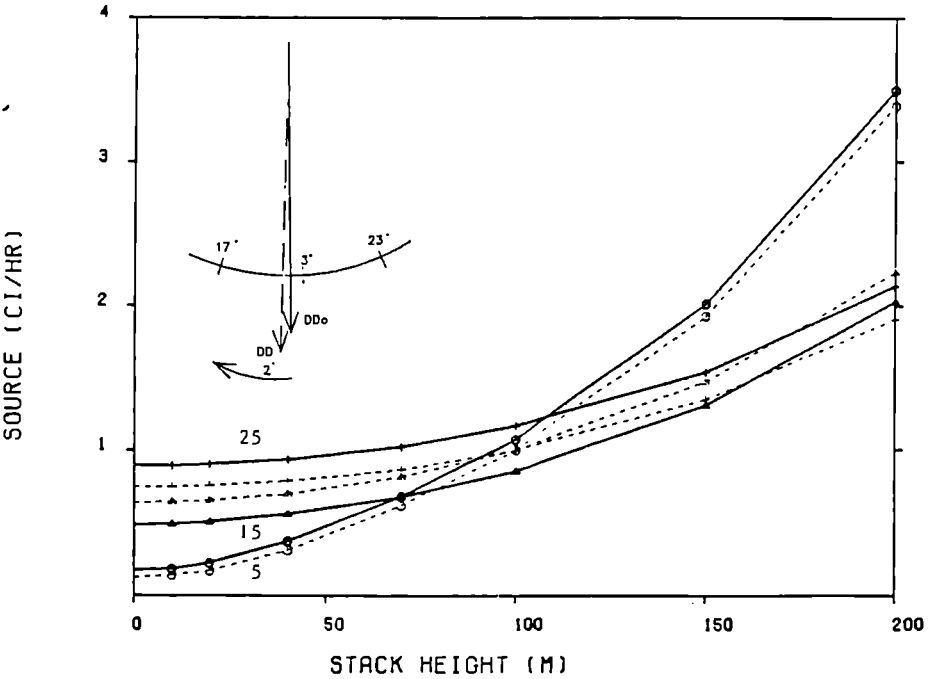
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Fig. 1

IV

KOORDINIERUNGSTÄTIGKEIT

COORDINATION ACTIVITIES

ACTIVITES DE COORDINATION

IV. Coordination

Study Group meetings, Workshops, Seminars and Symposia have proved to be a most effective means of coordination because they are naturally adapted to scientific work and easily accepted by scientists. These meetings, focusing on the evaluation of particular subjects areas of the Radiation Protection Programme, are attended by research workers involved in the contract programme, as well as scientists from non-participating laboratories or organizations and by scientific staff members of the Commission.

On the following pages the various meetings held in the period from 1 January 1990 - 31 May 1991 are listed:

A: Meetings of Study Groups, where scientists involved in the contract programme, independent experts and Staff Members of the Commission discuss specific subject areas of the programme.

B: Meetings organized, coorganized or cosponsored by the Commission of the European Communities on special subject areas of interest for radiation protection and where contacts among scientists from a wider range of discipline and countries might be established.

C: Meetings of experts appointed for the purpose of coordinating and stimulating efforts toward practical measures of radiation protection as foreseen in Chapter III of the EURATOM Treaty or convened by the Commission for special tasks.

☞ For full names of acronyms and abbreviatons please see chapter VII.

Meetings of Study Groups, Period: 1 January 1990 - 31 May 1991

Study Group on "RADE-AID: Decision Aiding System for Use in Emergencies", Post-Chernobyl Action

Apeldoorn (NL), 16-18 January 1990
4 participants from 3 countries and the Commission

Principal subjects:

- Review of the draft report and executive summary;
- Demonstration of software and its future direction.

Study Group on "Decision Aiding Software for Use in Emergency Planning and the Establishment of Intervention Levels"

Fontenay-aux-Roses (F), 25-26 January 1990
8 participants from 4 countries and the Commission

Principal subjects:

- Demonstration and comparison of software systems (RADE-AID, DACFOOD and VIG) for aiding the resolution of complex decision problems;
- Application to decisions on food restrictions and relocation following an accident.

Study Group on "Improvement of Reliable Long Distance Atmospheric Transport Models", Post-Chernobyl Action

Fontenay-aux-Roses (F), 31 January - 1 February 1990
6 participants from 4 countries and the Commission

Principal subjects:

- Completion of the final report and executive summary ;
- Specification of the software package to be made available to interested users.

Study Group on "Methods for Assessing the Radiological Impact of Accidents (MARIA)"

Luxembourg (L), 13 February 1990
4 participants from 2 countries and the Commission

Principal subjects:

- Completion of the COSYMA accident consequence code and procedures for its distribution;
- The role of formal expert judgement elicitation in uncertainty analyses;
- Training courses in the use of COSYMA.

Study Group on "Preparation of the Seminar on Dosimetry in Diagnostic Radiology"

Luxembourg, 20-21 February 1990
4 participants from Germany and the Commission

Principal subjects:

- Intercomparison action of dosemeters used in diagnostic radiology;
- Dosimetry for quality assurance and quality control of the radiological equipment.

Study Group on "Improvement of Potential Countermeasures in the Urban Environment", Post-Chernobyl Action

Harwell (UK), 6-8 March 1990
8 participants from 4 countries and the Commission

Principal subjects:

- Caesium retention behaviour in natural construction material;
- Caesium retention behaviour in man-made construction materials;
- Absorption and displacement of caesium from urban surfaces;
- Full scale decontamination experiments.

Study Group on "Transfer of Radionuclides in Forest"

Louvain-la-Neuve (B), 8-9 March 1990
14 participants from 7 countries and the Commission

Principal subjects:
- Deposition and interception of radionuclides by forest canopies;
- Run-off, migration and plant-availability;
- Animal contamination in forested areas.

Study Group on "Decision Support System for Nuclear Emergencies"

Fontenay-aux-Roses (F), 8-9 March 1990
16 participants from 8 countries and the Commission

Principal subjects:

- Review of the technical specification for the system structure;
- Atmospheric dispersion and meteorology, environmental transfer of radionuclides, countermeasures modelling, consequence assessment and decision aiding techniques.

Study Group on "Quality Criteria for Diagnostic Radiographic Images"

Paris-Cachan (F), 22-25 March 1990
6 participants from 3 countries and the Commission

Principal subjects:

- Preparation of the 2nd edition of the Working Document "Quality Criteria for Diagnostic Radiographic Images";
- Preparation of a trial of the consequences on image quality and patient exposure by using the proposed quality criteria;
- European training course on "Quality Assurance in Diagnostic Radiology".

Study Group on "Expression of Radiological and Non-radiological Detriment and the Comparative Assessment and Management of Risks Associated with Energy Systems"

East Anglia (UK), 10-11 April 1990
11 participants from 4 countries and the Commission

Principal subjects:

- Valuation of life, cost of unit radiation exposure, alternative methods for evaluating detriment;
- Risks from different energy systems and methodologies for their evaluation and comparison;
- Acceptability and tolerability of risk.

Study Group on "Reduction of Patient Exposure in Diagnostic Radiology"

Brussels (B), 23-24 April 1990
26 participants from 11 countries, IAEA and the Commission

Principal subjects:

- Discussion of key problems in the 18 specific research projects of the 1990-1991 Radiation Protection Programme;
- Establishment of cross links between the 3 multinational cooperative groups of contracts:
  1. Dosimetry and estimation of risk
  2. Quality assurance and referral criteria
  3. Dosimetric and quality assurance aspects of new radiological techniques
- Preparation of future common actions e.g. trial with the revised quality criteria and creation of knowledge data bases as well as of expert systems for quality assurance and dose reduction measures;
- Contacts with other Commission's programmes e.g. "Advanced Informatics in Medicine" (DG XIII), "Expert Systems for Quality Assurance of Nuclear Medicine Software" (Cost-B-2), DG XII) and with IAEA: Coordinated Research Programme "Radiation Dose in Diagnostic Radiology and Methods for Dose Reduction".

Study Group on "Radiation Protection Dosimetry"

Luxembourg (L), 10 May 1990
24 participants from 10 countries and the Commission

Principal subjects:

- Account of work on radiation protection dosimetry;
- Progress report on the revision of the technical recommendations;
- Intercomparison of dosemeters used for quality control in diagnostic radiology;
- Proposals for future action.

Study Group "Scientific Advisory Committee of the European RESSAC Programme"

Cadarache (F), 10-11 May 1990
21 participants from 8 countries and the Commission

Principal subjects:

- Presentation of the last developments of the RESSAC related programmes:
  - radiological impact of radionuclides accidentally released
  - preliminary evaluation of the radiological consequences of a PWR accident
  - improvement of practical countermeasures against nuclear contamination in the agricultural environment

- Presentation, discussion and evaluation of the RESSAC programme
  - analytic experiments
  - in situ experiments
  - global experiments
- Presentation of the associated contracts from Piacenza, Gembloux and Risø
- Presentation and discussion of the work planned for 1990, 1991
- Discussion about the identification and choice of the European soils to be involved in the EUR-RESSAC exercise

Study Group on "Biological Effectiveness of Different Radiations"

Darmstadt (D), 18-19 May 1990
20 participants from 4 countries and the Commission

Principal subjects:

- Physics of energy deposition and track structure
- Biophysical modelling of high LET radiation
- Cytological effects of protons, alpha particles and heavy ions.

Study Group on "Formal Expert Judgement Elicitation and its Use in the Assessment of Uncertainties in Accident Consequence Assessment"

JRC ISPRA (I), 11-13 June 1990
16 participants from 10 countries and the Commission

Principal subjects:

- Demonstration of and training in the use of a software package for expert judgement elicitation and evaluation;
- Specification of a pilot exercise to apply expert elicitation to one module of the COSYMA accident consequence code.

Study Group on "Optimisation in Radiological Protection"

Chilton (UK), 15 June 1990
9 participants from 2 countries and the Commission

Principal subjects:

- ALARA in nuclear installations, procedures guide for practical optimisation in design and maintenance, task specific dosimetry;
- Accident data base for radiation incidents and accidents in general industry;
- Application of ALARA to complex situations, eg, accidents and where there may be conflicting objectives.

Study Group on "Decision Support System for Nuclear Emergencies"

Neuherberg (D), 5-6 July 1990
15 participants from 9 countries and the Commission

Principal subjects:

- Technical specification for the structure of the demonstration system;
- Atmospheric dispersion models for various distances, interface between dispersion predictions and results of environmental monitoring;
- Inclusion of uncertainties in predictions and probabilistic forecasts.

Study Group on "Exposure Pathways in the Urban Environment and Decontamination"

Risø (DK), 10-12 July 1990
7 participants from 4 countries and the Commission

Principal subjects:

- Indoor exposure, deposition of radioactive material to urban surfaces and its subsequent fate, wash-off and weathering, resuspension;
- Decontamination of urban surfaces and future research requirements.

Study Group on "Research Needs on Urban Decontamination"

Risø (DK), 12 July 1990
10 participants from 5 countries and the Commission

Principal subjects:

- Chemical decontamination of building and road construction materials;
- Decision aiding systems for evaluation of decontamination efficiencies and costs;
- Full scale decontamination experiments.

Study Group on "Cell Transformation"

Dublin, (IRL), 28 September 1990
15 participants 7 countries and the Commission

Principal subjects:

- Development of human epithelial cell systems;
- Protocol for C3H10T1/2 cell system;
- Collaboration within and between projects.

