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Environment
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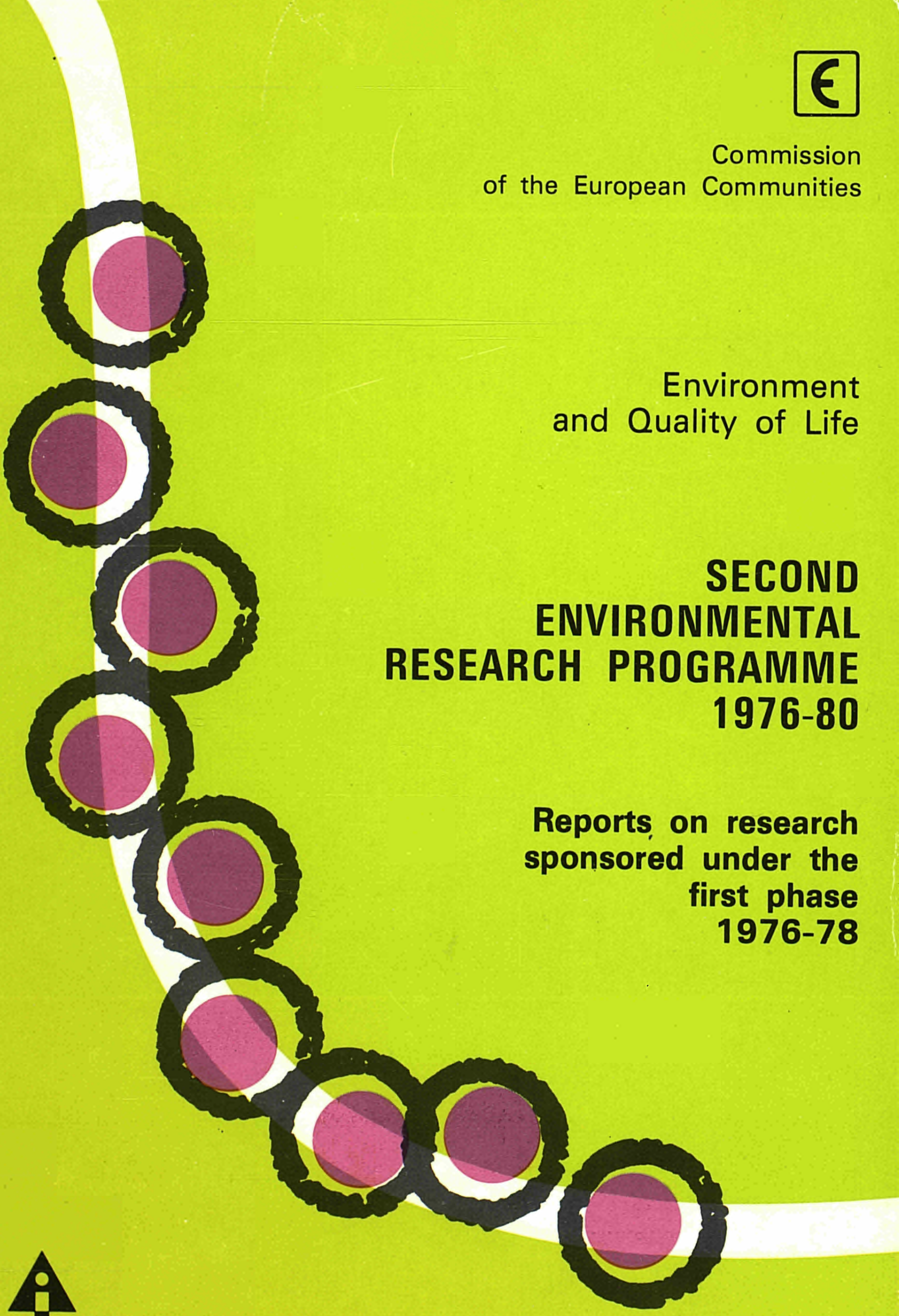

SECOND ENVIRONMENTAL RESEARCH PROGRAMME 1976-80

Reports on research sponsored under the first phase 1976-78

INDIRECT ACTION

EUR 6388 EN

COMMISSION OF THE EUROPEAN COMMUNITIES

environment and quality of life

Second environmental research programme
1976-80

Reports on research sponsored under the
first phase: 1976-78

INDIRECT ACTION

Directorate-General Research, Science and Education
Environment and Raw Materials Research Programmes

1980 EUR 6388 EN

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TABLE OF CONTENTS

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* n° of contract, country, contractor, project leader(s)

Table of contents (cont.d) Page

Table of contents (cont.d) Page

Table of contents (cont.d) Page

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* see page 610

Table of contents (cont.d) Page

Table of contents (cont.d) Page

Table of contents (cont.d) Page

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FOREWORD

The Second Environmental Research Programme, indirect action, was adopted by the Council of the European Communities on 15 March 1976[*] for a five year period, 1976-1980, with a total allocation of 16 million units of account.

As the First Environmental Research Programme, indirect action[**], carried out in the period 1973-1975, the current programme is aimed at providing scientific and technical support to the European Community policy on the environment. In fact the results of Community-sponsored research are exploited in the preparation, updating and implementation of Community regulations. These are needed to protect future populations and the environment against pollution and nuisances and to improve the quality of life.

The programme has been divided into two phases, the first one ending on 31st December 1978. The cost-sharing research contracts concluded with institutes and laboratories in the member countries deal with the various topics (see Table of Contents) included in the four research areas of the programme:

1. Research aimed at the establishment of criteria (exposure-effect relationships) for pollutants and environmental chemicals

2. Research concerning environmental information management

3. Research concerning the reduction and prevention of pollution and nuisances including clean technologies

4. Research concerning the protection and improvement of the natural environment

This volume contains the summary reports concerning the research carried out during the first phase of the programme. The texts are in the form submitted by the contractors.

The professional staff assigned to the management of the Second Environmental Research Programme are:

Ph. Bourdeau
Head of Division

E. Di Ferrante

R. Fantechi

P. Guillot

M. Lex

H. Ott

G. Weidenbach

Commission of the European Communities
Directorate-General XII
Research, Science and Education
200, rue de la Loi
1049 Brussels
Belgium

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[*] Official Journal of the European Communities nº L74 of 20.3.76.

[**] Official Journal of the European Communities nº C61 of 28.7.73.

The members of the Advisory Committee on Programme Management (ACPM) for Environmental Research, created on 10th December 1973, who assisted the Commission of the European Communities during the execution of the first phase of the Second Environmental Research Programme (indirect action) were:

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C. DE WISPELAERE
Programmation de la
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Bruxelles/Brussel

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Institut d'Hygiène
et d'Epidémiologie
Bruxelles/Brussel

M. RENSON or N. HILGERS
Cabinet du Ministère
Santé publique et Famille
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A. HEILIGENTHAL
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Copenhagen

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et du Cadre de Vie
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Ministère de l'Environnement
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en Milieuhygiëne
Leidschendam

J.G. WESSELS-BOER (Vice-Chairman 1977)
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en Milieuhygiëne
Leidschendam

P. WINKEL
TNO
Delft

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and Social Security
London

W.S. CLOUGH
Department of the Environment
London

M. CUTHBERT
Department of Health
and Social Security
London

L.E. REED
Department of the Environment
London

A.F.E. WISE (Chairman 1977)
Department of the Environment
London

COMMISSION OF THE EUROPEAN COMMUNITIES

Ph. BOURDEAU
Directorate General XII
Research, Science and Education
Brussels, Belgium

F. GEISS
Joint Research Centre
Ispra (Varese), Italy

RESEARCH AREA 1 : ESTABLISHMENT OF CRITERIA

TOPIC 11 : HEAVY METALS

Ecology

Contractor: Inst.f.Ingenieurbiologie, Universität Karlsruhe
Contract n°: 172 - 77 - 1 ENVD
Project leader: Prof.Dr.L.Hartmann
Title of project: "Synergistic effects of heavy metal ions on the activity of bacteria and other aquatic microorganisms"

The impact of heavy metals on the biological self-purification process consists in an inhibition of bacterial metabolism. In presence of heavy metals the degradation of organic substances by organotrophic bacteria is disturbed; the reaction velocity decreases. As shown in a former research project this inhibition can be mathematically described using the formulations valid for noncompetitive inhibition.

$$V^{*}max_{I} = V^{*}max \frac{Ki}{Ki + I} \qquad 1$$

By this the effect of single metals towards biocommunities of defined activity would be predictable. However, in pollution of natural waters normally several different heavy metals are present which may interfere with each other resulting in an enhancement or reduction of the individual inhibitory effects. If this is the case it is questionable whether a prognosis based on equation 1 will prove valid for the overall system.
The research was intended to estimate the extend to which synergistic or antagonistic effects occur in heavy metal inhibition and to interprete and mathematically describe those effects.
The term synergism is generally applied to an enhancement of the individual effect of one inhibitor in presence of another inhibitor; the term antagonism would mean a reduction of the individual effect. If the combined effect of two inhibitors is exactly what should be expected on the basis of their individual action the term "summation" is used.

As detailed information on the special characteristics of inhibition by Hg^{2+}, Pb^{2+} and Zn^{2+} ions was available from a former research project those metals were chosen to be tested in combination, Cd was chosen because of its great similarity in the

overall chemical properties compared with Zn.
The biological test material should be representative for natural biocommunities as well as reproducible in composition and activity. Therefore biocommunities grown in a lab model river were used. All investigations of the combined action of two heavy metals were conducted using the Warburg technique.

Self-purification activity was measured as substrate respiration with Na-acetate as substrate. Concomitantly theoretical investigations were carried out concerning the development of mathematical models for synergistic resp. antagonistic reactions on the basis of the standard formulations for noncompetitive enzymatic inhibition.
The investigations yielded the following results:

1. Not only the chemical properties but also the individual inhibitory effects of Zn and Cd ions proved to be very similar. Both cause either a linear noncompetitive inhibition (equation 1) or a partly noncompetitive inhibition. The combined effect of both metals is less than should be expected on the basis of their individual action; Zn and Cd seem to be antagonists.
   Under the assumption that both metals compete for the same place of reaction in enzymes ~~ac~~cording to the following scheme of reaction

$$\begin{tikzcd}
E + Zn \arrow[r, shift left] & EZn \arrow[l, shift left, "Ki_{Zn}"] \\
+ & \\
Cd \arrow[d, shift left] & \\
ECd \arrow[u, shift left, "Ki_{Cd}"'] &
\end{tikzcd}$$

   the overall inhibition may be written as

$$V\overset{*}{m}ax_{Cd,Zn} = V\overset{*}{m}ax \frac{Ki_{Zn}}{Zn + Ki_{Zn} + \frac{Ki_{Zn}}{Ki_{Cd}} Cd} \qquad 2$$

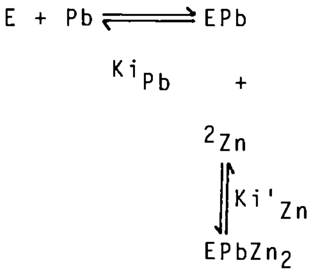
An antagonism of this type leads to a phenotypical increase of the Ki-values, the Ki-value of Zn-inhibition depending on the Cd concentration according to

$$Ki_{Zn,Cd} = Ki_{Zn}\left(1 + \frac{Cd}{Ki_{Cd}}\right) \qquad 3$$

The mathematical model could be verified by experiment. The average deviation of the actual data from the values calculated according to equation 2 was less than 2%.

2. With some special biocommunities Zn^{2+} and Hg^{2+} ions exhibit similar individual actions. A combination of both metals then yields an antagonistic effect that may be interpreted as a competition for the reaction partner and mathematically described according to equation 2.
   With most microbial systems, however, Hg-inhibition is characterized by a change in the rate limiting step whereas Zn-inhibition is of the linear noncompetitive type. In these cases the combined effect of both metals is exactly what should be expected on the basis of their individual action and therefore may be called a summation.
   In summation the sensitivity of the biological system to one inhibitor is not influenced by the second inhibitor; the Ki-values remain constant.

3. The combined action of Zn^{2+} and Pb^{2+} ions may be called synergistic. In presence of Pb two Zn^{2+} ions are bound to the enzyme. The reaction scheme may be written as:



A mathematical description of the overall inhibition is given by the equation

$$\overset{*}{V}max_{Zn,Pb} = \overset{*}{V}max \frac{Ki_{Pb}Ki'_{Zn}}{Pb\ Zn^2 + Ki'_{Zn}Pb + Ki_{Pb}Ki'_{Zn}} \qquad 4$$

in which Ki'_{Zn} has the dimension $(mMol/l)^2$ and is not identical with the Ki-value of Zn-inhibition in absence of Pb. The Ki'_{Zn}-values can be determined in the representation of Dixon, plotting $1/\overset{*}{V}max_{Zn,Pb}$ versus Zn^2. The straight lines obtained for different concentrations of Pb meet in one single crossing-point, the coordinates being $x= -Ki'_{Zn}$ and $y= 1/\overset{*}{V}max$. Increasing Pb concentrations result in a phenotypical decrease of Ki'_{Zn} according to

$$Ki'_{Zn,Pb} = Ki'_{Zn}\ (1 + \frac{Ki_{Pb}}{Pb}) \qquad 5$$

From the results obtained until now the conclusion may be drawn that interference between different heavy metals occurs quite frequently. Therefore as a rule, a valid prognosis cannot be based on the assumption of a simple summation of effects.The standard laws enzyme inhibition may be regarded as a model conception that proved quite helpful in the deduction of mathematival descriptions for synergistic resp. antagonistic reactions.

Definitions of Symbols

$\overset{*}{V}max$	mg O_2/g N,min	maximum reaction velocity related to biomass concentration
$\overset{*}{V}max_I$	mg O_2/g N,min	maximum reaction velocity related to biomass concentration in presence of inhibitor
I	mMol/l	inhibitor concentration
Ki´	mMol/l	inhibitor constant; concentration of inhibitor, that yields half maximum inhibition
Ki_{I_1,I_2}	mMol/l	phenotypical constant in presence of a second inhibitor

Ki'	mMol/l	inhibitor constant, that is only valid in presence of a second inhibitor and replaces Ki

References:

G. Engelmann "Antagonistic effects in heavy metal inhibitions"
Report presented at the second meeting of the "Freshwater Contact Group" at Metz

G. Engelmann "Antagonistische Effekte bei der kombinierten Hemmwirkung von Zink- und Cadmium-Ionen"
Kalrsruher Berichte zur Ingenieurbiologie, 1977

Contractor : Université Paris VII
Contract : 150-77-1 ENVF
Project Leader : J. FAUCHERRE
Title of project : Biogeochemical mechanism of contamination of plants by lead, cadmium and zinc.

OBJECTIVE OF THE RESEARCH

1) To study the biogeochemical mechanism of contamination by Pb, Zn and Cd of natural waters, soils and plants.

2) To examine the effects of this pollution on the population birth rate.

3) To reconstruct the last 70 years of the history of the pollution and the microclimate of a valley from the variations in the concentrations of Pb, Zn and Cd as recorder in the tree-rings of conifers.

MATERIALS AND METHODS

1) Determination of pH and of redox potential of polluted river water from the deposits of galena, blende, and pyrite. Analysis of Pb, ~~Zn,~~ Cd, Mn, Fe^{2+}, Fe^{3+}, AsO_2^- by atomic absorption or polarography for waters, soils (and interstitial waters), and plants.

Development of a geochemical model for :

a) the migration of Pb, Zn and Cd ions from the parent rock,
b) the transport of these ions in water flows,
c) the trapping of these ions by soils,
d) their remobilization in soils and subsequent introductionsto plants.

2) Completion of a survey on the local population in order to evaluate the effect of Pb, Zn, and Cd pollution on the number of children per family.

3) Analysis of Pb, Zn and Cd in tree-rings of conifers by flameless atomic absorption with a detection limit of 0.2 ppm for Pb, 0.05 ppm for Zn, and 0.01 ppm for Cd with our operating conditions. X-ray diffraction was used to

define the tree-ring in the conifer cores. Statistical treatment of the data was done with hc help of a computer program based on the factor analysis.

RESULTS

The contract program bears on the contamination of waters, soils, and plants by Pb, Zn and Cd from washout by precipitation runoff over mining areas of galena, blende, and pyrite commonly found at the Triassic and Hettangien levels around the Massif Central.

We have shown that

1°) Pollution of river, with waters from mining runoff, has two sources : open mine during dry weather, scummy hill during wet periods. These two sources pollute by different mechanisms :

a) Pb, Zn and Cd pollution from surface mining : it is mainly due to pyrite oxidation and the consequent acidification (pH=2) of the waters. It decreases regulary each year. The neutralization of waters at confluence with a non-polluted river is indicated by the precipitation of $Fe(OH)_3$ which takes out Zn, Pb, and Cd by adsorption or coprecipitation and thus acts as an important purifier. This $Fe(OH)_3$ suspension, rich in polluting elements, is decanted to a large extent along irrigation canals so that soils of prairies and gardens are only slightly contaminated.

b) Pb, Zn, and Cd pollution from scummy hill.

These sands, rich in pyrite, are physically transported by river during wet periods and are deposited on the soils of prairies and gardens and can achieve thicknesses of 20 cm. The oxidation mechanism of FeS_2 thus takes place and brings about the in situ acidification of percolating waters with the mobilization of Zn^{2+}, Pb^{2+}, and Cd^{2+}. The alkaline reserve of soils is therefore neutralized to a thickness of about 5 cm for a sand deposit of 20 cm. The survival of plants with shallow roots (prairy grasses) is greatly compromised. On the other hand, plants with deep roots survive although they highly polluted.

In the case of vegetable gardens, the sandy deposits are smaller but are leaked and dispersed in the soil by plowing. The acidification of the soil is therefore not sufficient to neutralize the alkaline reserve, the pH remains at about 6.5, and the alteration of the pyrite brings about only thiosulfates which forme soluble complexes with Pb, Zn and Cd.

In this case, the plants are highly polluted but their survival is not endangered.

2°) Extent of Pb, Zn, and Cd contamination of plants of current consumption :

The first mechanism (pollution from surface mining) results in only small amounts of plants Pb pollution (2 to 3 times the tolerable dose limits set by the world Health Organization), whereas pollution from the second mechanism (physical pollution from scummy sands) is much more significant (2 to 15 timesthe WHO limits).

These standards are exceeded much more frequently for Pb than for Cd. The above ground parts of the plants are systematically polluted to a much greater extent than the non chlorophyl parts (tubers, roots, fruits).

The maximum pollution occur during the summer when evaporation/transpiration is at optimum.

3°) The zinc and cadmium content of tree-ring of conifers are good chronological tracers of pollution in water-flows

We can therefore roughly reconstruct the local microclimate, the decrease of mining activity and the backround value of Zn and Cd indicating anthropogenic pollution. On the other hand Pb cannot be used as a tracer. It will be used as such for atmospheric pollutions close to main roads, but in a very limited way.

In the case of atmospheric pollution (the Roumare Forest near the industrial center of Rouen) the zinc of tree-rings of conifers remains a good chronological tracer of pollution. Its concentration correlates well with the global distribution of toxin-sensitive epithytic flora within a radius of 3 to 4 km from the emission source.

The extrapolation of pollution curves with respect to time allows the determination of the "zero level" of pollution prior to the industrial period. This level was 0,04 ppm for Zn and 0,03 for Pb in tree-rings of conifers.

4°) Effects of pollution of plants of current consumption on the birth rate

From 1926 to 1968 the number of children per family, about 2 on the national scale, is 2,1 for a non polluted community. It drops to 1,1 for a community irrigated by a river polluted of Pb and Cd.

From 1886 to 1906 before the appearance of this pollution the birth rate was normal for both communities (2,3 and 2,4). Its appears, therefore, that a correlation exists between the extent of Pb contamination in the river, and the birth rate.

CONCLUSIONS AND ADDITIONAL COMMENTS

The investigations that were foreseen have been accomplished, except for two aspects which, in our opinion, further study.

1°) To prove scientifically the relationships between the decrease in the birth rate and Pb and Cd pollution. Our statistical observations give a strong indication of such a relationship but do not constitute a formal proof. Would it not be feasible to perform experiments using rats or mice whose food contained concentration of lead analogous to those found in our study ? "See especially D. HOWARD and al. in Fertility and Sterility" 27-755, 1971, on Reproductivity ability and progeny of F_1 Lead toxic Rats". Cd could also be introduced in order to examine the eventual cumulativeeffects of Pb+Cd. But which laboratory could perform this type of research ?

2°) To better understand the nature of the phenomon responsable for the entrainment of Pb , Zn and Cd ions by the hydroxydes of iron and aluminium. Is it an adsorption or a coprecipitation of basic salts or mixed hydroxydes. This study would allow the parametres responsible for the spontaneous or induced decontamination of natural or waste waters and the stability of estuarine sediments and sewer muds to be controlled. We are heading further in this direction.

LIST OF RELEVANT PUBLICATIONS

G. MICHARD - J. FAUCHERRE

Etude géochimique de l'altération de minerais sulfurés. Chem. Geology 68-84, 1970.

M. TREUIL - J. FAUCHERRE - J.C. MACQUAR

Etude du comportement géochimique de Zn, Pb, Cd, Ni et Cu dans la sédimentogenèse. Mineral. Deposita 1-22, 1970.

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Quelques aspects de la géochimie des éléments traces Actual. Chim. 18-24, 1974.

J. FAUCHERRE

Exemple de pollution des eaux de rivière par une mine à ciel ouvert. Communic. Journées d'études sur l'enseignement de la Chimie. Soc. Chim. Fr 14-3, 1974.

R. VIE LE SAGE - J. FAUCHERRE

Méthode rapide et non destructive d'analyse du fer et du manganèse dans les végétaux par fluorescence X. Analusis, 4-25, 1976.

J. FAUCHERRE - A. DUTOT

Le zinc et le cadmium des anneaux de croissance des végétaux en tant que traceurs chronologiques de la pollution d'un cours d'eau. C.R. Acad Sci. 282, 1976.

J. FAUCHERRE

Les éléments traces indicateurs des climats anciens. Actual. Chim. p. 14-19, Déc. 1977.

J. FAUCHERRE - A.M. PINART - A. DUTOT

Contamination des eaux, des sols et des végétaux par le plomb, le cadmium et le zinc. C.R. Contrat Ministère de l'Environnement, Oct.1978.

A. DUTOT

Le plomb, le zinc, le cadmium des anneaux de croissance des conifères en tant que traceurs chronologiques de la pollution atmosphérique et des eaux naturelles; Thèse Univ. Paris VII, Janvier 1979.

Contractor : INSTITUT NATIONAL de la RECHERCHE AGRONOMIQUE
STATION D'AGRONOMIE - Centre de Recherches de Bordeaux
33140 PONT DE LA MAYE (FRANCE).

Contract n° : 153-77-1 ENV F

Project leader : JUSTE Christian.

Title of project : Study of organo-metallic compounds existing between cadmium lead, nickel and mercury and organic matter of soils. Effect of these compounds on the possible contamination of the food chain.

A/ OBJECTIVE OF THE RESEARCH.

The objective of this research is to state precisely :

1) influence of addition of Pb, Cd, Hg and Ni on the following properties of humic acids of a soil :
   a - mobility in an electric field ;
   b - infra-red absorption spectra ;
   c - solubility in water and soda ;
   d - biological stability ;
2) influence of the association of these metals with humic acids on their mobility in soils and their plant availability.

B/ MATERIALS AND METHODS.

1) Preparation of metallic humic compounds.

Humic acids are extracted with soda 0,5 N from a soil constituted by humic podzol top horizon developed in the sand of "Landes". After a cleaning dialysis, these humic acids are put in a normal solution of each of the metals (nitrates or chloride for Hg) at pH 4. The resulting precipitates are washed, dried at 45°C, then ground. Composition of these precipitates is given in the first table.

2) Mobility in an electric field.

The different humic compounds are put in solution in N soda. 50 µl of this solution are set on a strip of Wathman n°1 paper. At the extremities of this strip of paper, we put an electric field of 300 volts during a period of 4 hours. The electrolyte of transportation is made with a solution of sodium borate, at pH 9,2.