Study Group on "Evaluation of the Protective Measures taken in the USSR Following the Chernobyl Accident - Decision Conferences"

Moscow, Kiev, Minsk, (USSR), 8-19 October 1990
6 participants from 4 countries and the Commission

Principal subjects:

- Decision conferences to elucidate and clarify issues influencing Soviet policy on relocation of settlements contaminated following the Chernobyl accident;
- Compilation of dosimetric, economic and social information as an input to a cost benefit analyses of relocation.

Study Group on "Quality Criteria for Diagnostic Radiographic Images in Paediatrics"

München (D), 25-30 October 1990
14 participants from 6 countries and the Commission

Principal subjects:

- Evaluation of the European Trial on Quality Criteria for a series of conventional radiological examinations of 3 age groups of children (premature 1000 gr, 10 months, 2 year old infants);
- Establishment of the list of Quality Criteria analogous to that of the adult radiology (Doc XII/173/90).

Study Group on "IAEA Coordinated Research Programme on Optimisation of Diagnostic Radiology and Dose Reduction"

München (D), 30 October 1990
6 participants from 2 countries, IAEA and the Commission

- Definition of research priorities;
- Establishment of a working plan for the IAEA contractors for the 1st year;
- Collaboration with the CEC Radiation Protection Programme;
- Preparation of a common meeting of contractors of the CEC and IAEA research programmes in March 1991.

Study Group on "Quality Assurance and Dose Reduction in Fluoroscopy and Digital Radiography"

Dublin (IRL), 4-6 November 1990
10 participants from 4 countries and the Commission

Principal subjects:

- Presentation of Quality Assurance and Quality Control measures in the various Diagnostic Radiology departments of the St James' Hospital;
- Comparative dose assessment for patients and staff;
- Critical parameters for image quality and patient exposure;
- Overview on formation and training activities of the contractors' institutions on radiation protection in medicine.

Study Group on "Radiation Protection in Nuclear Power Plants"

Luxembourg (L), 12-13 November 1990
33 participants from 10 countries, OECD and the Commission

Principal subjects:

- Analysis of data provided by the questionnaire on job-related doses;
- Exchange of experiences and information on radiation protection particularly in decontamination field.

Study Group "Scientific Advisory Committee of the European RESSAC Programme"

Cadarache (F), 14-15 November 1990
21 participants from 8 countries and the Commission

Principal subjects:

- Presentation and evaluation of the RESSAC programme
  - analytical experiments
  - in situ experiments
  - global experiments;
- Furtherance of the RESSAC building;
- Reports of the associated contracts Piacenza, Gembloux, Risø;
- Selection of four European soils;
- Soil adhesion, soil type and transfer;
- Soil ingestion and bioavailability;
- Modelling of transfer in guts which ingested soils.

Study Group on "Unresolved Issues in the Development of a Decision Support System for Nuclear Emergencies"

Luxembourg (L), 28-29 November 1990
14 participants from 8 countries and the Commission

Principal subjects:

- Uncertainties in forecast meteorology and atmospheric dispersion and how they should be accommodated in the system;
- Methods for effectively combining the predictions of atmospheric dispersion models and environmental monitoring results.

Study Group on "Expression of Radiological and Non-radiological Detriment and the Comparative Assessment and Management of Risks Associated with Energy Systems"

Fontenay aux Roses (F), 6-7 December 1990
13 participants from 3 countries and the Commission

Principal subjects:

- Software packages to evaluate radiological detriment, alternative expressions of detriment;
- Methodologies for comparative risk assessments;
- Risk of energy production in the coal and nuclear fuel cycles.

Study Group on "Transfer of Radionuclides to Livestock"

Rome (I), 6-9 January 1991
17 participants from 7 countries and the Commission

Principal subjects:

- Speciation and bioavailability;
- Soil adhesion, digestion and digestibility, and transfer;
- Physiological state of the animal and its transfer of radionuclides.

Study Group on "Intercomparison of Probabilistic Accident Consequence Assessment Codes"

Paris (F), 16-17 January 1991
32 participants from 15 countries and the Commission

Principal subjects:

- Specification of problems to be evaluated in the intercomparison;
- Environmental, meteorological, demographic, etc data bases to be used;
- Project management and timescales.

Study Group on "Decision Aiding Software for Use in Emergency Planning and the Establishment of Intervention Levels"

Apeldoorn (NL), 22-23 January 1991
8 participants from 6 countries and the Commission

Principal subjects:

- Software developments and improvements for RADE-AID;
- Radiological data bases to be included;
- Comparison of techniques being developed in the EC and elsewhere;
- Promoting the use of decision analysis techniques in radiological protection.

Study Group on "Research Approaches to Low Dose Radiation Effects"

Bad Honnef (D), 28-31 January 1991
40 participants from 6 countries and the Commission

Principal subjects:

- Biophysics and molecular damage;
- DNA repair and mutagenesis;
- Cytogenetic effects;
- Cell transformation;
- Animal carcinogenesis.

Study Group on "Bioavailability of Radionuclides in Relation to Their Physicochemical Form in Soil Systems"

Leuven (B), 29-30 January 1991
8 participants from 6 countries

Principal subjects:

- Intrinsic relationship between the radioactivity concentration in the substrate and the uptake by the plant;
- Role of the solid phase on the radioactivity concentration in liquid phase;
- Theory and mathematical concepts behind the solid/liquid interaction.

Study Group on "Quality Assurance in Digital Radiology"

Mannheim (D), 14-15 February 1991
6 participants from 3 countries and the Commission

Principal subjects:
- Coordination of on-going research on dose reduction in fluoroscopy and digital radiography;
- Quality assurance requirements concerning image quality and patient dose, quality control measures for performance and constancy tests, impact of variations of parameters on patient dose;
- Preparation of 1st progress report with a view to harmonizing presentation of exposure data and working procedures.

Study Group on "Evaluation of the Protective Measures taken in the USSR following the Chernobyl Accident"

Brussels (B), 20-21 February 1991
4 participants from 2 countries and the Commission

Principal subjects:

- Drafting the conclusions and recommendations on the evaluation made of the protective measures taken in the USSR.

Study Group on "Optimisation in Radiological Protection"

Chilton (UK), 27 February 1991
8 participants from 2 countries and the Commission

Principal subjects:

- ALARA training courses, procedural guides, generic optimisation, data base on accidents in industry;
- ALARA applied to complex situations in radiological protection.

Study Group on "Exposure Pathways in the Urban Environment and Decontamination"

Vienna (A), 8 March 1991
5 participants from 3 countries, IAEA and the Commission

Principal subjects:

- Indoor exposure, deposition of radioactive material on skin;
- Deposition and subsequent transfer of material deposited on urban surfaces;
- Deposition of airborne material in foggy conditions;
- Transfer of deposited radionuclides through sewerage systems;
- Decontamination of surfaces contaminated by caesium.

Study Group on "Quality Assurance and Referral Criteria"

Vienna (A), 10 March 1991
6 participants from 4 countries, IAEA and the Commission

Principal subject:

- Discussion of ways and means for quality control measures, calibration-intercomparison and participation in the trial with the CEC "Quality Criteria for Diagnostic Radiographic Images".

Study Group on "Reduction of Patient Exposure in Diagnostic Radiology"

Luxembourg (L), 20 March 1991
30 participants from 13 countries, IAEA and the Commission

Principal subjects:

- State of preparation of the 1992-1993 Radiation Protection Research Programme;
- Coordination of the contributions to the 1st Progress Report of the 1990-1991 Programme;
- Participation in the 1991 Trial with the Quality Criteria for Diagnostic Radiographic Images;
- Elaboration of guidance to quality control in Mammography screening units.

Study Group on "Cyclotron-produced Radioisotopes and Radiopharmaceuticals; Minimisation of Radiation Hasards"

JRC-ISPRA (I), 11 April 1991
11 participants from 4 countries and the Commission

Principal subjects:

- Production procedures of radioisotopes;
- Radiochemical handling and radionuclear quality control: use of automatic systems for separation, labelling and quality control;
- Training for manipulators of isotopes.

Study Group on "Intercomparison of Probabilistic Accident Consequence Assessment Codes"

Brussels (B), 24-25 April 1991
30 participants from 14 countries and the Commission

Principal subjects:

- Specification of pilot intercomparison problems;
- Resolution of difficulties encountered in interpretation of data bases;
- Source terms to be used in the intercomparison.

Study Group on "Livestock: Soil as a Source of Radionuclides"

Dublin (IRL), 1-2 August 1991
14 participants from 7 countries

Principal subjects:

- Soil adhesion, soil type and transfer;
- Soil ingestion and bioavailability;
- Modelling of transfer in guts which ingested soils.

B

Meetings Organized or Coorganized by the CEC,
Period: 1 January 1990 - 31 May 1991

EULEP General Assembly

Reisensburg (D), 12-15 March 1990
70 participants from 14 countries and the Commission

Principal subjects:

- Task Group Meetings
- Follow-up of patients treated with whole body irradiation
- Use of modern molecular-biological methods in experimental pathology

Workshop "Statistics of Human Exposure to Ionizing Radiation"

Coorganized with US DOE, Washington, DC and AECB, Ottawa (Cd)
Oxford (UK), 2-4 April 1990
160 participants from 31 countries and the Commission

Principal subjects:

- Natural exposure to indoor radon in homes;
- Natural exposure at workplace;
- Occupational exposure in the nuclear fuel cycle;
- Exposure from effluents discharged by nuclear installations;
- Medical diagnostic and therapeutic exposures;
- Occupational exposure in the medical and para-medical professions;
- Exposure from consumer products;
- Airplan travel.

Seminar on "Draft New Recommendations of the International Commission on Radiological Protection"

Luxembourg (L), 5-6 April 1990
140 participants from 15 countries and the Commission

Principal subjects:

- Risk estimation;
- Judgment of detriment;
- Conceptual framework of protection.

EURADOS Working Group 9 "Critically Accident Dosimetry"

Paris (F), 23-24 April 1990
5 participants from 3 countries

Principal subjects:

- International intercomparison of critically accidents dosemeters;
- Spectrometry in mixed neutron-photon fields;
- Dosimetry in the leakage radiation field of the SILENE reactor;
- Discussion of the future programme.

Seminar on "Methods and Codes for Assessing the Off-site Consequences of Nuclear Accidents"

Coorganized with KfK Karlsruhe, NRPB Chilton and NCSR Athens
Athens (GR), 7-11 May 1990
170 participants from over 30 countries and the Commission

Principal subjects:

- Probabilistic accident consequence codes and associated software;
- Atmospheric dispersion, environmental transfer, dosimetric and health effects models, economic impact of accidents, countermeasures, demographic and agricultural data bases, uncertainty analyses;
- Assessment of accident consequences in real time.

Symposium on "Status of Paediatric Radiology in Europe"

Organised by the University of Munich, under the patronage of the CEC
München (D), 16 May 1990
100 participants from 15 countries and the Commission

Principal subjects:

- Special requirement to paediatric radiology in contrast to adult radiology;
- Differences in patient parameters, behaviour, disease type and incidence, in imaging modalities and radiation risks;
- Organizational structure of paediatric radiology training programmes in Europe;
- Optimisation of paediatric radiology with a view to reduction of patient exposure.

EURADOS General Assembly and Working Group Meetings

Lisboa (P), 23-24 May 1990
63 participants from 14 countries

Principal subjects:

- Discussion on the new ICRP recommendations;
- Overview on work of EULEP, EUROMET and US DOE;
- Presentation of the Work of LNETI (Portugal);
- New activities of the ICRU;
- Meetings of Working Groups on:
  - Skin Dosimetry (WG 2);
  - Numerical Dosimetry (WG 4);
  - Assessment of Internal Dose (WG 6);
  - Development of Individual Dosemeters (WG 8);
  - Basic Physical Data and Characteristics of Radiation Protection Instrumentation (WG 10).