After drying, the electrophoregrams are analysed with a densitometer. This analysis give for each metal, by integration, the rate of mobile and immobile humic acids (this calculation is made on 8 repetitions).

Then the electrophoregrams are cut perpendicularly to the humic acids movement. After dry or wet ashing (Hg) the trips are analysed to determine the quantity of metal which have moved with humic acid.

3) Infra-red absorption spectra.

Spectra of pellets of humic compounds (1 % in KBr) is made using a Perkin Elmer apparatus, model n° 297, (from 4 000 to 600 cm^{-1}).

4) Solubility in water and soda.

100 mg of each humic compound are put in 50 ml of water or N/10 NaOH, in centrifugal tubes. After one hour of shaking, the tubes are put in centrifuge during 10 minutes at 4 000 RPm. The liquid is filtered, then analysed : carbon, nitrogen and metal are determined. On the dryed solid fraction, only the metal is determined.

5) Biological stability.

This determination is made using a respirometer : humic compounds are put in flasks (equivalent of 20 mg C), mixed with 1 g of soil and wet with 1 ml of water. It works five days during 8 yours at 30°C. Absorption of oxygen is determined. Fresh air is given each night to the dispositive. Replacement of water by a glucose solution (1 ‰) gives precision on the mechanism of biological stabilization of humic compounds.

6) Metals mobility in soils.

Metallic humic compound or equivalent mineral salt is put in top part of cylindrical tube. These tubes are filled with pure sand or with a high cation exchange capacity calcareous-clay soil. A quantity of water, equivalent to 800 mm of rain, is leached through the tubes in a period of four weeks. In the leachates, concentration of metal and optical density are determined. The optical density gives an idea of the organic matter in the leachate. At the end of this period of four weeks, the tubes receive 10 ml of 1 ‰ glucose solution with soil inoculum. After a waiting week, the tubes are leached one week more.

7) Plant absorption of metals.

The test plant is maize in the third leaf state. After a period of 84 hours of growing in a nutritive aerated solution, the plant is put 72 hours in a solution with or not metallic-humic compounds or corresponding mineral salts. The different determinations are made :

- variation of fresh weight of plants ;
- dry matter of leaves and roots ;
- phosphorus and metals present in leaves and roots ;
- concentration of staying phosphorus and metal in the last solutions.

C/ RESULTS.

1) Composition of metallic humic compounds

In the working conditions of preparation of the different humic compounds, the quantity of Cd, Ni and Pb bound with humic acids is about 250 meq/100 g and for Hg is 120 meq/100 g.

2) Mobility in an electric field.

The mobility of the mercuric humic compound is the greatest (mobility rate : 1,95) ; only 26,1 % of the metal don't move. On the opposite, Cd and Pb humic compounds move very few : 87,2 % of metal don't move. Mobility of Ni humic compound is between Cd and Hg humic compounds mobilities.

3) Infra-red absorption spectra.

The spectra of Cd, Ni and Pb humic compound show that carboxylic radicals are in salt form : disappearance of the C = 0 absorption band at 1 710 cm^{-1}. The spectra of Hg humic compound is like the pure humic acid.

4) Solubility in water and soda.

Only 25 % of the carbon of the pure humic acid and 1 or 2 % of Ni and Pb of humic compound are dissolved by water.

With soda, 65 to 75 % of the carbon of humic compounds and 50 to 72 % of metal of humic compound are put in solution. Ni is the most easily dissolved metal.

5) Biological stability.

Decomposition of the organic matter of the reference soil is stopped in presence of Hg, Cd, Ni and, in a weak way, Pb humic compounds during the two first days. After this time, this effect disappears with Cd, Ni and Pb humic compounds, but not with Hg. In all cases, the organic matter of the humic compound is protected by the metal.

Decomposition of added glucose is stopped by the Hg humic compound but not by Pb, Ni and Cd humic compounds. In the case of the Cd humic compound the destruction of the humic organic matter of the compounds is stimulated similarly to that of the pure humic acid.

6) Metals mobility in soils.

In the case of sand, metals from metallic salts get out from the soil tubes in the first week. On the opposite, the metallic humic compounds (Pb, Cd, Hg) don't get out. Ni humic compound move slightly : 2,3 % of Ni got out of the tube at the end of the four weeks. Mobility of Cd and Ni is increased by the addition of glucose solution with soil inoculum.
In the case of calcareous clay soil with mineral salts or humic coumpounds added, no metal get out of the tubes.

7) Plant absorption of metals.

In the case of Ni, Hg and Cd mineral salt the maize growth is depressed in 72 hours, this effect don't occure with Pb ; the most phytotoxic metals are Hg and Cd. The pure humic acide have no effect on the maize growth in the time of experimentation ; all the humic metallic compounds, even Pb humic compound, depress the maize growth. The Hg humic compound is less depressive than the mineral salt. The uptake of Cd is decreased by its association with humic acid, this is not the same for Pb and Ni. In weight the uptake of Cd, Pb and Ni, in mineral salt form, is the same. Translocation of Cd from roots to leaves is better when the metal is in humic compound form. The best translocation occurs with Ni and the worst with Pb. Ni in each form and Pb in humic compounds form decrease the uptake of phosphorus.

D/ CONCLUSIONS.

Ni because of its mobility and Hg because of its curious properties in humic compounds need a supplement of studies to give precisions about their properties in soils. Because of its great phytotoxicity (the same that Hg) and its faculty to be put in solution after a biological activation, Cd needs new studies. Pb which is characterized by no phytotoxicity and a great inertia in soils and nutrient solution cultures don't need further studies.

CONTRACTOR : Université de Bordeaux I - Faculté des Sciences - 351, cours de la Libération - 33405 - TALENCE - France.

CONTRAT N° 211 - 77 - 1 ENV. F.

PROJECT LEADER : Prof. R. MARTY - Directeur du Laboratoire d'Ecologie et Ecophysiologie animales.

TITLE OF PROJECT : Experimental trophic chain in freshwater environment : fundamental and practical research

---

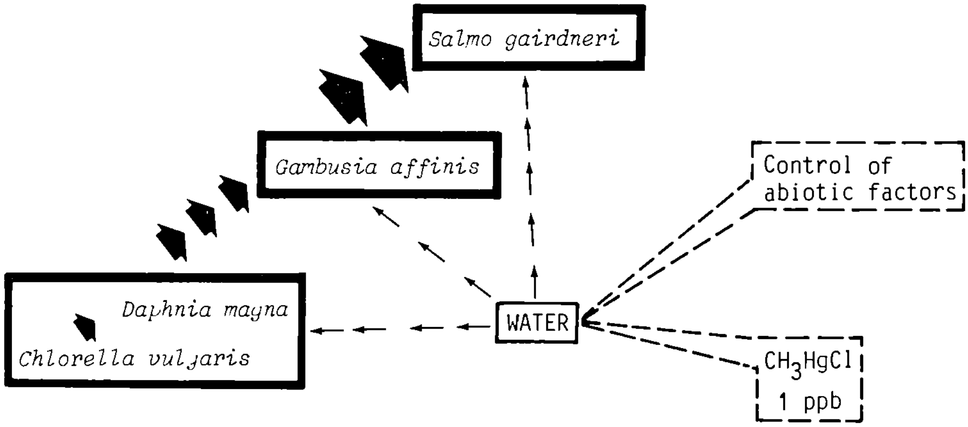
OBJECTIVE OF THE RESEARCH :

The contamination of the natural systems by mercurial compounds is most often due to a chronical rejection of very small quantities of heavy metal in the environment. From an ecotoxicological point of view, two major processes exist in aquatic environment : the biological methylation of inorganic compounds and the bioconcentration of the contaminant in vegetables and animals. This latter process is the difference between the pollutant penetration in and excretion from the organism. With the combined action of the parameter time and the transferts between the different trophic levels, a bioamplification can be seen when the trophic levels rise.

In order to analyse the bioaccumulation and the bioamplification of the mercurial compounds as well as the ecotoxicological incidences of these contaminants on the aquatic organisms, for model study at the laboratory level, we have chosen the experimental trophic chain. The researches made have also aimed at estimating the respective part of the different sorts of contamination (direct and trophic) on each level as well as the synergists or antagonists effects of the environment temperature.

MATERIAL AND METHODS :

- Experimental trophic chain.



The setting up of this model demands careful control and regulation of experimental conditions and should yield precise results which can easily be reproduced. Much preliminary research has been proved necessary to define the trophic sequence between the four levels, the means of contamination and the renewal cycles of the environments.

The quantification of the respective parts of the two sorts of contamination, on the "fish levels", is realized by the use of three food chains:

- CHAIN 1 : global contamination, direct and trophic.

- CHAIN 2 : direct contamination, only the environment is contaminated (1 ppb of CH_3HgCl).

- CONTROL CHAIN : environment and food uncontaminated.

- Experimental conditions :

- renewal cycle of the environment (fish levels) : 2 days.
- water temperature : 10, 18 and 26°C.
- contamination length of *Chlorella vulgaris* : 24 hours.
- contamination length of *Daphnia magna* : 96 hours.
- contamination length of *Gambusia affinis* and *Salmo gairdneri* :10 and 30 days.
- mercurial dosage : Hg^{203} and radioactivity counting - Atomic absorption without flame.
- expression of the results : µg of $Hg.g^{-1}$ (wet or dry weights) and concentration factor with regards to the dose in the environment.

- Ecotoxicological incidence analyse of the mercurial contamination on the terminal consummer : *Salmo gairdneri*.

- metal concentration in the principal organs : liver, brain, muscle, gills, posterior intestine, serum, red globules.
- on the hepatic level : structural analyse by optic and electronic microscopy-enzymatical dosages (LDH, MDH, GOT, GPT).
- on the blood level ; enzymatical dosages, study of the circulating proteins (dosage, polyacrylamide gel electrophoresis).

PRINCIPAL RESULTS :

- Quantification of the dynamic of the mercurial bioaccumulation by each trophic level, in function of the contamination length, of the two penetration ways (for the superior levels) and of the water temperature (10, 18 and 26°C). Illustration of these results is given on the four graphs.
- Study of the global mercurial transfer along the trophic chain, from the productor level to the terminal consumer.
- Ecotoxicological incidences analyse on the terminal level (*Salmo gairdneri*), in relation with the increase of the water temperature - Detailed study of the contamination mechanisms in function of the penetration ways of the toxic in the organism.

CONCLUSION :

Our researches are actually continuing, following two principal axes :

- complexification of the experimental protocols : compared study of $HgCl_2$ ans CH_3HgCl - influences of other abiotic parameters - synergic or

antagonism action of several contaminants...
- fondamental approach of the contamination processes by the mercurial compounds ; action on the membrane barriers - realisation and use of cell culture models (human hepatocytes).

This researches enter at the same time in a fondamental aim, in order to increase our Knowledge on the ecotoxicological contamination mechanisms which occur in natural environments as well as in an applied aim, searching the sitting up of "contamination indicators" which may interfere in the definition of most elaborated toxicological tests.

LIST OF REFERENCES TO PUBLICATIONS AND ORAL COMMUNICATIONS :

1 - Chaînes trophiques expérimentales (études écotoxicologiques fondamentales et appliquées) - Symbioses IX, n° 1, 1977, 57-70.

2 - Modèles expérimentaux en écotoxicologie : chaînes trophiques en milieu limnique. Bull. Ecol. 8(4), 1977, 401-414.

3 - Modèles expérimentaux en écotoxicologie - Symposium "Contamination des chaînes biologiques" - PARIS - 1978.

4 - Experimental trophic chains in freshwater environment : their ecotoxicological interests both fundamental and practical. Research seminar (ecological tests relevant to the implementation of proposed regulations concerning environmental chemicals : evaluation and research needs) - BERLIN Ouest, décembre 1977.

5 - Ecotoxicological studies on an experimental trophic chain in freshwater environment - Workshop European Communities - Water Research Center - Windermere (Angleterre) - 1977.

6 - Ecotoxicological action of mercury by-products on different animal tissues - International workshop on monitoring environmental materials and specimen banking - BERLIN-Ouest, octobre 1978.

7 - Bioaccumulation an Bioamplification of mercury compounds in an experimental trophic chain - Workshop European Communities - Centre Européen Ecologie - METZ - 1978.

8 - Bioaccumulation and bioamplification of mercury compounds in a second level consumer, *Gambusia affinis* - Bull. Contam. Toxicol. 22(6), 1979.

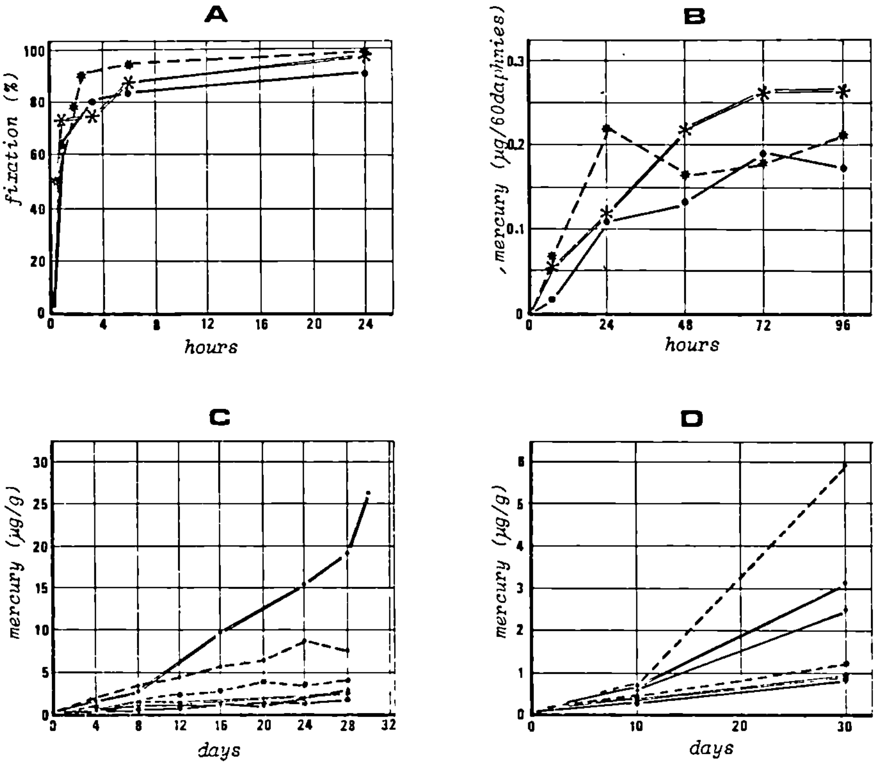
9 - Transfer of methyl-mercury in an experimental freshwater trophic chain - temperature effects - (in press).

10 - Ecotoxicological effects of methyl-mercury on the liver tissue of a third level consumer, *Salmo gairdneri* - Synergic temperature effect - (in press).

11 - Contamination d'une chaîne trophique expérimentale par le méthylmercure. Importance du système "producteur - consommateur primaire" (in press).

12 - Changes in transition temperature of DPPC liposomes caused by methylmercury and inorganic mercury (in press).

13 - Chaîne trophique expérimentale en milieu limnique (A. DELARCHE et F. RIBEYRE) - Thèses de 3ème cycle - Université de Bordeaux I, 1978, n° 1436, 265p..



Fixation dynamics of mercury by *Chlorella vulgaris* , *Daphnia magna* (A and B —•—10°C,--✱--18°C,═✱═26°C), *Gambusia affinis and Salmo gairdneri* (C and D • global contamination, ✱ trophic contamination ; —10°C,--18°C ▬ 26°C).

Contractor: C.N.R. - Istituto Italiano di Idrobiologia - Pallanza
Contract n° 157-77-1 ENV I
Project leader: Dr. Piero Guilizzoni
Title of project: Heavy metals distribution and their effects on photosynthetic rates of two submersed macrophytes of Lake Mezzola (N. Italy).

Summary

Objective of the research

Lake Mezzola is located at north-east of one of the largest Italian lakes, Lake Como, to which it is connected through the River Mera. From north to south, the Lake Mezzola hydrographic system is formed by a small lake, Pozzo di Riva, connected with L. Mezzola by a channel (Fosso di Riva), by the River Mera inflow and by some tributaries which often dry during the year. Before reaching Lake Pozzo di Riva, two small branching streams (Merette) pass through an industrial and agricultural area.

In addition to the potential sources of pollution represented by industries, farms and field fertilization activities, a great pollution hazard consists in the abundance of scums that a metallurgic factory is accumulating since long very close to the Pozzo di Riva lake. The analysis of this waste material shows high concentrations of chromium, iron, zinc, nickel and manganese, together with many other metallic elements.

The objectives of this study were to provide information on: 1) general trophic conditions of the two lakes; 2) the role of the major inputs in the heavy metals mass balance; 3) distribution, accumulation and relationships between metals in the submersed macrophyte tissues, lacustrine sediments, interstitial water and the water layer near the bottom; 4) relationships between chemical parameters and photosynthetic activity of Potamogeton crispus and Potamogeton perfoliatus.

Materials and methods

Lake water samples were collected monthly during 1977 in the deepest part of Lake Mezzola and Pozzo di Riva and analyzed for several chemical parameters (O_2, pH, alkalinity, nutrients, silicates, specific conductance, Ca, Mg, Na, K, Fe, Mn, Zn, Cu, Cr, Co, Ni, Pb, Cd, Mo).

In the study area, the submergent macrophyte vegetation is dominated by two species, namely Potamogeton crispus and Potamogeton perfoliatus. During the year, these species plus two others (Callitriche stagnalis and Ranunculus sp.) were routinely collected with the surrounding water and sediments from five littoral weed bed sites.

In the laboratory, fresh sediment samples were thoroughly mixed and subsamples were taken by a teflon squeezer to determine the heavy metal concentrations in the interstitial water.

Plant materials were dried at 105°C , ground to a fine powder, ashed at 500°C and then acid digested in a teflon bomb.

Subsamples of sediment cores were dried, sieved (180 x 230 µm mesh) and solubilized in the same manner.

All samples were analyzed for ten metals by atomic absorption spectrophotometry.

Results

The water chemistry shows striking differences between Lake Pozzo di Riva and Lake Mezzola: in fact, in the first one we have observed higher nutrient content, larger oxygen deficit, and high concentrations of ammonia and nitrates during the summer stratification period. These results and some other biological data allow us to conclude that Pozzo di Riva is a mesotrophic lake, while Lake Mezzola is classifiable as oligo-mesotrophic.

The nutrient loadings for Lake Mezzola were measured as 26490 kg P/year and 632738 kg N/year, and for Pozzo di Riva 1741 kg P/year and 27247 kg N/year. These current phosphorus loadings are 2 and 5 times the "permissible" input rate according to the 1976 Vollenweider loading criteria.

The heavy metal contents of the two lakes were low for all the investigated elements except for iron, manganese and zinc.

The quantities of iron (kg/yr) that enter Lake Pozzo di Riva through its tributaries and those entering Lake Mezzola by Fosso di Riva channel and Mera river are not balanced by the quantities that exit by the respective outflows. This result supports the hypothesis of an appreciable sinking of iron into the lake sediments.

Copper, manganese and chromium follow a similar trend in Lake Mezzola, while in Pozzo di Riva the outputs are about twice the measured inputs. These apparent deficits could be explained by the runoff of the metallurgic factory scums accumulated along the lake shorelines.

The zinc mass balance shows a completely different picture; whereas for the other elements the rainfall contributions to the budget was negligible, rainwater appears to be the major source of zinc for the two lakes.

From the heavy metal concentrations of rainwater, and from the examination of several enrichment factors, we assume the existence of an artificial input in the atmosphere for lead, cadmium and to a lesser extent for copper and chromium.

Compared with the littoral sediments, higher heavy metals concentrations were found in the open lake cores possibly due to the high quantities of finely subdivized organic matter. In fact, positive significant correlations were found between organic matter content and iron, manganese, zinc, copper and potassium sediment concentrations.

The comparison of heavy metals content between the five littoral stations shows an apparent influence of the metallurgic factory wastes in addition to that due to the Mera river inflow.

The general order of enrichment by heavy metals in the study area is: $Cu < Ni < Zn < Cr < Mn < Fe$.

Negative significant correlations were found between sediment and interstitial water content of iron and copper, while manganese is positively correlated.

As it concerns the aquatic vegetation, many differences arose from the statistical analyses of each element, plant species, sampling site and seasonal collection. However, no strong variations were evident for the same species growing in different areas.

Significant negative correlations have been found between the content of Ni, Mn, Zn, Fe and Cr in plant tissues of Potamogeton crispus and P. perfoliatus and their dry weights. The comparison of tissue metal concentrations of the four aquatic macrophytes collected at various sites demonstrates that metal levels are regulated by physiological and ecological factors.

Several significant correlations were found between metal content in plant tissues and sediment metal content; the more interesting are those for chromium, manganese and potassium (inverse) and for copper, calcium and sodium (direct).

Potamogeton crispus and P. perfoliatus from two different sites were sampled also to provide field data on natural photosynthetic profile and laboratory studies on photosynthetic rate-light curves.

The same species growing at different sites shows variations in the carbon assimilation ($mg\ C \cdot g\ dry\ wt^{-1} \cdot hr^{-1}$) according to the different chemical environmental parameters. In particular, alkalinity seems to play an important role in photosynthetic production of P. perfoliatus during experiments in which temperature, light and nutrients were controlled.

From the field and laboratory experiments in which plants from other six lakes were considered, it appeared that copper, chromium, manganese, iron and nickel adversely affect the P. perfoliatus production.

Apparent "net" photosynthesis of P. crispus is negatively correlated with sodium, potassium, manganese and nickel tissues content.

Conclusions

The differences in water, macrophyte and sediment metallic concentrations between the sites reflect some of the differences in catchment geology of the region and in the allochthonous inputs from industrial activities. Human influence on the aquatic system appears in the nutrient concentrations in water, and in chromium and iron values in sediments and plants.

The evaluation of the actual concentration of heavy metals able to influence macrophyte photosynthesis production directly under field conditions is complicated by the overlapping of several environmental and physiological factors; nevertheless, the existing data indicate that copper and chromium tissue content is actively involved in the reduction of Potamogeton production.

Contractor : SNAMPROGETTI/ASSORENI

Contract n° : 293-76-1 ENV I

Project leader : Dott. P.P. GARIBALDI

Title of project : Isotopic Lead Experiment

Objectives of the Research

The main object of the research is to define the contribution of automotive traffic to the lead concentration in the blood of a population living in a wide area of N.W. Italy. The method used is to follow the variation in time of the isotopic composition of the blood lead. Measurements are also made in air, soil, plants, water. To this end most of the petrol (>98%) distributed in a wide area of N.W. Italy (Piedmont, Val d'Aosta, Liguria and Lombardy) has, as an additive, exclusively alkyl lead produced from Australian lead (Broken Hill mine), with an isotopic ratio clearly distinguishable both from the lead already present in the environment and from the lead now employed for other purposes.

Isotopic ratio (Pb 206/Pb 207) of some environmental samples taken in Piedmont (mean value)

Atmospheric particulates	1.17
Blood	1.17
Petrol	1.18
Soil	1.17
Vegetation	1.16
Australian lead	1.04

The work started in July 1974 and is expected to end in late 1979. Therefore, following the distribution rate of the Australian lead in the petrol present in this region, the experiment can be subdivided into the following four periods :

- Phase "0" - background definition - July 1974-July 1975
- Phase "1" - transition period from the old lead to the Australian lead in petrol - August 1975-April 1977
- Phase "2" - use of 100% Australian lead (Pb-206/Pb-207=1.04) as additive in the petrol - May 1977-June 1979
- Phase "3" - restoration of the initial isotopic conditions-July 1979-December 1979.

The analysis of the alkyl lead isotopic ratio in the refineries of the area studied, confirms that the distribution of Australian lead did follow the schedule previously given.