EURADOS Council

Lisboa (P), 25 May 1990
26 participants from 12 countries

Principal subjects:

- Discussion on EURADOS affairs (constitution, membership, officers);
- Discussion on Working Group activities;
- Proposal for a new Working Group on Neutron Spectrometry;
- Discussion about cooperation with EULEP and US DOE.

"Third International Workshop on Respiratory Tract Dosimetry"

Coorganized with US DOE, Washington DC and ITRI, Albuquerque
Albuquerque, New Mexico (USA), 1-3 July 1990
86 participants from 15 countries and the Commission

Principal subjects:

- Physicochemical properties of radioactive aerosols;
- Deposition/retention in head airways;
- Deposition/retention in lung;
- "Super class Y" lung retention;
- Long-term retention of particles in conduction airways;
- Nonuniform irradiation of the lung - "hot particles";
- Lung dose assessment from improved worker in vivo and excreta analysis.

EURADOS Working Group 8 "Development of Individual Dosemeters for External Penetrating Radiation"

Marburg (D), 8 September 1990

18 participants from 8 countries

Principal subjects:

- Track etch detectors;
- Intercomparison of etch and electrochemical etch procedures;
- Discussion of results of a joint irradiation project;
- Background characteristics of various plastic materials;
- Progress reports from laboratories.

First International Conference on "Biological and Radioecological Aspects of the Chernobyl Accident"

Jointly organized by the Institute of Animal Evolutionary Morphology and Ecology, Moscow (USSR) and IUR; cosponsored by the CEC
Zeleny Mys (USSR), 10-18 September 1990
150 participants from 15 countries and the Commission

Principal subjects:

- Radioecology of plants;
- Radioecology of terrestrial animals;
- Radioecology of hydrobionts;
- Radiation genetics;
- Radiobiology;
- Agricultural radioecology.

EULEP Symposium on "Role of the Alveolar Macrophage in the Clearance of Inhaled Particles"

Oxford (UK), 19-21 September 1990
80 participants from 12 countries and the Commission

Principal subjects:

- Protection of lungs by pulmonary alveolar macrophage;
- Phagocytosis transport and dissolution of particles;
- Variations between species.

Annual Meeting of the "European Society for Radiation Biology"

Session on Low dose/Low dose rate effects coorganized by the CEC
Dublin (IRL) 24-27 September 1990
230 participants from 30 countries and the Commission

Principal subjects:

- Dosimetry and risk assessment;
- New developments in radiation therapy;
- Radiation mutagenesis;
- In-vivo effects of radiation;
- DNA damage and repair;
- Environmental radiation;
- Low dose/low dose rate effects ;
- Human cell models for carcinogenesis studies;
- Radiation transformation in-vitro.

IUR Working Group on Soil-to-Plant Transfer

Uppsala (S), 27-29 September 1990
43 participants from 16 countries

Principal subjects:

- Resuspension and crop contamination;
- Radiocaesium association with soil components: the application of sequential extraction technique;
- Soil adhesion to plants and its importance for plant-to-animal transfer.

Seminar on "Comparative Assessment of the Environmental Impact of Radionuclides Released during Three Major Nuclear Accidents: Kyshtym, Windscale, Chernobyl"

Coorganized with IUR
Luxembourg (L), 1-5 October 1990
188 participants from 26 countries, IUR and the Commission

Principal subjects:

- Accident source terms;
- Atmospheric dispersion, resuspension, chemical and physical forms of contamination;
- Environmental contamination and transfer;
- Radiological implications for man and his environment;
- Countermeasures;
- Radiological studies and modelling;
- Impact assessments, remedial action.

EULEP/EURADOS Task Group Meeting on "Human Lung Dynamics"

Chilton (UK), 22-23 October 1990
12 participants from 4 countries

Principal subjects:

- Discussion on the objectives of the group;
- Respiratory tract models;
- Assessment of radiation doses after inhalation of radioactive material;
- Dependence of regional deposition on particles size and physiological parameters;
- The new lung model of ICRP.

Symposium on "The Relevance of Animal Models of Radiation Carcinogenesis and Developments in Molecular Biology"

Coorganized with the ITRI, TNO Rijswijk
Rijswijk (NL), 22-23 October 1990
70 participants from 12 countries and the Commission

Principal subjects:

- Can in vivo studies be replaced by in vitro investigations?
- Which animal models continue to be relevant and indispensable?

EULEP Task Group on "Bone-seeking Radionuclides"

Chilton (UK), 6-7 November 1990
14 participants from 7 countries and the Commission

Principal subjects:

- Distribution, dosimetry and effects of bone seeking radionuclides;
- Non-uniformity of the microdistribution of transuranics;
- High solubility of Radon in bone narrow;
- Epidemiological studies of Radium 224 patients.

EULEP Task Group on "Fetal Dosimetry and Effects of Incorporated Radionuclides"

Freiburg (D), 12-13 November 1990
15 participants from 4 countries and the Commission

Principal subjects:

- Reduction of risk by decorporation;
- Effect of microbeam irradiation on the pre-implantation embryo;
- Effect of neutron irradiation on the development of the central nervous system;
- Spread of plutonium and americium in the fetus.

EURADOS Working Group 4 "Numerical Dosimetry"

Bologna (I), 26-28 November 1990
15 participants from 4 countries

Principal subjects:

- Numerical dosimetry at the laboratories of ENEA;
- Status of electron transport for dosimetry;
- Experiences with the Monte Carlo neutron and photon transport code system;
- Cross sections for thermal neutrons;
- Bonner sphere response functions;
- Dose equivalent calculations in phantoms.

Information Meeting with "National Societies of European Radiographers and Radiological Technicians"

Luxembourg (L), 3-4 December 1990
40 participants from 16 countries and the Commission

Principal subjects:

- Radiation protection directives: recent evolutions in implementation;
- A general system for the recognition of higher education diplomas;
- Quality criteria for diagnostic images;
- Training in radiation protection for radiographers and radiological technicians.

EURADOS Working Group 10 "Basic Physical Data and Characteristics of Radiation Protection Measurements"

Dudweiler (D), 5-7 December 1990
28 participants from 8 countries

Principal subjects:

- W values for neutron dosimetry and microdosimetry;
- Theoretical investigation of W values;
- W values for charged particles;
- Instrumentation for radiation protection based on cavity chamber principles;
- Progress on tissue-equivalent proportional counters.

EURADOS Working Group 2 "Skin Dosimetry"

Oxford (UK), 6 December 1990
5 participants from 4 countries

Principal subjects:

- Preparation of the Skin Dosimetry Workshop;
- Dose rate meters for skin dosimetry measurements;
- Extrapolation chambers;
- Report on intercomparison measurements.

Third meeting of IAEA/CEC Coordinated research programme on "The Validation of Models for Predicting Radionuclide Transfer in Terrestrial, Urban and Aquatic Environments (VAMP)"

Coorganized with IAEA
Vienna (A), 4-7 March 1991
96 participants from 22 countries

Principal subjects:

- Validation of terrestrial transfer models;
- Validation of urban transfer models;
- Validation of multiple pathways transfer models;
- Transfer and countermeasures.

Workshop on "The Future of Human Radiation Research"

Coorganized with IARC Lyon, US DOE Washington DC and RERF Hiroshima
Schloss Elmau (D), 4-8 March 1991
54 participants from 11 countries and the Commission

Principal subjects:

- Epidemiology review of atomic bomb survivors;
- Epidemiology of leukaemia, lung cancer, breast cancer, thyroid cancer;
- Combined exposures with other carcinogens;
- Research approaches and analytical methodology.

EULEP General Assembly

Reisensburg (D), 10-14 March 1991
70 participants from 15 countries and the Commission

Principal subjects:

- Late effects of total body irradiation;
- Molecular markers of differentiation;
- Uranium miners in Saxonia;
- Cell transformation systems.

1st meeting of the Coordinated Research Programme (C.R.P.) of IAEA and the CEC Radiation Protection Programme/Reduction of Patient Exposure

Coorganized with IAEA
Vienna (A), 11-12 March 1991
25 participants from 13 countries, IAEA and the Commission

Principal subjects:

- Establishment of the working protocol for the 10 contractors of the IAEA-C.R.P. on radiation doses in diagnostic radiology;
- Discussion of ways and means for quality control measures, calibration-intercomparison;
- Possible participation in the trial with the CEC "Quality criteria for diagnostic radiographic images".

EULEP/EURADOS Task Group Meeting on "Human Lung Dynamics"

Reissenburg (D), 13 March 1991
14 participants from 5 countries

Principal subjects:

- The new lung model of ICRP;
- Sensitivity studies of parameters of the new model;
- Discussion on the future programme of the group;
- Assessment of radiation doses after inhalation of radioactive material;
- Dependence of regional deposition in the lung on particles size and physiological parameters.

International Seminar on "Dosimetry in Diagnostic Radiology"

Coorganized with PTB, Braunschweig, ICRU and WHO-EUROPE
Luxembourg (L), 19-21 March 1991
220 participants from 19 countries and the Commission

Principal subjects:

- International and national standards;
- International and national surveys;
- Instruments, techniques, spectrometry;
- The 1990 CEC intercomparison programme of dosemeters for diagnostic radiology equipment;
- Patient doses;
- Quality assurance.

IUR Seminar on Radioecology and Countermeasures

Kiev (USSR), 27 April - 4 May 1991
65 participants from 6 countries

Principal subjects:

- Mapping of radioactivity levels and intercalibration between and ground methods;
- Dissolution of hot particles and availability;
- Countermeasures and protection of watercourses;
- Interception of radionuclides by forests.

Workshop on "Skin Dosimetry: Radiological Protection Aspects of Skin Irradiation"

Coorganized with NEB Dublin; EURADOS and US DOE, Washington DC
Dublin (IRL), 13-15 May 1991
76 participants from 11 countries and the Commission

Principal subjects:

- Theoretical and experimental determination of skin doses;
- Hazards to skin;
- Requirements for controlling skin exposure in the workplace;
- Implications of new ICRP recommendations on individual monitoring of skin;
- Experiences gained from clearing work after accidental events.

EURADOS Council

Dublin (IRL), 15 and 18 May 1991
24 participants from 10 countries

Principal subjects:

- Discussion on revision of EURADOS constitution;
- Presentation of Working Group activities;
- Discussion on future EURADOS activities;
- Discussion about a workshop on individual dosimetry with respect to the new operational quantities and dose limits.

EURADOS General Assembly and Working Group Meetings

Dublin (IRL), 16-17 May 1991
75 participants from 17 countries

Principal subjects:

- Discussion on ICRP publication 60;
- Proposal for the revision of the EURADOS constitution;
- Presentation of the Work of Nuclear Energy Board of Ireland;
- Results of the Skin Dosimetry Workshop;
- Meetings of Working Groups on:
  - Skin Dosimetry (WG 2);
  - Numerical Dosimetry (WG 4);
  - Assessment of Internal Dose (WG 6);
  - Radiation Spectrometry in Working Environments (WG 7);
  - Development of Individual Dosemeters (WG 8);
  - Critical Accident Dosimetry (WG 9);
  - Basic Physical Data and Characteristics of Radiation Protection Instruments (WG 10).

IUR Working Group on Plant-to-Animal Transfer

Uppsala (S) 16-19 September 1991
23 participants from 7 countries

Principal subjects:

- Factors influencing gastro-intestinal absorption of radionuclides;
- Importance of food selection and dietary habits on final intake of radionuclides;
- Techniques to reduce the transfer to animal products.