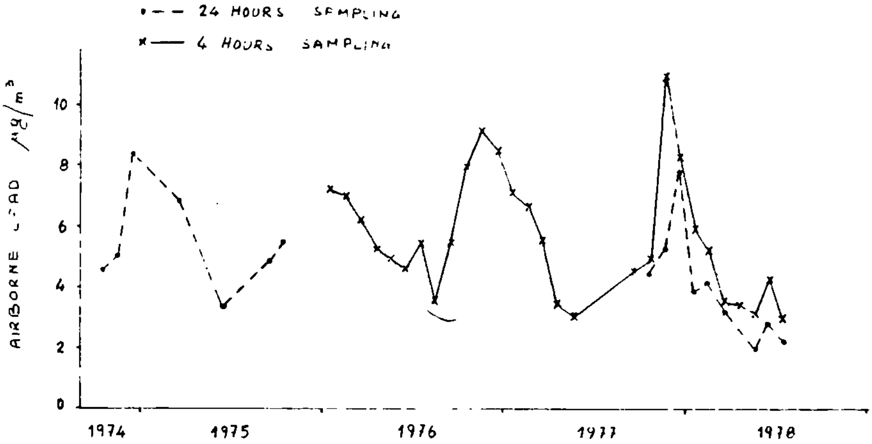
Results

The research work is being carried out in cooperation with the Ispra Establishment of the Commission's Joint Research Centre, which is dealing especially with isotopic ratio measurements. ASSORENI has been involved in the following activities :

- Monitoring of the petrol distribution in the area receiving isotopically labelled petrol.
- Periodic blood sampling in suitable populations and analysis of lead concentrations in blood.
- Sampling of atmospheric particulates in the city of Turin and analysis of lead concentrations.
- Collaboration with J.R.C. Ispra in obtaining and analysing soil, water and vegetation samples.

Isotopic ratio analyses performed by the J.R.C. Ispra, have not been completed yet, and the data so far obtained need further study and confirmation.

The results of measurements of lead content in airborne particulate samples, collected at 8 stations in the town of Turin, are summarized in the figure.



Conclusions

It is not yet possible to draw conclusions from the results of the study, since this report is to be integrated with the results obtained by the J.R.C. Ispra, especially as regards the isotopic composition values, which are fundamental to this study. The work carried out may be summarized as follows :

- since May 1977 almost all the petrol in the area studied and in the surrounding regions contained Australian lead ;
- the atmospheric lead exposure in the city of Turin has been determined ;
- successive blood samples have been taken from single subjects ;
- all the blood samples have been analysed for lead content ;
- for each subject enough information has been collected to allow a complete picture to be assembled from the isotopic composition and lead concentration data ;
- the analytical techniques employed for lead levels in blood and in atmospheric particulates have been subjected to periodic circuit tests.

Contractor : Association Euratom ITAL.

Contract n°: 185-77-1 ENV N

Project leader: P. Poelstra, M.J. Frissel, N. van der Klugt

Title of project: The contamination of vegetation, surface water and deep groundwater (drinking water) by heavy metals unintentionally released into soils.

Objective of the research:
Because of various activities of men heavy metals are released to the environment. These metals will accumulate in soils. Main problems are:

- Food crops may take up more heavy metals than desired (without decrease of actual yield)
- Production maybe hampered by the presence of heavy metals (of importance for food crops, ornamental plants, nature)
- Heavy metals may be released from the area where they were accumulated and thus contaminate surface water or deep ground water

The particular aim of this project is to study objects where significant quantities of heavy metals are released to soils, i.e. refuse dumps and applications of sewage sludge. Studies on sewage sludge have been concentrated on the presence of Cd within the sludge. Part of the project on sludge was carried out in cooperation with the Bundesforschungsanstalt für Landwirtschaft Braunschweig Völkenrode F.R.G.

The study on the refuse dumps is about half way many samples have been analysed, however, the data have not yet been interpreted. Therefore, on this activity is not reported. The study to the fate of Cd is partly rounded off, this report contains the main result of the latter study.

MATERIALS AND METHODS

Soils: Sandy soil Braunschweig. Slightly acid sandy soil used since 1895 for dumping sewage water with an average of 900 mm annually Total Cd content: top layer 3.5 mg Cd kg^{-1}, decreasing to 0.75 mg Cd kg^{-1} at a depth of 40 cm, below that almost constant.

Peat soil Schoonebeek: N.E. part of the Netherlands. Peaty soil used as permanent pasture. Total Cd concentration 1.4 mg kg^{-1}, decreasing to 0.2 mg kg^{-1} at 20 cm depth, and below this 0.2 mg kg^{-1} with a peak of 0.5 mg kg^{-1} at a depth of 90 cm.

Clay soil Valburg: Soil from the foreland of the Rhine river, used as permanent pasture. Total concentration at soil surface 6.5 mg kg^{-1} almost linearly decreasing to 1 mg kg^{-1} at a depth of 35 cm, and decreasing further to 0.5 mg kg^{-1} at a depth of 100 cm.

Adsorption measurements: 1 g air dry soil samples (with known moisture content) were dispersed in 10 ml 0.01 M $CaCl_2$ solution in plastic bottles; $CdCl_2$, labelled with ^{115m}Cd, was added via micropipettes.

Column experiments: Column experiments were carried out in perspex columns, internal diameter 120 mm, external diameter 132 mn, lenght 120 cm. To prepare a soil monolith a perspex column is pressed into the soil to a depth of 100 cm dug-out and transported to the laboratory.

To avoid water saturation of the soil the leaching solution was supplied as droplets, which were obtained by a rain simulator.
For the soil of Valburg and Schoonebeek a 0.005 N a leaching solution and for the Braunschweig soil a 0.0244 N solution was used.

If anaerobic conditions are required the soils are kept in a nitrogen atmosphere, the last traces of oxygen are removed by micro-organisms.

RESULTS

A typical result of the adsorption experiments is shown in Fig. 1: The isotherms after 24 h equilibrium time. For comparison with the column experiments use is made of the reciprocal values of the slope of the adsorption isotherm, the adsorption coefficient α:

$$\alpha = Cd_{sol}/Cd_{ads} \quad (\text{mg Cd ml}^{-1} \text{ soil solution/mg Cd g}^{-1} \text{ dry soil})$$

The results of the column experiments are shown in the Figs. 2-4, values of α are shown in the legends.
The calculations are based on a mathematical simulation model, which takes into account 25 layers in which both air-filled and moisture-filled pore volumes vary with depth. It is assumed that instantaneous quilibrium exists between solid and solution phase and between stable Cd and ^{115m}Cd; the adsorption isotherm varies with depth.

Variables as pore volumes and soil densities were measured at the end of the experiment, values for the waterflux were derived from daily observations. The values of the adsorption coefficients α were evaluated by eye fitting from calculated and observed values. Some ones may be disappointed by the rather poor fit of the curves, but it must be kept in mind that it was not tried to fit each individual curve (which allows an almost hundred percent fit), but to fit all curves obtained with a particular soil simultaneously.
Since undisturbed soil columns, each with small local differences were used, a hundred percent fit cannot be expected. The values are tabulated in table 1.

The results of the leaching experiments of the Cd contaminated soil from Braunschweig are shown in the Figs. 5 and 6. The calculated curves in Fig. 5 were based on the same assumptions as described for the Cd inflow curves. This means that also for Cd which was already adsorbed for decennia, instantaneous absorption was assumed. The adsorption coefficients, α, required to obtain the fit between calculated and observed curves are tabulated in table 1. For Fig. 6, no calculations were performed, this should require a rather precise estmate of the redox potential as a function of time and depth, and a determination of the adsorption coefficient as a function of redox potential and time. Apparently it lasted several weeks (the whole leaching experiment shown in Fig. 6 lasted 110 weeks) before the redox potential was so low that no Cd losses occurred.

CONCLUSIONS

Table 1 shows a comparison between the adsorption coefficients α, obtained after 15 min. equilibration time and the α's derived from column leaching experiments. It appears that for the upper 20 cm of the (aerobic) soil from Braunschweig good argreement between the two values of α exists.

The experiments with the anaerobic soils (Figs. 4 and 6) show irreversibility characteristics. Both ^{115m}Cd and stable Cd ions penetrate into the soil rather well; for the mathematical description even an α of 0.023 can be used, which is only slightly lower than under aerobic conditions. But, in contrast, the leaching of Cd ions under aneaerobic conditions is almost absent. From the E_H, pH diagram it must be expected that in the presence of sulfide (concentrations for dissolved CO_2 and S species 10^{-3}moles/l, pH 6.2) precipitation of CdS occurs below about -80mV. In our observations leaching of Cd ceased already at redox potentials below 100 mV. This may be explained by higher S concentrations in the soil than expected (S concentrations were not measured) and by lower redox potentials down in the column.

The adsorption of Cd-ions on the clay soil of Valburg is so high that the penetration of Cd into the soils is only a few cm, this makes an evaluation of α from the inflow pattern rather inaccurate. The inflow curves of the Figs. 2 and 3 are therefore calculated with a value of α of 0.0014, which was derived from the adsorption experiments.
The conclusion which can be drawn from Figs. 2 and 3 is that there is no indication for a difference between α in adsorption experiments and column experiments, but, as mentioned, small differences will not be visible.

For the peat soil of Schoonebeek there is finally good agreement between the value of α obtained from adsorption measurements after 24 h and column experiments.

One of the main conclusion of this study is therefore that adsorption/desorption experments, as they were developed, can be used for the calculation of the migration and accumulation of Cd in actual soils, indeed.

Table 1.

Values of the adsorption coefficient α in different soils.

soil	α derived from 15 min-adsorption measurements	α derived from column experiments	
		inflow of stable Cd and ^{115m}Cd	leaching of stable Cd
	g ml^{-1}	g ml^{-1}	g ml^{-1}
Sewage field Braunschweig			
aerobe 0-20 cm	0.030	0.030	0.030
anaerobe 0-20 cm		0.023	0*
Clay soil Valburg	0.0015	0.0014	
Peat soil Scoonebeek	0.044	0.0031	

*Strongly dependent on redox potential

Fig.1.

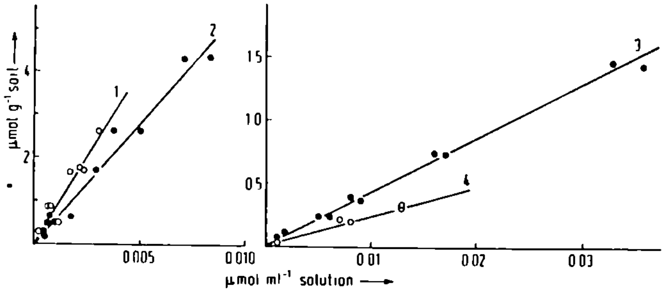


Fig.2.

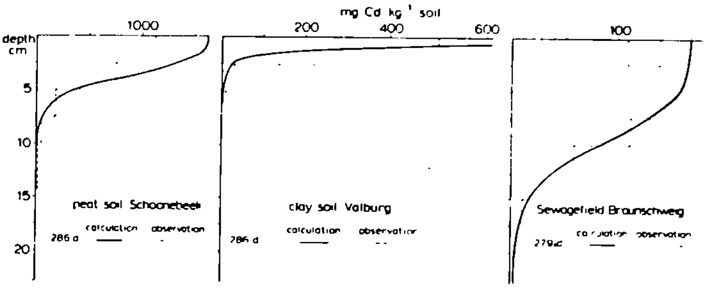


Fig.3.

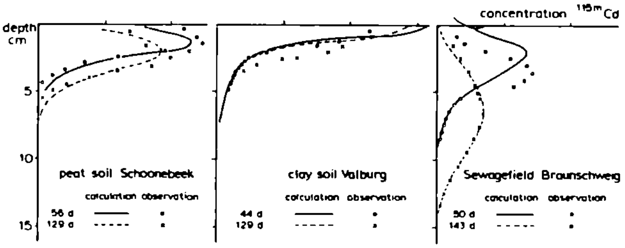


Fig. 1. Adsorption isotherms of Cd ions on soil. 1: Clay soil, Valburg, 2: Peat soil, Schoonebeek, 3: Sandy soil, layer 0-20 cm, Braunschweig, 4: Sandy soil, layer 30-40 cm, Braunschweig. Equilibration time 24 h.

Fig. 2. Calculated and observed distribution of Cd in undisturbed soil columns with time elapsed since the beginning of the application of Cd. Cd concentration of the influent: 5 mg l^{-1}. Further details are: Peat soil: influent rate approx. 1 cm d^{-1}, α = 0.0031; Clay soil: influent rate approx. 0.7 cm d^{-1}, α = 0.0014; Sewage field: influent rate approx. 2 cm d^{-1} during the first 146 d, thereafter approx. 0.4 cm d^{-1}, α = 0.030.

Fig. 3. Calculated and observed distribution of ^{115m}Cd in undisturbed soil columns. The ^{115m}Cd was added at the beginning of the application of stable Cd. Horizontal scale: 1 unit equals 0.02 mCi ^{115m}Cd per cm^3 bulk soil at time 0.

Fig.4.

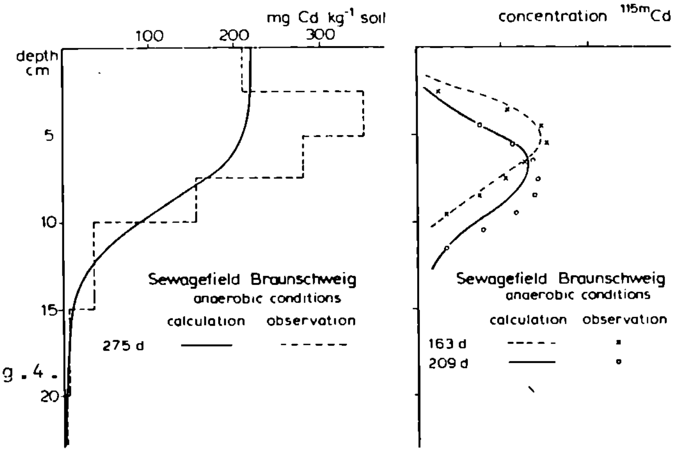


Fig.5.

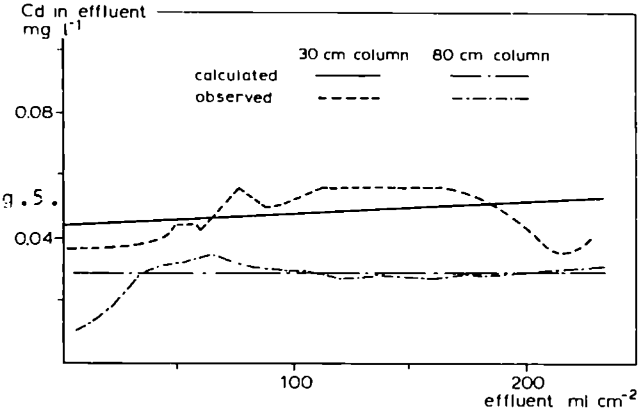


Fig.6.

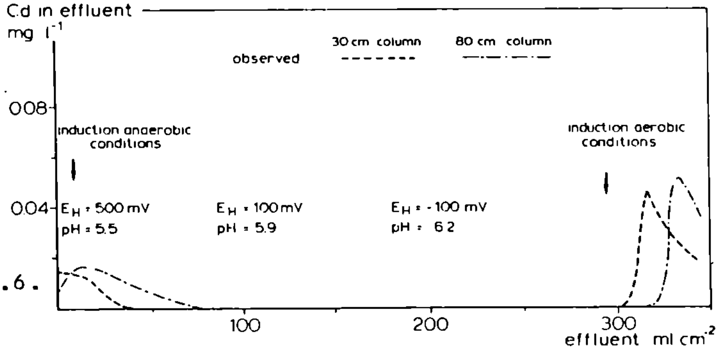


Fig. 4. Calculated and observed distribution of stable and radioactive Cd in undisturbed soil columns. For general information see Figs. 2 and 3. Specific details: Cd concentration in influent 5 mg Cd l^{-1}, influent rate approx. 2 cm d^{-1}, α = 0.023.

Fig. 5. The concentration of Cd in the effluent of soil columns, the observed values are means of two duplicate leaching experiments. 'Rain', supplied via a rain simulator, approx. 0.5 ml d^{-1}, influer Cd-free.

Fig. 6. Same as Fig. 5, but precautions were taken to induce anerobic condition in the column, these precautions were terminated when 300 ml cm^{-2} had passed the columns, this was after approx. 100 weeks.
Place of E_H and pH electrode 10 cm below the soil surface.

Publications and lectures.

Publication.

Poelstra, P., M.J. Frissel and N. El-Bassam. Transport and accumulation of Cd-ions in soils. Accepted for publication Z. Pfl. u. Bodenkunde.

Lectures.

M.J. Frissel: Transport en accumulatie van Cd in de bodem.
Werkgroep: Transport en Accumulatie processen in de bodem, Wageningen, Netherlands.

P. Poelstra: Transport en accumulation of heavy metals in soils.
E.C. Contractors meeting Metz, France.
P. Poelstra: Transport ans accumulation of Cd in soils.
ESNA meeting Brno, Czeckoslovakia.

M.J. Frissel: Behaviour of Cd in soils and its uptake by crops. Meeting on "Siedlungsabfallkomposte", Braunschweig - Völkenrode, F.R.G.

Contractor : The Foundation Institute for Soil Fertility, Haren

Contract N^{o} 199-77-1 ENV N

Project Leader : dr. W. Salomons

Title of the Project: "Dynamics of heavy metals in fluvial and limnic sediment-water systems".

Objective of the Research

A number of large lakes in Western Europe are fed by more or less contaminated rivers. Examples are Lake Constance, Lake of Geneva and Lake IJssel. Especially Lake IJssel (IJsselmeer) has become seriously contaminated with heavy metals. It is fed by a distributary of the river Rhine. In this lake a number of processes affect the distribution of the heavy metals over the dissolved and solid state (attached to the suspended matter or to the deposited sediment). Lake IJssel will be used as a model in order to describe the changes in occurrence of the heavy metals when a contaminated river flows into a lake.
Over a two year period samples will be taken from the surface waters, the suspended matter, the deposited sediments and from the interstitial water. In these samples the contents of mercury, lead, cadmium, copper, nickel, zinc and chromium will be determined. The pH variations in the lake, which determine to a great extent adsorption-desorption processes will be studied with the methods of stable isotope geochemistry. Laboratory experiments will be conducted on the adsorption behaviour of heavy metals onto the suspended matter.
Both the results from field and laboratory experiments will be used to construct a hydrodynamical-geochemical model, which simulates the behaviour of the dissolved metals in a lake.

Materials and Methods

A large variety of techniques is used in this combined field and laboratory study. For the sampling of surface waters specially constructed all-teflon made filtration apparatus is used. Suspended matter is sampled in teflon-lined continuous centrifuges. Heavy metals in the sediments, water and suspended matter are analysed with atomic absorption and with neutron activation analyses. Mass spectrometry is used in the stable isotope

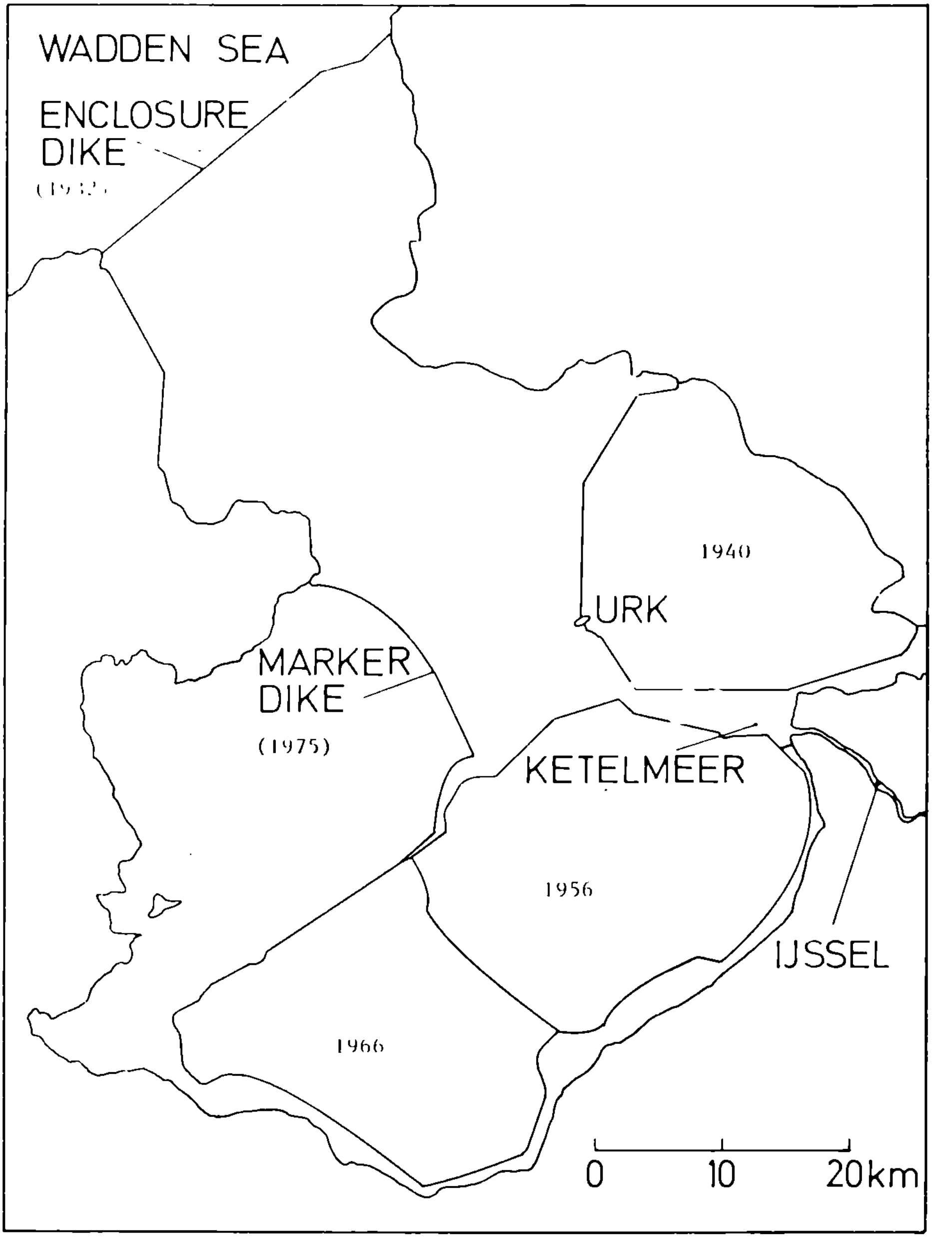


Figure 1. Lake IJssel (IJsselmeer)

studies. For the adsorption studies radiochemical techniques are applied.

Results

Lake IJssel came into existence in 1932 when the Zuiderzee was cut off from the North Sea. The lake surface area has gradually decreased as a consequence of reclamation projects (figure 1). The present surface area is 1320 km^2, including the Ketelmeer (the mouthing area of the river IJssel). The mean depth of the lake is 4.5 m, the volume of the water body is 5.5 km^3. The river IJssel is the main source of water. The residence time of the water is about 6 months. Water leaves the lake through two outlets in the Enclosure Dike.

Trace metals are transported both in solution and attached to the suspended matter to Ketalmeer and Lake IJssel. Table 1 gives the concentration of trace metals in the surface waters of the Ketelmeer.

	o-P	Zn	Cu	Cr	Cd	Ni	Mn
Dissolved	225	17	4.8	1.5	0.4	6.8	41
Particulate	177	59	7.9	17	1.1	2.5	55
% Dissolved	56	22	38	8	27	73	43

Table 1. Concentrations of trace metals and phosphorus (µg/l) in the Ketelmeer.

Phosphorus and nickel are transported mainly in solution, chromium is transported predominantly in particulate form.

The concentration of suspended matter in the river IJssel is about 40 mg/l; this amounts to an input of 400.000 tons of suspended matter each year. About half of the suspended matter and its associated particulate metals is deposited in the Ketelmeer. The remainder is spread out over the lake. Due to the shallowness (mean depth 4.5 m) frequent erosion of the deposited sediments takes place: winds having a force of 5-6 on the Beaufort scale can easily stir up the sediments. The recent contaminated sediments are mixed intensively with the older less contaminated sediments. As a consequence hardly any metal gradients in the lake sediments (with the exception of the Ketelmeer area) are found: the whole lake system can be described as two compartments (Ketelmeer and Lake IJssel). Concentrations of trace metals in the sediments (corrected for grain size differences) are presented in table 2.