C

Meetings of Experts, Period: 1 January 1990 - 31 May 1991

"Assistance in the Event of a Nuclear Accident or a Radiological Emergency"

Luxembourg (L), 25 January 1990
32 participants from 12 countries and the Commission

Principal subjects:

- Presentation of Community activities in the field of assistance in the event of a nuclear accident or a radiological emergency;
- Legal and administrative aspects of assistance: review of the existing agreements between Member States;
- Technical aspects of assistance.

Implementation of Council Decision 87/600/EURATOM of 14 December 1987 on "Community Arrangements for the Early Exchange of Information in the Event of a Radiological Emergency (ECURIE)"

Luxembourg (L), 9-10 April 1990
19 participants from 11 countries, IAEA and the Commission

Principal subjects:

- Development of the ECURIE system;
- Outline planning of exercises.

Directive 89/618/EURATOM on "Informing the Public about Health Protection Measures in Radiological Emergency"

Luxembourg (L), 28 May 1990
20 participants from 12 countries and the Commission

Principal subjects:

- Presentation of the provision of the Council Directive;
- Obligations of Member States under Article 33 of the EURATOM Treaty;
- Analysis of transposition of article 8, on regular notification of the public, of the EEC Directive 82/501 concerning the accident with chemicals of Seveso;
- Regulatory and practical aspects of informing the public in the Member States.

Ad Hoc Committee to Establish a List of Products Excluded from Council Regulation: EEC n° 737/90 of 22 March 1990

Luxembourg (L), 15 June 1990
26 participants from 11 countries and the Commission

Principal subject:

- Establishment of a list of products excluded from the application of Council Regulation: EEC n° 737/90 of 22 March 1990, on the conditions governing imports of agricultural products originating in third countries, following the accident at the Chernobyl nuclear power station.

Group of Experts referred to in Article 31 of the EURATOM Treaty

Luxembourg (L), 11-12 July 1990
23 participants from 12 countries and the Commission

Principal subjects:

- Activities in progress within the ICRP and other international organisation concerned with radiation protection;
- Incidence of leukaemia among children of exposed workers;
- Health protection in connection with the use of industrial radiography;
- Guide for the use of teachers for radiation protection in primary and secondary education;
- Radiological protection criteria for the recycling of materials from the dismantling of nuclear installations.

Implementation of Council Decision 87/600/EURATOM of 14 December 1987 on "Community Arrangements for the Early Exchange of Information in the Event of a Radiological Emergency (ECURIE)"

Luxembourg (L), 24-26 July 1990
15 participants from 11 countries, IAEA and the Commission

Principal subject:

- Discussion of information format for ECURIE communications.

Group of Experts referred to in Articles 35 and 36 of the EURATOM Treaty: Environmental Radioactivity

Luxembourg (L), 6-7 September 1990
26 participants from 11 countries and the Commission

Principal subjects:

- Presentation and discussion of the last report (1984-1986);
- Presentation and discussion of a JRC report entitled: Monitoring of Environmental Radioactivity in the EC: inventory of methods and development of data quality objectives;
- Improvement of data communications and data intercomparability.

Working Group "Exercises" in the Frame of ECURIE

Luxembourg (L), 10-12 September 1990
11 participants from 11 countries and the Commission

Principal subject:

- Detailed planning of exercises.

Ad Hoc Committee to Establish a List of Products Excluded from Council Regulation: EEC n° 737/90 of 22 March 1990

Luxembourg (L), 24 September 1990
21 participants from 12 countries and the Commission

Principal subject:

- Establishment of a list of products excluded from the application of Council Regulation: EEC n° 737/90 of 22 March 1990, on the conditions governing imports of agricultural products originating in third countries, following the accident at the Chernobyl nuclear power station.

Group of Experts referred to in Article 31 of the EURATOM Treaty

Luxembourg (L), 26-27 November 1990
20 participants from 8 countries and the Commission

Principal subjects:

- The radiological protection criteria for the recycling of materials from the dismantling of nuclear installations;
- Updating and extension of Publication: Radiation Protection n° 43.

"Assistance in the Event of a Nuclear Accident or a Radiological Emergency"

Luxembourg (L), 27 November 1990
29 participants from 12 countries and the Commission

Principal subjects:

- Elaboration of a catalogue, collecting the information necessary for requesting and giving assistance;
- Exchange of experience in the field of emergency exercises.

Directive 89/618/EURATOM on "Informing the Public about Health Protection in Radiological Emergency"

Luxembourg (L), 9 January 1991
20 participants from 12 countries and the Commission

Principal subjects:

- Draft Commission Communication on the implementation of Directive 89/618/EURATOM;
- Progress by Member States in transposing the Directive;
- Exchange of opinions on the nuclear event scale;
- Results of the survey on "European public opinion and radiation protection".

Group of Experts referred to in Article 31 of the EURATOM Treaty, Working Group on Relocation

Brussels (B), 15 January 1991
7 participants from 7 countries and the Commission

Principal subject:

- Review of the current approach in Member States and elsewhere on intervention levels for relocation.

Group of Experts referred to in Article 31 of the EURATOM Treaty

Luxembourg (L), 14-15 February 1991
21 participants from 12 countries and the Commission

Principal subjects:

- Revision of the basic safety standards;
- Information of recent developments of Community Radiation Protection Provisions;
- Intervention levels for relocation.

Working Group "Exercises" in the Frame of ECURIE

Luxembourg (L), 26-27 February 1991
12 participants from 12 countries and the Commission

Principal subject:

- Detailed planning of exercises.

Group of Experts referred to in Article 31 of the EURATOM Treaty, Working Group

Fontenay-aux-Roses (F), 6 March 1991
6 participants from 5 countries and the Commission

Principal subject:

- Discussion of the scenarios and parameters pertaining to the recycling of steel products.

Ad Hoc Committee to Establish a List of Products Excluded from Council Regulation: EEC n° 737/90 of 22 March 1990

Luxembourg (L), 27 March 1991
22 participants from 12 countries and the Commission

Principal subject:

- Establishment of a list of products excluded from the application of Council Regulation: EEC n° 737/90 of 22 March 1990, on the conditions governing imports of agricultural products originating in third countries, following the accident at the Chernobyl nuclear power station.

Application of Commission Recommendation 90/143/EURATOM "Protection of the Public against Indoor Exposure to Radon"

Luxembourg (L), 7-8 May 1991
31 participants from 12 countries and the Commission

Principal subjects:

- Initiatives and guidelines at national level for the protection of the public against radon;
- International developments;
- Possible new Commission initiatives.

Group of Experts referred to in Article 31 of the EURATOM Treaty, Working Group

Brussels (B), 30 May 1991
7 participants from 5 countries and the Commission

Principal subject:

- Discussion of the scenarios and parameters pertaining to the recycling of steel products.

V

ERPET

EUROPÄISCHE AUS- UND FORTBILDUNG AUF DEM GEBIET DES STRAHLENSCHUTZES

EUROPEAN RADIATION PROTECTION EDUCATION AND TRAINING

ENSEIGNEMENT ET FORMATION EUROPEENS EN RADIOPROTECTION

V. Training Activities, Period: 1 January 1990 - 31 May 1991

The CEC is promoting education and training activities in radiation protection in order to maintain and extend Community expertise in radiation protection, in particular in view of the forthcoming developments in the Community. These education and training activities are in compliance with Article 33 of the EURATOM Treaty.

Education and training activities are organised by the Commission's services in charge of Radiation Protection: DG XI, DG XII and, where appropriate, the service for EURO Courses of the JRC-ISPRA, together with competent institutions in Member States, existing cooperative groups or other groups created for this purpose.

The education and training activities involve:

- Organisation of training courses;
- Development and provision of information and training packages;
- Exchange of scientists and promotion of participation in scientific conferences.

On the following pages training courses and other activities, organized in the period from 1 January 1990 - 31 May 1991, are listed. The courses provided coordinated, up-to-date programmes on key problems in radiation protection, for which a consistent Community approach is crucial. Some of these courses will be repeated after evaluation and updating in order to ensure a larger number of interested persons to become acquainted with the most advanced knowledge in radiation protection.

☞ For full names of acronyms and abbreviatons please see chapter VII.

First Summer School on Radioecology

Jointly organized by the CEC, IUR, CEN/SCK, Mol
Mol (B), 8-20 July 1990
29 participants from 14 countries

Purpose:

To fulfil the needs of qualified graduates in environmental radioactivity in the following fields:

- Basic principles applied to radioecology;
- Radionuclides transfer in terrestrial ecosystems, in aquatic ecosystems, in food chains and modelling;
- Ecological effects of radiations and consequences;
- Dose limits and legislation;
- Accidental situations and emergency procedures in relation to dose assessment and remedial actions;
- Application of radiological knowledge to waste management problems.

Target group:

Young scientists working at research centres, universities and government bodies, as well as in private industry involved in radioecology.

The Probabilistic Accident Consequence Code COSYMA

Jointly organized by CEC/KfK, Karlsruhe
Karlsruhe D, 17-21 September 1990
33 participants from 14 countries

Purpose:

- to familiarise potential users with the main features of the code;
- to assist them in its implementation;
- to provide guidance on the preparation of input data and on the interpretation of output.

Target Group:

potential users of probabilistic accident consequence codes, either in a research capacity or more formally as an input to risk assessment and evaluation.

"Training in Radiation Protection in Primary and Secondary Education"

Luxembourg (L), 13 November 1990
20 participants from 12 countries and the Commission

Principal subjects:

- Development of a European manual on radioactivity and radiation protection for teachers;
- Distribution within the Member States of such a Manual.

Optimisation of Radiological Protection in the Design and Operation of Nuclear and Industrial Facilities

Jointly organized by the CEC, CEPN, Fontenay-aux-Roses and NRPP, Chilton Saclay (F), 19-23 November 1990
24 participants from 7 countries

Purpose:

Presentation of tools and structures that can help implementing the concept of optimisation at a practical level.

Target group:

Plant designers, system planners, maintenance operation planners, operation managers, engineers and health physicists.

Management Board of the European School of Radiation Protection

Luxembourg (L), 13 February 1991
6 participants from the 3 participating Institutes ISH Salzgitter/Neuherberg (D) NRPB Chilton (UK), INSTN Saclay (F) and the Commission

Principal subjects:

Finalization of course programmes and selection of speakers for the 3 courses:

- Radiation Protection in the Event of Accidents
  Saclay, 18-22 March 1991:

- Basic Principles of Radiation Protection
  Chilton, 10-14 June 1991

- Radiation Protection of the Patient
  Neuherberg, 14-18 October 1991

Evaluation procedure, establishment of questionnaires;
Projects for future courses.

Radiation Protection in the Event of Accidents

Jointly organized by the CEC, CIR, Fontenay-aux-Roses/INSTN, Saclay, ISH Salzgitter/Neuherberg and NRPB, Chilton
Saclay (F), 18-22 March 1991
26 participants from 10 countries

Purpose:

To provide a common level of understanding of the practical intervention in the case of nuclear accidents or radiation emergencies, with emphasis on radiation physics and biology, effects of radiation on living matter, risk assessment, assessment of accidental exposure, principles of intervention, emergency planning, protection measures, medical management, diagnosis and treatment, technical visits.

Target group:

Medical staff and other officials involved in health management in the event of a radiation accident.

Quality Assurance for Image Quality and Dose Reduction in Medical Diagnostic Radiology

Jointly organized by the CEC, CAATS/INSERM Cachan and IRS Ltd, Liverpool: Course held in French language
Paris (F), 3-7 June 1991
45 participants from 8 countries

Purpose:

To provide up-to-date operational aspects and measures of radiation protection practice in diagnostic radiology, with emphasis on background information to operational and legislative framework for radiation protection of the patient as well as the staff, quality assurance in diagnostic radiology: quality requirements with regard to radiographic images and patient dose; quality control programmes: organization, implementation, measurements,; role of technical developments, practical demonstrations.