	Zn	Cu	Cr	Pb	Cd	Ni	Hg	n
Ketelmeer 1977	1960	250	570	320	34	67	5	60
IJsselmeer 1977	430	40	94	73	2.8	30	0.8	85
IJsselmeer 1933	133	19	88	39	0.4	39	---	12

Table 2. Concentration (μg/g) of trace metals in sediments from the Ketelmeer and from Lake IJssel (Corrected for differences in grain size). n refers to the number of samples analysed.

Samples from the lake taken in 1933 have recently been analysed for trace metals. Large increases over the period 1933-1977 are observed for cadmium and zinc. The increases for lead, copper and chromium are smaller. Concentrations of trace metals in the pore waters of the sediments are presented in table 3. Hardly any differences are observed between the seriously polluted Ketelmeer area and the less polluted Lake IJssel: The concentrations in the sediments are not reflected in the pore waters.

	Zn	Cu	Cr	Pb	Cd	Ni	Hg
Ketelmeer	21	8	8	5	2	29	0.1
IJsselmeer	13	5	16	15	1.4	20	0.1

Table 3. Concentrations (μg/l) in pore waters from the Ketelmeer and Lake IJssel.

Compared with surface waters, the concentrations of nickel, lead and chromium are high in the pore waters; concentrations of zinc in pore waters are lower (compare table 3 with table 1).

Large differences in dissolved trace metals concentrations are observed between the Ketelmeer area and the Northern part of the lake. Apparently, trace metals are lost from solution on their way from the river to the outlets in the Enclosure Dike. Table 4 gives the results of a balance study.

	Zn	Cu	Cd	Cr	P
Metals transported into the lake in solution	344	47	6.8	20	2500
Metals leaving the lake through the outlets in the Enclosure Dike	56	35	2.6	3.9	923

Table 4. Balance study for dissolved trace metals and phosphorus in lake IJssel (tons/yr).

Two processes may account for the accumulation of dissolved trace metals in the lake: biological processes (uptake by algae) and abiological processes (adsorption and/or precipitation).

The concentrations of trace metals in the algae were determined by analysis of algal material. Concentrations for Zn, Cu, Cd and Cr are 200, 25, 1.5 and 0 µg/g, respectively. Based on an accumulation of 160.000 tons of algal material in the lake, the amounts of trace metals accumulated in algal material are 32, 4, 0.24 and 0 tons. Only for copper the accumulation by algae is important: about 30%. The influence of algal production on the accumulation of the other trace metals is less than 10%.

The concentrations of dissolved trace metals (zinc, cadmium and chromium and to a lesser extent nickel) decrease in the direction of the Enclosure Dike. The decrease in the concentrations correlates with the pH of the surface waters, which increases from 7.3 (river IJssel) to a value of 9.0 (Enclosure Dike). Apparently pH-dependant adsorption processes are taking place. These processes have additionally been studied in the laboratory. Some results are given in table 5. Over the pH range observed in the IJsselmeer the adsorption of cadmium, zinc and nickel depends on the pH to a high extent.

	% in solution of added metal		
pH	Cd	Zn	Ni
6.0	90	90	100
7.0	70	65	90
8.0	50	35	80
9.0	30	5	65

Table 5. The adsorption of trace metal onto suspended matter in laboratory experiments.

pH variations in Lake IJssel, extremely important for the modelling of trace metals, are induced by reaction occuring within the carbon cycle of the lake. These reactions (e.g. the carbon dioxide loss to the atmosphere, carbon uptake by algae, carbonate precipitation and the release of bicarbonate from pore waters) have been studied with both conventional chemical techniques and with stable isotope geochemistry. In summer the influence of algae on the carbon cycle is predominant; in the northern part of the lake the ^{13}C concentration in the dissolved bicarbonate increases continuously from -7 ‰ (February) to more than 0 ‰ (October) (all concentrations are given as relative deviations (in ‰) from that of a standard). Erosion of sediments with subsequent release of pore waters takes place in the stormy winter period. This pore water has a high bicarbonate content and low concentrations of ^{13}C (about - 14‰), moderately high trace metal concentrations and high phosphorus concentrations. The decrease in the ^{13}C concentrations from 0‰ (October) to -7‰ (February) is caused mainly by the release of pore water.

Conclusions and additional comments

The field studies have resulted into a clear insight into the main processes which affect the concentrations of dissolved trace metals in Lake IJssel. The main processes are the uptake by algae (for copper) and the pH dependant adsorption processes for the other trace metals which accumulate in the lake. The adsorption processes are also determined by the presence of complexing agents in the water (NTA and chloride) which cause a smaller adsorption. The time series obtained over the two year period together with the laboratory experiments, which will be continued in 1979/1980, will be the basis for the modelling of the geochemical-hydrodynamic processes affecting the dissolved trace metals in Lake IJssel.

Publications and oral communications

W. Salomons (1978). Processes affecting trace metals in Lake IJssel. Contribution to the symposium "The effect of human activities on the ecology and sedimentation of lakes and reservoirs". 10th International Congress on Sedimentology. Israël 1978.

W. Salomons (1978). Phisical and chemical processes affecting metals in Lake IJssel Meeting of the Freshwater Contact Group. Metz France.

W. Salomons and W.G. Mook (1978). Physical and chemical processes affecting metal concentrations in lake sediments. Submitted to Enviromental Geology.

Contractor:	Institute for Soil Fertility, Haren, NL
Contract n°:	274-78-1 ENV N
Project leader:	R.G.Gerritse
Title of project:	Biogeochemical aspects of organic heavy metal complexes

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1. Objective of the research

The objective is to gain more insight into the factors affecting the mobility of heavy metals in soil systems especially in relation to the migration of heavy metals as organic heavy metal complexes in sludge amended or treated soils. Relevant effects of or on microbiological and biochemical processes in the soil will also be studied.

2. Methods.

Due to the very low solution concentrations of heavy metals in the environments studied (often less than 1 µg/liter) direct methods (carbon rod AAS and differential pulse polarography) in combination with preconcentration techniques are difficult to use in evaluating adsorption under conditions usually encountered in practice.

A relative method, using radioactive isotopes of the metals, has proved quite useful in establishing the extent of adsorption in soil systems and the effect changes in the matrix (pH, Eh, ionic strength and composition) have on adsorption of metals. Fractionation of the labelled metals in soil or sludge solutions by gel permeation chromatography has provided some information on the nature of speciation.

Two types of soil were used, one an acid sandy top soil and the other a slightly alkaline sandy loam top soil. The adsorption was measured after shaking 5 g soil for 2-3 days with 25 ml solution containing the metal(s) under investigation. Either solutions of Ca, Na and K chlorides at various ionic strengths or the liquid phases of various sewage sludges were used to equilibrate the soil with. After 2-3 days the soil/solution was centrifuged for one hour at 40,000 g. The distribution constant of the metals is then calculated from the total radioactivity in the soil/solution system and the radioactivity remaining in solution after centrifugation.

Adsorption isotherms have been obtained in this way for Pb, Cd, Zn and V in a concentration range of 0-100 ppb. Similar work will be done with As, Cr, Co, Hg, Sn, Ag, Ni, Sb and possibly other metals. Cu presents a problem as its available radioactive isotope has a very short half-life time.

3. Results

In tables 1-4 distribution constants are given for Pb, Cd, Zn and V for the various soil/solution systems studied. The metals were added in solutions containing respectively Pb^{2+}, Cd^{2+}, Zn^{2+} and VO^{2+} ions. The distribution constants (K_o) are given as averages for the concentration range of 0.01-1 µg/liter above the background concentrations of the metals in the soil/solution. For the metals mentioned the adsorption isotherm is fairly linear in this range. The distribution constant is defined as the ratio between the amount of metal adsorbed per unit weight of soil (µg/g) and the concentration of that metal in the soil solution (µg/ml). The units of K_o are thus ml/g.

In table 5 the cationic compositions of the solutions from which Pb, Cd, Zn and V were adsorbed are given. In the sludge solution of course part of Ca and Mg can be complexed by dissolved organic matter.

From the speciation studies by gelchromatography of sewage sludge solutions it was found that of Pb, Zn and Cd more than 95% and of V between 25 and 50% was present as complexed species different from those present in purely inorganic solutions. 0.1-1% of Zn and Cd, 1-5% of V and more than 50% of Pb was present in species of high molecular weight.

4. Discussion

From the adsorption data it can be concluded that Pb is generally adsorbed much stronger than Cd, Zn and V and that Cd and Zn are fairly similar in their adsorption characteristics. Only Pb and V adsorption in soils appears to be decreased by the organic matrix of the sludge solutions (tables 2-3) when compared with data for purely inorganic solutions (table 1) at comparable ionic strengths and pH. The adsorption of Pb, Cd and Zn in soils is decreased with increasing ionic strength and decreasing pH, while that of V is increased. This opposite behaviour of V can also be gathered from data given by Tyler for a purely organic spruce forest top soil. Distribution constants calculated from his data are given in table 6.

The distribution constant (K_o) as measured here after an equilibration time of 3 days applies to the exchangeable metal fraction.

If this fraction is known the corresponding solution concentration of the metal is found from:

$$C_m = \frac{C_s}{K_o} \qquad (1)$$

where: C_m = solution concentration

C_s = exchangeable metal content of soil.

The data given in tables 1-4 can be used to estimate the potential mobilities of Pb, Cd, Zn and V in the environment by applying the following equation:

$$V_{rel} = \frac{1}{1 + \omega \cdot K_o} \cdot 100\% \qquad (2)$$

in which: V_{rel} = the mobility of the metal relative to that of water in the soil;

ω = the phase ratio of soil and soil solution in a unit of soil.

The distribution constant (K_o) is a measure for average distribution of the various possible metal species:

$$K_o = \sum_i X_i \, K_o^i \qquad (3)$$

in which: X_i = molar fraction in solution of species i

K_o^i = related distribution constant.

The mobility of a metal is given by equation 1, provided the various species are in mutual equilibrium. Otherwise each species should be treated separately with equation 1 using the proper K_o^i.

In fig.1 equation 2 is represented graphically for the situation of 350 mm annual surplus precipitation and 30% moisture ($\omega \sim 4$). Migration of Cd and Zn in the sandy soil can under unfavourable conditions, be as high as a few cm per year. Migration of Pb is low under all circumstances (a few mm's per year at the most) in both soils studied.

In the loam soil the mobilities of Pb, Cd, Zn and V are very low under all conditions studied.

Oral communication:

title: Heavy metal speciation in sludge. Possible effects on the mobility of heavy metals in sludge amended soils.

Presented in the Freshwater Contact Group meeting at Metz 18-20 October 1978.

Table 1

Distribution constants for Pb, Cd and Zn in soil/water systems. 5 g soil was shaken with 25 ml water containing the amount of salts indicated, Soil A is an acid soil (pH-KCl = 5.5, 4% organic matter). Soil B is a slightly alkaline sandy loam (pH-KCl = 7, 2-3% organic matter, 20% clay). **The C.E.C's are 21.6 and 16.0 meq/100 g soil resp.** The distribution constants are given for „zero" solution concentration of the metal (K_0 for $C \rightarrow 0.01$ µg/l)

phase system	pH	K_o^{Pb}	K_o^{Cd}	K_o^{Zn}	K_o^{V}
water + soil A	5.65	1400	500	300	70
water + soil B	8.0	5300	3800	3300	350
0.005 N Ca+Na+K chlorides (3:1:1)					
+ soil A	4.9	500	30	20	180
+ soil B	7.8	5000	1200	1800	330
0.05 N Ca+Na+K chlorides (3:1:1)					
+ soil A	4.35	350	4	7	250
+ soil B	7.6	3400	500	1200	600

Table 2

Distribution constants of Pb, Cd and Zn in soil/sludge-solution* systems 5 g soil was shaken with 25 ml solution. K in ml/g.

phase system	pH'	K_o^{Pb}	K_o^{Cd}	K_o^{Zn}	K_o^{V}
sludge solution 1					
+ soil A (21A)	5.35	300	38	26	15
+ soil B (21B)	7.1	1000	650	2600	60
sludge solution 2					
+ soil A (22A)	5.1	120	8	5	**22**
+ soil B (22B)	7.25	400	450	1000	**120**
sludge solution 3					
+ soil A (23A)	5.5	150	30	11	45
+ soil B (23B)	7.6	1100	2000	3000	110

* soils as in table 1

Sludge solution 1 was obtained from industrial anaerobic sewage sludge after centrifugation at 40,000 g for 1 h.