Target groups:

All those actively involved in the day to day practice of diagnostic radiology, as well as those responsible for education and training of radiographers and radiological technicians.

VI

AUSWAHL EINIGER AUF VERANLASSUNG DER KOMMISSION ERSCHIENENER VERÖFFENTLICHUNGEN

SELECTION OF PUBLICATIONS ISSUED ON THE INITIATIVE OF THE COMMISSION

CHOIX DE PUBLICATIONS EDITEES SUR L'INITIATIVE DE LA COMMISSION

Publications, Period: 1 January 1990 - 31 May 1991

The scientific research results of the Commission's Radiation Protection Programme are presented in articles published in scientific journals. References to these are given in the corresponding Progress Reports. In certain cases the Commission initiated surveys of detailed results of specific activities in the field of radiation protection and published them as monographs or proceedings. Short descriptions of those publications, published or prepared in the period from 1 January 1990 - 31 May 1991, are given on the following pages.

For full names of acronyms and abbreviatons please see chapter VII.

MONOGRAPHS

Community Radiation Protection Legislation

Report edited by DG XI: Environment, Nuclear Safety and Civil Protection

Composure of the provisions of the European Communities in the area of radiation protection. This document contains the full text of the provisions of the European Communities in the area of radiation protection. It is designed to help those who work in the area of radiation protection and those interested in these problems by providing them with an easily understood collection of the relevant texts which have been issued on the basis of the EURATOM Treaty.

The collection is arranged as follows:

I. Provisions of the EURATOM Treaty;
II. Regulations, Directives and Decisions of the Council or the Commission;
III. Recommendations and Communications of the Commission.

It has been prepared in English, French and German versions by the Radiation Protection Division of the Directorate-General for the Environment, Nuclear Safety and Civil Protection in Luxembourg, it contains all official texts in force as of 1 August 1991.

Doc. XI/3539/90 EN, FR, DE, 1990; 285 pages.
The second edition of this document is in press and will be available in September 1991

To be ordered through:
Commission of the European Communities
Radiation Protection Division
Mr H. Lellig
Centre Wagner
Rue Alcide de Gasperi
L-2920 Luxembourg

Monograph on Environmental Radioactivity in the European Community (1984-1985-1986)

Report edited by DG XI: Environment, Nuclear Safety and Civil Protection and JRC, Institute for the Environment (ISPRA)

Under the terms of Article 36 of the EURATOM Treaty, Member States shall periodically communicate to the Commission information on environmental radioactivity levels. Compilations of the information received have been published by the Commission as a series of reports beginning in the early 1960's; the current report is the 23rd in the series and covers the years 1984, 1985 and 1986.

With respect to previous editions, this report differs in that:

- It now contains data from twelve Member States, including Portugal and Spain, which joined the European Community in 1986;
- The data format has been harmonized;
- During the period covered by the report, the Chernobyl accident temporarily increased environmental radioactivity levels to a considerable degree introducing wide geographical and temporal variations in several Member States. The report, therefore, incorporates a special chapter illustrating these effects.

A further, important, innovation has been the introduction of all the environmental monitoring results received from the Member States into the REM databank by the Institute for the Environment of the CEC at ISPRA as part of its DG XI supported programme.

In general, five different sampling media and five categories of radioactivity are covered:

Sampling media:
Air
Total ground deposition (dry + wet)
River water
Drinking water
Milk

Radioactive categories
Total alpha
Total or residual beta (excl. tritium)
Caesium-137 (^{137}Cs)
Strontium-90 (^{90}Sr)
Tritium (^{3}H)

Report EUR 12254 EN, 1990, 145 pages, Radiation Protection Series n° 46

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 11.25

The Radiological Exposure of the Population of the European Community from Radioactivity in North European Marine Waters. Project "Marina"

Report prepared by a group of experts convened by the CEC and edited by DG XI: Environment, Nuclear Safety and Civil Protection

Project "Marina" was set up by the CEC in 1985 to look at the radiological impact of radionuclides, both natural and anthropogenic, in northern European marine waters. This report is a summary of project Marina's work and its conclusions.

Report EUR 12483 EN, 1990, 571 pages, Radiation Protection Series n° 47

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 45

Radioactivity Transfer to Animal Products

Report prepared by Associated Nuclear Services Ltd for the Commission of the European Communities, DG XI: Environment, Nuclear Safety and Civil Protection

Information on the behaviour of strontium, caesium, ruthenium, plutonium and americium in a range of domestic animals is reviewed to form a basis for the specification of time-dependent mathematical models describing uptake, distribution and retention in various domestic animals. Transfer factors relating concentration in animal products to daily radioactivity intake are derived after 100 d continuous intake and at equilibrium. These transfer factors are compared with the available published literature and used as a basis for the derivation of feedingstuffs conversion factors relating limiting concentrations in animal feedingstuffs to limiting concentrations in human foodstuffs for application to animals receiving commercial feedingstuffs after a nuclear accident. Recommended transfer factors for animal products in conditions of continuous discharge and models for application to field conditions after a nuclear accident are also presented. Transfer of caesium to animal products is more effective than that for the other elements considered here. Transfer to meat of lamb, fattening pig, and chickens is generally more effective than that for other animals and other products.

Report EUR 12608 EN, 1990, 145 pages.

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 12.50

Impact Radioécologique de l'Accident de Tchernobyl sur les Ecosystèmes Aquatiques Continentaux (Radioecological Impact of the Chernobyl Accident on Continental Aquatic Ecosystems)

Report prepared for the CEC by IUR
Edited by DG XI: Environment, Nuclear Safety and Civil Protection

The pooling of knowledge on water, sediments, aquatic plants and fish allowed an evaluation report to be drawn up on the impact of the Chernobyl accident and to extract data on the mechanisms in the transfer of certain radionuclides in rivers and lakes. The radioactivity is directly related to the level of deposits which were, essentially, in wet form. Differences in radioactivity levels are noted owing to the distance from Chernobyl, the atmospheric streams and pluviometric conditions. The most commonly detected radionuclides are: ^{131}I, ^{132}Te, $^{134+137}Cs$, $^{103+106}Ru$, ^{110m}Ag and, to a lesser degree, ^{89}Sr and ^{90}Sr. Very quickly, ^{137}Cs becomes dominant everywhere. The peak of radioactivity in river water occured very soon after the accident. It was of short duration and the decrease in radioactivity was very quick due to dilution. In lakes, this decay was much slower. In sediment, which is the ideal storage location for radionuclides, the radioactivity varied in time owing either to new deposits or to the migration of those deposits downstream in the river basins. The radionuclides present in fallout can be quickly detected using aquatic plants. In fish, above all the presence of $^{134+137}Cs$, $^{103+106}Ru$, ^{110m}Ag and ^{90}Sr are noted. They allow an easier comparison of the concentrations of radionuclides in space and in time. The fixing dynamics can be followed by ^{137}Cs only. River fish were only subjected to water and food with a high radioactivity for a very short time and their ^{137}Cs concentration remained constantly low. The effective half-life of ^{137}Cs observed *in situ* for fish is in about from 100 to 200 days. For lacustrine fish, differences are observed in the levels of radiocontamination, according to the regions and species. The higher activities were found in a first time in the planktivorous species (vandace), then the carnivorous fish (perch), which have a slower accumulation process. The effective half-life observed *in situ* is considerably higher than in rivers and can reach three years for trout of mountain lakes in Norway. The level of radiocontamination in the aquatic environment, reached after the Chernobyl accident, is higher than that observed after the atmospheric nuclear tests between 1956 and 1964; at those times, the levels of radioactivity found in fish, for example, were between 10 and 100 times less than in 1986 to 1987. In the first case we have an acute contamination, when in the second it was more chronic.

Internal Document DG XI/3522/90 FR, 1991, 222 pages, Radiation Protection Series n° 50
Preliminary report to be replaced by the final version in 1992.

To be ordered through:
Commission of the European Communities
Radiation Protection Division
Mr F. Luykx
Centre Wagner
Rue Alcide de Gasperi
L-2920 Luxembourg

Survey on Education and Training of Radiation Physicists in the Member States of the European Community

Report edited by DG XI: Environment, Nuclear Safety and Civil Protection

Article 5 of Directive 84/466/EURATOM mentions the availability of a qualified expert in radiophysics to sophisticated departments of radiotherapy and nuclear medicine.

Since the qualified expert has a major and direct role to play in the protection of the patient undergoing medical examination or treatment involving ionizing radiation, his presence in the hospital and the training he has received are considerable aspects of radiation protection in the medical domain.

The application of Article 5 of Directive 84/466/EURATOM is of great importance for the protection of the patient undergoing medical examination or treatment involving ionizing radiation.

This report, developed to evaluate the actual application of this article, reveal that although in several Member States the concept of the qualified expert in radiophysics has already been introduced into national law, in practice a need for further harmonization clearly emerges.

On the availability of training facilities, the situation in the Community is rather positive, but the formal recognition of education and training of medical physicists by government bodies is still in a developing stage.

Report EUR 13298 EN, 1991, 29 pages, Radiation Protection Series, n° 51

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 7.50

COSYMA - a New Programme Package for Accident Consequence Assessment

Prepared by scientists from KfK Karlsruhe (D) and the NRPB, Chilton (UK)

This report describes the suite of programmes and data libraries which comprise COSYMA, a new probabilistic accident consequence assessment code that was developed within the framework of the CEC MARIA research progamme. The basic features of each of the models included in the programme are described. Guidance is also given on the range of applications for which the code can be used and the types of results which can be generated.

Report EUR 13028 EN, 1991, 96 pages.

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 8.75

COSYMA Users' Guide

Prepared by scientists from KfK Karlsruhe (D) and the NRPB, Chilton (UK)

The COSYMA probabilistic accident consequence code system, which was developed within the CEC MARIA research programme, has been made available to institutes actively working in this field. A training course has been held on the use of COSYMA and a comprehensive Users Guide has been prepared to assist in its effective use.

This Users' Guide is periodically updated and is only available to those organisations that have formally obtained the code system from the CEC or its contractors.

Report EUR 13045 EN, 1991, ~ 650 pages.

Available on request from:
Kernforschungszentrum Karlsruhe GmbH
Postfach 3640
D-7500 Karlsruhe 1

Radiological Aspects of Nuclear Accident Scenarios

Post-Chernobyl Action Report
Prepared by scientists from CNEN, Roma (I); CEA, Fontenay-aux-Roses (F); ICSTM, London (UK); GSF, Neuherberg (D); NRPB, Chilton (UK); KfK, Karlsruhe (D) and TNO, Rijswijk (NL)

Two distinct topics are addressed in this report : firstly, the development of a number of key components of a computerised real time emergency response system for use in the event of an accident and, secondly, the development of a system to aid decisions on the introduction of countermeasures to mitigate the consequences of an accident. In both cases software packages, DART (Dose Assessment in Real Time) and RADE-AID, have been prepared.

The models included in the DART package are described and include methods for predicting atmospheric dispersion over short, medium and long ranges, for the estimation of source terms and for dose assessment following an accidental release of radioactive material. The software package is available to institutes actively working in this field and is accompanied by appropriate user guides.

The basic features of the RADE-AID system are described and a prototype version of the system has been released. The system is based on multi-attribute value analysis which was selected as the most appropriate approach to use for the type of problems under investigation. Illustrative applications of the system to the relocation of people and restriction of foodstuffs after an accident are described. The techniques developed are equally applicable to aid decisions on other complex problems.

Report EUR 12552 EN, 1991, vol. I, 161 pages., vol. II, 196 pages.

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: vol. I: ECU 12.50; vol. II: ECU 15

Improvement of Practical Countermeasures: Preventive Medication

Post-Chernobyl Action Report
Prepared by scientists from ITRI-TNO, Rijswijk (NL); ULB, Brussels (B); Univ. degli Studi, Firenze (I); Univ. des Saarlands, Homburg (D) and St. James Hospital, Dublin (IRL)

The Chernobyl releases of ^{131}I were particularly important and the pattern of deposition in the EC countries very variable according to wind and weather conditions. In case of ingestion of radioiodine, the target organ is the thyroid. The human thyroid uptake depends on the relative supply of stable Iodine in the diet. The risks of radiation are the induction of benign and malignant tumours, and hypothyroidism. Age, sex, ethnic origin and level of thyroid stimulation are influencing the risk importance.

Five laboratories from five different Member States participated in a research programme aiming to make an evaluation of risks of radioiodine release of the population and of iodine treatment and to define criteria for preventive treatment for a whole population and a protocol for the treatment of patients after radioiodine ingestion. A survey of iodine supply in the diet and radioiodine uptake in various European regions showed important variations within the same country and even within the same region. These data on thyroid uptake can be used to set decision criteria for iodine prophylaxis after a nuclear accident.

The radioiodine kinetics in foetal and newborn thyroid have been studied in chimpanzees. Considering the life expectancy and the probably higher thyroid radiosensitivity of the foetus, the relative risks may be as 30 times higher in the foetus than in the adult. Recommendations to public health services in case of nuclear accidents are formulated and the interest of a generalized iodine prophylaxis emphasized.

Report EUR 12556 EN, 1991, 174 pages.

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
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Price: ECU 13,75

Treatment and Biological Dosimetry of Exposed Persons

Post-Chernobyl Action Report

Prepared by scientists from ITRI-TNO, Rijswijk (NL); Univ. Ulm (D); C.E.A., Fontenay-aux-Roses (F); Inst. Curie, Paris (F); CEN/SCK, Mol (B); NRPB, Chilton (UK) and Univ. of Leiden, Leiden (NL)

At least 200 people were involved in the initial efforts to mitigate the consequences of the Chernobyl reactor accident and to get the situation under control and were exposed to radiation levels that required immediate medical care. In the majority of the cases the body dose received by individuals could not be reliably derived from personal dosemeters carried by the victims. On the basis of peripheral blood counts, bone marrow puncture and chromosomal analysis, 19 patients were treated with bone marrow or foetal liver cell transplants, but the effects of the transplantations have been disappointing in that a beneficial influence was not observed. The main problem in treating such victims was the great uncertainty in establishing the risk of the individual patient developing a fatal radiation syndrome. Therefore, it was decided to reanalyse the predictive value of the changes in blood cell counts and to perform in vitro studies on dose effect relations of chromosome aberrations with emphasis on the influence of partial body irradiation. In addition, a new approach to biological prognostic dosimetry was initiated by studying the possible application of the new Hemopoietic Growth Factors (HGF) for determining the amount of surviving stem cells of the blood-forming system.

There is usually an urgent need to provide an estimate of dose as quickly as possible. The present techniques are not ideal in this respect because for dicentric analysis the microscopy cannot begin until 2 days after receipt of the blood sample. The relatively new method of Prematurely Condensed Chromosomes (PCC), however, opens up the possibility of scoring aberrations within a few hours of blood sampling. It has the potential for overcoming the complications of interphase death and mitotic delay which apply to conventional metaphase analysis with no loss of sensitivity or accuracy. The Chernobyl experience was notable for highlighting two important features of biological dosimetry that are particularly relevant to high, life-threatening exposures. Firstly, for highly over-exposed subjects, lymphocyte cultures yield a low mitotic index. Secondly, accidental irradiation is usually inhomogeneous and this can often be detected cytogenetically. Culture techniques were extensively compared and this has resulted in notable improvements and optimal standardisation. Two relatively new methods were also investigated in depth: the micronuclei technique and the assay based on prematurely condensed chromosomes.
Recommendations were made with respect to diagnostic procedures with prognostic relevance, improvement of therapeutic means and organisation and logistics.

Report EUR 12558 EN, 1991, 230 pages.

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of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 18,75

Improvement of Reliable Long Distance Atmospheric Transfer Models

Post-Chernobyl Action Report
Prepared by scientists from NCSR, Athens (GR); University of Pavia (I); CEA, Fontenay-aux-Roses (F) and SRD, Warrington (UK)

Models of long range pollutant transfer are useful in providing an indication of when and where contamination might be expected to appear, and what its severity might be, following an accidental release of radioactive material. The merits and limitations of different modelling approaches are investigated, in particular the uncertainties inherent in the predictions and potential biases their use might introduce. The use of such models, together with measured levels of environmental contamination, is also investigated as a means of estimating the magnitude of the released material and for improving and updating model predictions. The investigations provide a valuable framework for decisions on the most appropriate modelling approaches to be used commensurate with the reliability demanded of the application. As such they are of considerable value in optimizing the design of off-site emergency response systems, especially for application at long distances.

Report EUR 12549 EN, 1991, 452 pages.

To be ordered through :
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Boîte Postale 1003
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Price: ECU 37.50

Improvement of Practical Countermeasures against Nuclear Contamination in the Urban Environment

Post-Chernobyl Action Report
Prepared by scientists from KUL, Leuven (B); University of Glasgow, (UK); Risø National Lab., Roskilde (DK); UKAEA, Harwell (UK)

The caesium isotopes (^{134}Cs and ^{137}Cs) represent the most important risk to the population of a contaminated urban area following an accidental release.

An **inventory of exposed materials** was needed to assess the risks of contamination in the urban environment. A case study in the United Kingdom developed a methodology to establish inventories of urban surfaces based on statistical data for manufacture and use of construction materials and on historical growth maps. This methodology can now be applied by national authorities of Member States.

When caesium is deposited on urban surfaces, it migrates, in part, into the pores of the material before being bound. The vast majority of sites binding caesium hold on to it only weakly. However, about 1% of sites found on virtually all building materials and also in soil can bind caesium strongly and in an almost irreversible manner.

Physical methods for decontamination such as domestic cleaning equipment, street cleaning equipment, agricultural and road maintenance machinery, high pressure water jets and sand-blasting methods can prove effective as countermeasures.

Laboratory and field studies indicate that chemical decontamination will be ineffective and that caesium sorption will be largely irreversible. Physical methods, through the removal of street dust, mosses and lichens, or physical abrasion techniques through the removal of a tiny top layer of material would, however, be useful. Moreover, areas likely to be contaminated such as roofs or a road might be pretreated with a dilute solution of readily available surface-active substances. This prevents, for some days, diffusion of caesium into the pores and can reduce caesium retention by about 80%.

Report EUR 12555 EN, 1991, 343 pages.

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 27.50.

Evaluation of Data on the Transfer of Radionuclides in the Foodchain

Post Chernobyl Action Report
Prepared by scientists from KFA, Jülich (D); CEA, Cadarache (F); RIVM, Bilthoven (NL); ITE, Grange-over-Sands (UK); CEN/SCK, Mol (B); MLURI, Midlothian, NRPB, Chilton (UK); ENEA-DISP, Roma (I); IFE, Ambleside (UK); LNETI, Lisbon (P); MAFF, Lowestoft (UK)

From the long-term point of view only the long-lived are of importance for assesment of the late aftermath risk-assesment of the Chernobyl accident. As a result of this study it turned out that several transfer parameters were insufficiently or not considered in the calculation models used leading to uncertainty in prediction. Research had, before the Chernobyl accident, been mainly concentrated on agricultural ecosystems, which clearly did not provide all the necessary infirmation to evaluate the local, specific conditions. As a mater of fact transfer factors for the pathway soil-fodder-animal products had been poorly defined, little attention had been given to natural and semi-natural both as sources for food and as sources of run-off water to water-basins. In this investigation a number of parameters of importance have been brought to emergence which will allow a better description and evaluation of their role in the transfer of radionuclides to man. It has been a kind of a new starting point of this research area to deepen the scientific insights and to improve its practical use.

Report EUR 12550 EN, in press

To be published by:
Office for Official Publications
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Boîte Postale 1003
L-2985 Luxembourg

Underlying Data for Derived Emergency Reference Levels

Post-Chernobyl Action Report
Prepared by scientists from CEA, Fontenay-aux-Roses (F); JSH, Neuherberg (D); RIVM, Bilthoven (NL); GSF, Neuherberg (D); NRPB, Chilton (UK)

Restrictions in the consumption of contaminated foodstuffs can be an appropriate countermeasure and this was extensively used after the Chernobyl accident.

Food consumption was evaluated for different subgroups of the population. As expected, diet varies markedly with age from the infant, the 1-year-old up to the 18-year-old, with only minor changes at later ages. Within a Member State, regional consumption of certain classes of food may vary from 70% to 130% of the national average. The assessment of food distribution in and outside the EC showed that, on average, 84% of food is provided by national production, 13% is obtained from intra-Community trade; non-EC countries contribute only 3%. Obviously, these values vary widely, dependent on the type of food; highly urbanized and industrialized regions obtain, in general, more food from outside the country.

Existing dynamic models for the transfer of radioactivity through the terrestrial food chain were improved and used for verifying the assumptions made for maximum permitted contamination levels. Two models of differing complexity were compared: the simpler FARMLAND model is more suited to be used with default values whereas the more complex ECOSYS model is more appropriate for evaluating more site-specific situations.

Based on generally accepted radiation protection concepts and the ALARA principle (As Low As Reasonably Achievable) a methodology for deriving intervention levels for foodstuffs assuming several scenarios could be developed for which the efficiency of countermeasures (doses saved) was assessed. All this information is now being made available in a readily interpretable way to decision-makers. It is hoped that this will contribute to more transparent and better harmonized emergency management.

Report EUR 12553 EN, 374 pages, in press

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of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Improvement of Practical Countermeasures against Nuclear Contamination in the Agricultural Environment

Post Chernobyl Action Report
Prepared by scientists from RNL, Risø (DK); NEB, Dublin (IRL); CEN, Cadarache (F); CIEMAT, Madrid (E)

After the Chernobyl accident it became evident that not much attention had been given to techniques which could allow reclamation of agricultural areas although some limited research had been conducted. A number of scenarios were tested in places where nuclear tests had been carried out or where high radioactive contamination occurred with a subsequent impact on the agricultural environment. An assessment of the applied scenarios was carried out, regarding their efficiency and their eventual costs, their practicability. An analysis of research in related fields such as studies on soil to plant transfer or food processing lead to useful conclusions about agricultural countermeasures but even more led to recommendations about research priorities. This information could be used for selecting a scale of techniques for countermeasures in order to deal with different kinds and levels of contamination.

Report EUR 12554 EN, 208 pages, in press

To be published by:
Office for Official Publications
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Boîte Postale 1003
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Monitoring and Surveillance in Accident Situations

Post Chernobyl Action-Report

Prepared by scientists from ENEL, Roma (I); Univ. Politécnica de Catalunya, Barcelona (E); SCRPI, Le Vesinet (F); NRPB, Chilton (UK); PTB, Braunschweig (D); Risø National Laboratory, Roskilde (DK).

Monitoring and surveillance after an accidental release of radionuclides to the environment can be considered in four categories:

- The detection of the accident;
- Early measurements to determine the need for counter measures;
- Continuing surveillance of radionucledes in the environment, in food and in people until the situation returns to narmal to assess doses, to demonstrate that countermeasures have provided adequate protection and to provide public reassurance;
- Research studies, to follow the behaviour of radionuclides in the environment, in food and in people so as to improve predictive models.

Six laboratories form six different Member States participated in research addressing several of the above topics. The report contains sections on accident detection networks, on suitability of dose rate measuring instruments for monitoring in accident situations, on measurements of surface continuation with beta-emitting radionuclides, on in situ gamma-ray spectrometry, rapid methods of radionuclide analysis and in-vivo measurements. The report includes conclusions and recommendations.

Report EUR 12557 EN, in press

To be published by:
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PROCEEDINGS

Real-time Computing of the Environmental Consequences of an Accidental Release to the Atmosphere from a Nuclear Installation - Decision Aids to Offsite Emergency Management

Proceedings of the Second International Workshop jointly organized by CEC and IUR
Luxembourg (L), 16-19 May 1989

Edited by DG XI: Environment, Nuclear Safety and Civil Protection

Since the first international Workshop in 1985, the Chernobyl accident has led to greatly increased interest in the use of computers as an aid to modelling the actual situation in the event of an accident on the basis of the information then available in order to extrapolate in time and space, to obtain a better understanding of what actions might be appropriate and to investigate the potential influence of specific countermeasures. The proceedings provide an overview of the state of the art as it existed in early 1989, including as they do, not only contributions from Western Europe but also from the USA, Japan and Eastern Europe.

Report EUR 12320 EN, 1990, Vol. I and II, 423 and 377 pages.

To be ordered through:
Office for Official Publications
of the European Communities
Boîte Postale 1003
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Price: Vol. I: ECU 35; Vol. II: ECU 32,50

Symposium on Microdosimetry

Proceedings of the 10th Symposium jointly organized by CEC, US DOE, Washington DC; ENEA Roma (I) and KFA Jülich (D)
Rome (I), 21-26 May 1989

Edited by J. Booz, J. Dennis, H.G. Menzel

This Symposium was the 10th in a series organized by the Commission since 1967. It was attended by 175 scientists and more than 90 papers were presented in oral and poster sessions. The research in microdosimetry includes physical, chemical and biological aspects and is aimed at improved understanding of physico-chemical, biochemical and biological effects and mechanisms induced by radiation of different qualities. Papers on physical aspects included experimental and calculational results for descriptions of the energy deposition pattern on molecular and cellular level, on primary radiation interaction and detector responses, on effects of Auger-Electron cascades and on experimental techniques. Based on physical information the topics of molecular changes and radiation quality, of mechanisms of DNA strand breaks and of initial radiation effects in general were discusses. Several contributions focused on the central problem of microdosimetry, the understanding of the dependence of radiation effects on radiation quality and of the problem of low doses and low dose rates discussing biophysical models and radiation quality concepts. These aspects are of immediate relevance to radiological protection. Application of microdosimetry in radiation therapy was another topic of practical importance. The role of microdosimetry in assessments of radiation risks for both external irradiation and incorporated radionuclides was documented. Of particular interest were papers on the microdosimetry of radon decay products in the respiratory tract.

Report EUR 12864 EN, published in Radiation Protection Dosimetry, Vol. 31, n° 1-4, 1990, 460 pages.

To be ordered through:
Nuclear Technology Publishing
P.O. Box n° 7
Ashford
GB-Kent TN25 4NW

Price: £ 80

Transfer of Radionuclides in Natural and Semi-Natural Environments

Proceedings of a Workshop jointly organized by the CEC, ENEA Rome (I) and CRSA Udine (I)
Udine (I), 11-15 September 1989

Edited by G. Desmet, M. Belli, U. Sansone, P. Nassimbeni

After the Chernobyl accident, studies on the natural and semi-natural ecosystems have pinpointed a number of pathways that lead directly to man, primarily by way of meat and dairy products. Relatively little was known regarding the cycling of radionuclides and of their bioavailability, their migration in undisturbed natural profiles and transfer of contamination to adjacent areas under such conditions. Due to the heterogeneity of these systems, problems arise when transfer factors need to be determined. The usual radioecological definitions needed to be correspondingly adapted and the possibility of extrapolation of results obtained in the laboratory and in agricultural fields investigated.

The proceedings contain five chapters:

1. Transfer of Radionuclides in Natural and Semi-Natural Environments;
2. Radionuclides Cycling in Forest Ecosystems;
3. Contamination of Wildlife;
4. Radionuclides in Upland Pasture Ecosystems;
5. Effects of Fertiliser and other Chemicals on Radionuclides Contamination Levels.

An attempt was made to evaluate the relative contribution of such ecosystems to the contamination of the environment.

Report EUR 12448 EN, published by Elsevier Applied Science, 1990, 693 pages.

To be ordered through:
Elsevier Applied Science Publishers
Crown House
Linton Road
GB-Barking, Essex IG11 8JU

Price: £ 95

Uncertainty Analysis

Proceedings of a Workshop jointly organized by the CEC and US DOE, Washington D.C.
Santa Fe, New Mexico, (USA), 13-16 November 1989

Edited by C.E. Elderkin and G.N. Kelly

This Workshop was organised jointly by the CEC and the USDOE with the following objectives: the provision of a forum for an effeective and productive exchange of views and experience among experts in uncertainty analysis; the identification of the major limitations of current approaches in uncertainty analysis and how these might be circumvented in the future; and the opportunity to create an effective interface and establish better understanding between those active in the development of methods for uncertainty analysis and the users of such methods, particularly those in the area of accident consequence assessment.

The proceedings contain an introduction to the problems of uncertainty analysis and a summary of the outcome and conclusions of the workshop. This is followed by summaries of the conclusions of a number of working groups concerned with the following topics:

- Concepts and methods;
- Expert judgement in uncertainty analysis;
- Assessment of the consequences of potential accidents;
- Emergency response systems;
- Dose reconstruction.

The Proceedings also contain abstracts of individual presentations by participants in the Workshop.

Report EUR-13044 EN, published by Pacific Northwest Laboratory, PNL-SA-18372, CONF-8911195, 1990, 71 pages.

To be ordered through:
National Technical Information Service
US Department of Commerce
5285 Port Royal Rd
USA-Springfield, Virginia 22161

Price to be obtained from National Technical Information Service

Standing Conference on Health and Safety in the Nuclear Age

Proceedings of the Conference organized by the CEC
Brussels (B), 5-6 December 1989

Edited by DG XI: Environment, Nuclear Safety and Civil Protection

The subject of the meeting was informing the public on improvements in emergency preparedness and nuclear accident management. The Standing Conference was created by the CEC in 1986 and its goal is to convey to the European Public, via the mass media, factual information on nuclear subjects of current interest. About 100 experts and representatives of the mass media, competent authorities and socio-economic organizations followed the meeting, the specific objective of which was to discuss, from the point of view of radiation protection of the public and the environment, the improvements that nuclear accident management and intervention plans have undergone in recent years.

Invited contributions underlined the presently improved levels of international and Community cooperation in radiation and nuclear safety, particularly in the fields of emergency preparedness and planning, where recent years have witnessed significant developments.

The conference requested the Commission to pursue its public information efforts aimed at improving the understanding that the citizens of the Community have on the potential risks and the protective measures which were implemented in the nuclear sphere.

Report EUR 12682 EN, FR, DE; 1990; 266 pages, Radiation Protection Series n° 49

To be ordered through:
Office for Official Publications
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Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 25

Statistics of Human Exposure to Ionising Radiation

Proceedings of a Workshop jointly organised by the CEC; US DOE, Washington DC; AECB, Ottawa (Cd) and NRPB, Chilton (UK)
Oxford (UK), 2-4 April 1990

Edited by M.C. O'Riordan and J. Sinnaeve

The purpose of the Workshop was to bring together those engaged in the determination of human exposure to ionising radiation, and its control and consequences, with the following objectives: to establish current data on various sources of exposure for workforce and public, to discuss the methods of collecting and assessing data, to consider whether extra data is required, to explore the possibilities of pooling data, to examine the utility of data for dose limitation and to relate the data to epidemiological studies of radiation effects.

Some 160 scientists from 23 countries attended the workshop and 55 papers were presented. Apart from the introductory paper by the Director of NRPB on the causes and consequences of human exposure to ionising radiation, there were ten sessions under the following titles: selected aspects of human exposure, exposure to radon, occupational exposure from nuclear power, public exposure from nuclear power, occupational exposure in medicine, patient exposure in medicine, determination of dose distributions, control of dose distributions, epidemiological estimates of risk and round table summaries and discussions.

The round-table discussions included contributions from participants from the USA, UK, Canada, France, the UN Scientific Committee on the Effects of Atomic Radiation in Vienna, and the WHO, International Agency for Research on Cancer in Lyon. A summary of the discussions is given in the proceedings. The proceedings present the current state of knowledge on human exposure to ionising radiation and its consequences and provide firm pointers to the areas where improvements are required.

Report EUR 13781 EN, published in Radiation Protection Dosimetry, Vol. 36, n° 2-6, 1991, 343 pages.

To be ordered through:
Nuclear Technology Publishing
PO Box n° 7
GB-Ashford-Kent TN25 4NW

Price: £ 60

The Relevance of Animal Models of Radiation Carcinogenesis in the Light of Developments in Molecular Biology

Proceedings of a Symposium jointly organized by the CEC and IARI, TNO Rijswijk (NL).
Rijswijk (NL), 22-23 October 1990

Edited by J. J. Broerse and D. W. van Bekkum

The proceedings present abbreviated papers which considered the need for further animal studies of radiation carcinogenesis taking into account the progress made in the fields of cell culture, cell transformation, in-vitro/in-vivo models, molecular biology and the process of carcinogenesis. There is a large body of data available which could be re-examined using modern statistical analytical techniques and there are also many pathological specimens which could be re-analyzed using modern molecular biological techniques. Modern studies using large numbers of animals would be prohibitively expensive. The general conclusions reached are that while modern techniques can replace animal experiments to a considerable extent certain specific animal experiments using small rodents will continue to be needed to answer well defined questions.

Published in Radiation and Environmental Biophysics, Vol. 30, n°3, 1991, 257 pages.

To be ordered through:
Springer International
Heidelberger Platz 3
D-W-1000 Berlin 33

Price: 162 DM

Methods and Codes for Assessing the Off-site Consequences of Nuclear Accidents

Proceedings of a Workshop organized by the CEC
Athens (GR), 7-11 May 1991

Edited by F. Luykx and G.N. Kelly

This Workshop was concerned mainly with the research carried out within the CEC MARIA programme which has culminated in the development of the COSYMA probabilistic accident consequence code.

The proceedings contain the following chapters:

- An overview of the MARIA programme;
- Atmospheric dispersion and deposition;
- Food-chain transfer and ingestion pathways;
- External exposure and inhalation pathways;
- Countermeasures and economic consequences;
- Health effects models, land use and demographic data;
- Uncertainty estimation.

A special chapter is devoted to other national and international developments in accident consequence codes and in their application.

Report EUR 13013 EN, 1991, 1237 pages.

To be ordered through:
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Price: ECU 80

The Future of Human Radiation Research

Proceedings of a Workshop jointly organised by the CEC; IARC Lyon (F); US DOE, Washington DC and RERF Hiroshima (Japan)
Schloss Elmau (D), 4-8 March 1991

Edited by: G. Gerber, J.W. Thiessen

The theme of the Workshop concerned an assessment of the current knowledge of epidemiological studies on the current knowledge of epidemiological studies on low dose effects of radiation on man and the development of a future strategy for research in this area. Each session dealt with a specific form of cancer e.g., leukaemia, lung cancer, breast cancer, thyroid cancer, etc. with two later sessions dealing with basic research approaches and analytical statistical techniques. The recent analyses of the cancers and of other health effects occurring in the Atom Bomb survivors were thoroughly reviewed. General conclusions were that more work was needed on relative/absolute risk and transport of association between lung cancer and radon exposure, the combination of epidemiological risk models with the biological models of carcinogenesis, and a detailed follow up of the Atom Bomb survivors over the next 10 years will be most important.

Report EUR 14096 EN

To be published as a Report of the British Institute of Radiology by:
The British Journal of Radiology
36 Portland Place
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Skin Dosimetry

Proceedings of a Workshop jointly organized by the CEC; EURADOS-CENDOS and NEB, Dublin (IRL); with the sponsorship of the US DOE, Washington DC
Dublin (IRL), 13-15 May 1991

Edited by H.G. Menzel, P. Christensen, J.A. Dennis

Radiological protection of the skin implies a complexity of dosimetric problems. It was the purpose of the Workshop to make an inventory of the knowledge gained in the field of skin dosimetry since the Workshop on Dosimetry of Beta Particles and Low Energy X-rays held in Saclay (France) in 1985. The main subjects dealth with and discussed in six sessions at the Workshop were: Biological aspects, the hot particle problem, implication of national and international recommendations for monitoring procedures, standards and calibration, monitoring techniques/instrumentation and monitoring experiments and problems.

Results of biological experiments reported at the Workshop showed that the production of skin lesions is highly complex and that, depending on the circumstances of the irradiation, cells of quite different depths may become involved. Several papers and posters focused on the radiological protection problems of highly radioactive particles "hot particles" in nuclear workplaces, e.g. the problems concerned with the assessment of the dose and the establishment of the dose limit as well as thos related to the identification and removal of the particles from the workplaces. Various aspects of standardization and calibration problems of skin dosimetry were dealt with. There are now several dosemeter possibilities available, mainly those based on thermoluminescence dosimetry and thermal stimulated exoelectron techniques, which enable the assessment of the dose to the skin at different depths to be made. There is still a need for improvement of the design of field instruments for the assessment of skin dose rates at short distances from the source.

Report EUR 14092 EN, 220 pages.

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Respiratory Tract Dosimetry

Proceedings of the third international Workshop jointly organized by the CEC, US DOE, Washington DC and ITRI Albuquerque (USA)
Albuquerque, New Mexico (USA), 1-3 July 1990

Edited by R.A. Guilmette and B.B. Boecker

The date of this Workshop has coincided with the final state of preparation of the ICRP and NCRP reports on lung models and consequently sessions on repiratory tract models were the centre piece of the Workshop. Other sessions focused on recent experimental research, development in dosimetry, in-vivo measurements following accidental intakes and human bioassay techniques and the interpretation of the obtained data. The organisers were succesful in bringing together researchers and operational health physicists and the proceedings are reflecting both points of view. In view of the importance of inhalation as a route of intake the proceedings provide valuable information for the research on respiratory tract doses and associated risks.

Report EUR EN, 14108, ~ 250 pages.

Radiation Protection Dosimetry
Volume 38, Nos. 1-3, 1991

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Price: £ 60

Role of the Alveolar Macrophage in the Clearance of Inhaled Particles

Proceedings of a Workshop jointly organized by the CEC and EULEP
Oxford (UK), 19-21 September 1990

Edited by A. Morgan

The Workshop dealt with the problems of inhaled radioactive particles. The Workshop was devoted primarily to a review of the state-of-the-art of the knowledge of the pulmonary alveolar macrophage, its structure, kinetic behaviour and function. The pulmonary alveolar macrophage plays a fundamental role in the protection of the lung against microorganisms and contamination by particulates. The Proceedings highlight the importance of phagocystosis transport and dissolution of the particles, the use and limitations of bronchoalveolar lavage, variations in results from different species, and the effect of radiation on the alveolar macrophage, which is not considered to be a precursor cell for lung tumours. The general conclusions point out that continued research is still needed because of the important role the pulmonary alveolar macrophage has for the protection of the lung that some improvement of the techniques could be made and contacts with immunologists might be rewarding.

Report EUR ..... EN, in press

To be published by:

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National Institute of Health
Research Triangle Park
USA-North Carolina 20079

Comparative Assessment of the Environmental Impact of Radionuclides released during Three Major Nuclear Accidents: Kysthtym, Windscale, Chernobyl

Proceedings of a Seminar jointly organised by the CEC and the IUR
Luxembourg (L), 1-5 October 1990

Edited by DG XI: Environment, Nuclear Safety and Civil Protection

Since the beginning of the nuclear age, several accidents have occured in nuclear installations. Three of them had severe consequences for the environment: Kyshtym (USSR) on 29 September 1957, Windscale (UK) on 11 October 1957 and Chernobyl (USSR) on 26 April 1986. Many studies have investigated the nature and the consequences of these three accidents. This seminar provided the opportunity to present and to compare the nature of the radioactive releases, their atmospheric dispersion and deposition, the subsequent transfer of contamination through terrestrial and aquatic ecosystems and the resulting implications for man and his environment.

The specific conditions of each accident being quite different, the Seminar gave the opportunity to put the enormous amount of radioecological data gathered after the Chernobyl accident in perspective to the results obtained after the earlier accidents. About 45 Soviet scientists coming from different Republics involved or concerned by the 2 accidents in the USSR, have presented the latest information available, reproduced the Proceedings.

Report EUR 13574, Vol. I and II, 1229 pages, Radiation Protection Series n° 53, in press

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VII

LISTE DES ACRONYME UND ABKÜRZUNGEN

LIST OF ACRONYMS AND ABBREVIATIONS

LISTE DES ACRONYMES ET DES ABREVIATIONS

AECB	Atomic Energy Control Board, Ottawa (Canada)
AECL	Atomic Energy of Canada Limited (Canada)
AFPPE	Association Française du Personnel Paramédical d'Electrocardiologie, Paris (F)
AIRM	Associazione Italiana di Radioprotezione Medice (I)
AIRP	Associazione Italiana di Protezione contro le Radiazioni (I)
ALARA	As Low As Reasonably Achievable
CAATS/INSERM	Centre d'évaluation pour l'Assurance de qualité des Applications Technologiques dans le domaine de la Santé/Institut National de la Santé et de la Recherche Médicale, Cachan (F)
CEA	Commissariat à l'Energie Atomique, Fontenay-aux-Roses (F)
CEC	Commission of the European Communities, Brussels (B)
CEDHYS	Centre de Développement des Etudes et Applications en Hygiène et Sécurité, Paris (F)
CEN/SCK	Centre d'Energie Nucléaire/Studie Centrum voor Kernenergie, Mol (B)
CEPN	Centre d'étude sur l'Evaluation de la Protection dans le domaine Nucléaire, Fontenay-aux-Roses (F)
CIEMAT	Centro de Investigaciones Energéticas, Medioambientales y Technológicas, Madrid (E)
CIR	Centre International de Radiopathologie, Fontenay-aux-Roses (F)
CNEN	Comitate Nazionale per la Ricerca e per lo Sviluppo dell'Energia Nucleare e delle Egnergie Alternative, Rome (I)
COSYMA	Code System Maria
CRSA	Centro Regionale per la Sperimentazione Agraria per il Friuli-Venezia-Guilia, Udine (I)
EBMT	European Bone Marrow Transplant Group
ECURIE	Early Exchange of Information in the Event of Radiological Emergency
EFOMP	European Federation of Organisations of Medical Physics, York (UK)
ENEA/DISP	Comitato Nazionale per la Ricerca e per lo Sviluppo dell'Energia Nucleare e delle Energie Alternative, Direzione Sicurezza Nucleare e Protezione Sanitaria, Rome (I)
ENEL	Ente Nazional per l'Energia Elletrica, Roma (I)
ERPET	European Radiation Protection Education and Training CEC DG XI/A/1, Luxembourg (L) & DG XII/D/3, Brussels (B)
EULEP	European Late Effects Project Group
EURADOS/CENDOS	European Radiation Dosimetry Group/Collection and Evaluation of Neutron Dosimetry Data
EUROMET	European Metrology
DG	Directorate General of the CEC
GSF	Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (D)
IAEA	International Atomic Energy Agency (A)
IARC	International Agency for Research on Cancer, Lyon (F)
ICRP	International Commission on Radiological Protection
ICRU	International Commission on Radiation Units and Measurements
ICSTM	Imperial College of Techn. and Medicine Science, London (UK)
IFE	Institute of Freshwater Ecology, Ambleside (UK)
INTECHMER-CNAM	Institut National des Techniques de la Mer - Conservatoire National des Arts et Métiers-Cherbourg (F)

INSTN	Institut National des Scienes et Techniques Nucléaires, Saclay (F)
IOMP	International Organisation of Medical Physicists
IRS	Integrated Radiological Services Ltd, Liverpool (UK)
ISH	Institut für Strahlenhygiene/Bundesamt für Strahlenschutz, Salzgitter/Neuherberg (D)
ITE	Institute of Terrestrial Ecology, Grange-over-Sands (UK)
ITRI	Inhalation Toxicology Research Institute, Albuquerque, NM (USA)
ITRI/TNO	Instituut voor Toegepaste Radiobiologie en Immunologie, TNO Rijswijk (NL)
IUR	International Union of Radioecologists
JRC	Joint Research Center of the CEC at ISPRA
KFA	Forschungsanlage, Jülich (D)
KfK	Kernforschungszentrum Karlsruhe (D)
KUL	Katholieke Universiteit Leuven (B)
LNETI	Laboratorio Nacional de Engenharia e Tecnologia Industrial, Lisboa (P)
MAFF	Ministry of Agriculture, Food and Fisheries, Lowestoft (UK)
MARIA	Methods for Assessing the Radiological Impact of Accidents
MLURI	McAulay Land Use Research Institute, Edinburgh (UK)
NEB	Nuclear Energy Board of Ireland, Dublin (IRL)
NIRP	National Institute of Radiation Protection, Stockholm (S)
NCSR	Democritos, National Centre for Scientific Research, Athens (GR)
NRPB	National Radiological Protection Board, Chilton (UK)
OECD/NEA	Organisation for Economic Cooperation and Development/Nuclear Energy Agency
ORNL	Oak Ridge National Laboratory, Knoxville, Tennessee (USA)
PTB	Physikalisch-Technische Bundesanstalt, Braunschweig (D)
RADE-AID	Radiological Accident Decision Aiding System
RBE	Relative Biological Effectiveness
REM	Radioactivity Environmental Monitoring
RERF	Radiation Effects Research Foundation, Hiroshima (Japan)
RIVM	Rijks Instituut voor Volksgezondheid en Milieu, Bilthoven (NL)
SCRPI	Service Central pour la Protection contre les Rayonnements Ionisants, Le Vésinet (F)
SEPR	Sociedad Española de Proteccion Radiologica (E)
SFEN	Société Française d'Energie Nucléaire, Paris (F)
SFRP	Sociedad Española de Proteccion Radiologica (E)
SRD	Safety and Reliability Directorate, Warrington (UK)
SUAS	Swedish University of Agricultural Sciences, Umea (S)
TEPC	Tissue-Equivalent Proportional Counter
TNO	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek, Rijswijk (NL)
UKAEA	United Kingdom Atomic Energy Authority, Harwell (UK)
ULB	Université Libre de Bruxelles, Brussels (B)
US DOE	US Department of Energy, Washington DC (USA)
US EPA	US Environmental Protection Agency
US NCI	US National Cancer Institute, Bethesda (USA)
US NIES	US National Institute of Environmental Sciences
USL	Unità Sanitaria Locale, N° 7, Udine (I)
WHO	World Health Organisation

VIII

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