Sludge solution 2 was obtained from the same sludge after aeration for 2 months.

Sludge solution 3 was obtained from the solids remaining after centrifugating the aerated sludge, making it up to the same dry weight % as before, and aerating another month.

Table 3

Distribution constants of Pb, Cd and Zn in soil/sludge systems. 5 g soil was shaken with 25 ml solution. Conditions as in table 2 except that domestically derived sewage sludge was used instead of industrially derived sewage sludge.

phase system	pH	K_o^{Pb}	K_o^{Cd}	K_o^{Zn}	K_o^{V}
sludge solution 1					
+ soil A (31A)	5.8	500	50	35	26
+ soil B (31B)	7.3	500	100	350	75
sludge solution 2					
+ soil A (32A)	5.1	90	8	5	15
+ soil B (32B)	7.1	300	230	400	50
sludge solution 3					
+ soil A (33A)	4.9	600	17	9	40
+ soil A (33B)	7.2	1000	900	1900	75

Table 4

Distribution constants and concentrations of Pb, Cd and Zn in sewage sludges, treated as explained in table 2 and 3.

sludge solution	21 A/B	22 A/B	23 A/B	31 A/B	32 A/B	33 A/B
D.M. (%)	1.95	1.8	1.1	5.3	3.9	2.4
pH	7.7	6.4	5.2	7.6	5.8	5.65
org.N (ppm)	100	40	7	40	23	2
$N(NH_4^+)$(ppm)	850	156	4	915	96	2
$N(NO_3^-)$(ppm)	0	13	43	0	255	111
$N(NO_2^-)$(ppm)	0	82	0	0	15	0
K^{Pb} (l/g)	103	69	245	63	52	98
K^{Cd} (l/g)	73	1.6	4.4	47	1.2	3.5
K^{Zn} (l/g)	57	2.4	2.2	38	1.8	4.2
K_m^{V} (l/g)	0.5	0.4	-	1.1	0.5	-
C_m^{Pb} (µg/l)	3.5	5	1.5	11.3	14	7
C_m^{Cd} (µg/l)	1	50	18	0.3	11	4
C_m^{Zn} (µg/l)	25	650	600	70	1500	600
C_m^{V} (µg/l)	60	75	-	30	60	-

Table 5

Cationic (Ca, Mg, Na, K, NH_4) composition of the solutions used in the adsorption experiments of tables 1-3 (numbers refer to systems in tables 1-3), in meq/liter.

	10 A/B	11 A/B	12 A/B	21 A/B	22 A/B	23 A/B	31 A/B	32 A/B	33 A/B
NH_4^+	0	0	0	60	11	0.23	65	7	0.12
Ca^{2+}	0	3	30	5	16	5	5.2	30	12.8
Mg^{2+}	0	0	0	0.6	3.2	0.8	0.7	6	1.4
Na^+	0	1	10	4.8	5.2	0.6	8	7.3	1.1
K^+	0	1	10	2.1	2.3	0.3	2	2	0.44
Σ	0	5	50	72.5	37.7	6.9	87.8	52.3	15.9
$\frac{Ca+Mg}{Na+K+NH_4}$:	-	1.5	1.5	0.08	1.04	5.1	0.08	2.2	8.5

Table 6

Average distribution constants calculated for an organic spruce forest top soil from data of G.Tyler*

	K in unpolluted soil pH = 4.2 ionic strength 0.35 meq/l		K in polluted soil pH = 6.2 ionic strength 0.25 meq/l	
Pb	5700	(ml/g)	52000	(ml/g)
Cd	380		1350	
Zn	490		700	
V	1190		310	
Mn	280		3000	
Ni	280		1100	
Cu	1000		7400	
Cr	1400		8300	

* G.Tyler. Leaching rates of heavy metal ions in forest soil. Water Air and Soil Pollution (1978) 137-148

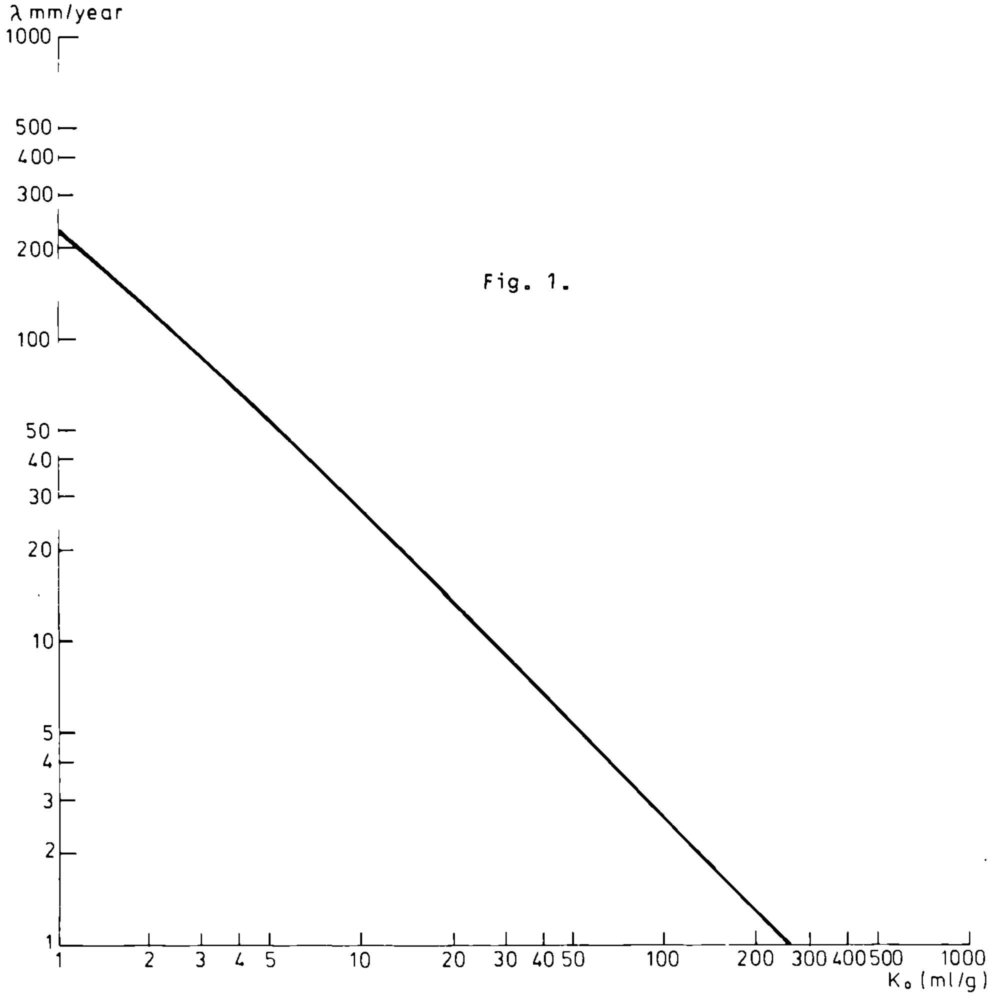
λ mm/year
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
1
2
3
4
5
10
20
30
40
50
100
200
300
400
500
1000
K_0 (ml/g)

Fig. 1.

Université Libre de Bruxelles
Service de Géologie et Géochimie Nucléaires
Faculté des Sciences

Number of contract : 134-76-7 ENVB
Project leader : E. PICCIOTTO (Senior scientist : D. PETIT)
Title of project : Continuation of the studies on isotopic composition of lead present in the Belgian environment. Cycle and absorption by the human body of lead emitted by automobiles.

In our preceeding study (contract n° 039-74-1 ENVB), we have been able to demonstrate, at least in Belgium during the period 1974-1977, that the isotopic composition of lead emitted by automobiles is very characteristic. The annexed figure, sums up the results obtained. In order to clarify matters, only the ratio Pb-206/Pb-207 is represented. One sees that lead produced by the automotive exhausts differ clearly from lead coming from natural sources of from other anthropogenic sources present in the environment. This is due to the fact that in our country, lead alkyls added to gasoline comes principally from Canadian and Australian ores of pre-Cambrian ages and thus of very low radiogenicity. This results have allowed us to evaluated the contribution of the lead coming from automobile traffic in some parts of the Belgian environment : atmosphere and precipitations, lakes, rivers and sea. We have been able to establish that the near totality of lead present in this parts originated from the automotive exhausts.

This present study has been applied to the estimation of the "automobile lead" contribution to the human body of the Belgian population.

Towards this aim, the isotopic composition of lead has been analyzed in blood of about ten subjects not professionally exposed, during the period 1976-1978. Those once forming two groups :

a) a group of six male adults submitted to lead coming from "normal sources" in urban (Brussels) and in rural (Vielsalm) environments (average blood lead concentration : 15 µg/100 cc);

b) a group of three persons submitted to non professional expositions by additionnal lead sources (average blood lead concentration : 40 µg/100 cc).
In this occurence, two children living in the vicinity of the foundry of Hoboken, and one rural worker using drinking water distributed by lead pipes.

The isotopic composition of lead have been measured by mass spectrometry at the "Service de Géochimie et Cosmochimie" of the University of Paris VII on a THOMSON-HOUSTON TSN 206 mass spectrometer and at the University of Brussels on a VARIAN MAT TH5 mass spectrometer belonging to the "Centre Belge de Géochronologie". The isotopic ratios have been corrected in order to take into account the isotopic fractionation (∽ 1 ± 0,5 ‰ by mass unit) and the contamination originating from the chemical analysis of the samples (this correction introduced an uncertainty of an order of magnitude lower than the isotopic analyses itself). The accuracy of an individual isotopic ratio is comprised between 10^{-4} and 10^{-3}.

The isotopic composition of lead in blood of the first group show remarquable constancy in time as well as in space (see figure). Its mean value is a little higher than the mean isotopic composition of lead coming from automobile traffic; the contribution of "automobile lead" in blood is calculated from a simplified mixed model. If the reasonable hypothesis is made that the lead present in blood comes from two sources : gasoline and industry, which the mean ratio Pb-206/Pb-207 are respectively: 1.12 and 1.18, this contribution can be estimated between 50 and 60 %.

The Pb-206/Pb-207 ratio as well as the blood lead concentration is higher in blood of the second group individuals (abnormal subjects) than in this of "normal subjects" (see figure). This is explained by a larger contributing in the first group of "industrial lead", more radiogenic. If one applies again a two sources model, one can deduce that between 40 and 60 % of the lead blood of the second group is due to its additional exposure. According to the same two sources model, their blood would countain the same amount of "automobile lead", but its relative proportion would be reduced to 20-30 %.

One must keep in mind that all the "automobile lead" wich appear in the blood did not necessarily enter in the body by inhalation. Measurements carried out on the feaces and the blood of a same subject show that the isotopic ratios of lead in feaces, wich contained about 90 % of ingested lead, and in blood are very similar (see figure). This is taken as an indication that a large proportion of "automobile lead" penetrate by ingestion through the contamination of the whole food chain.

Information obtained in this present study as well as by other authors seem to show that the particular situation met in Belgium can probably applied to other European countries (England, Spain, France, Netherlands and Switzerland). A few isotopic measurements made by our laboratory, by FACCHETTI et al. (1972) and by CHOW et al. (1975) on samples wich can be considered as representative of automotive exhausts (gasoline, atmospheric aerosols, soils and plants exposed to intense automobile traffic), correspond indeed to low radiogenic leads, very near of Belgian "automobile lead" (see figure). Moreover, direct measurements have been carried out at the University of Paris VII on two adults living in Paris.

The isotopic ratios of this leads are very similar to those of the Belgian subjects (see figure). It is likely that this present approach may be extended to a great part of Western Europe. It seems that England would be particularly well suited for such study in view of the very low Pb-206/Pb-207 ratio measured by FACCHETTI et al. (1972). On the other hand, similar studies by MANTON (1973, 1977) show that the situation is unfavourable in the United-States. This can be explained by the higher radiogenicity and the great variability of the lead ores used for manufacturing anti-knock additives added in the local gasolines.

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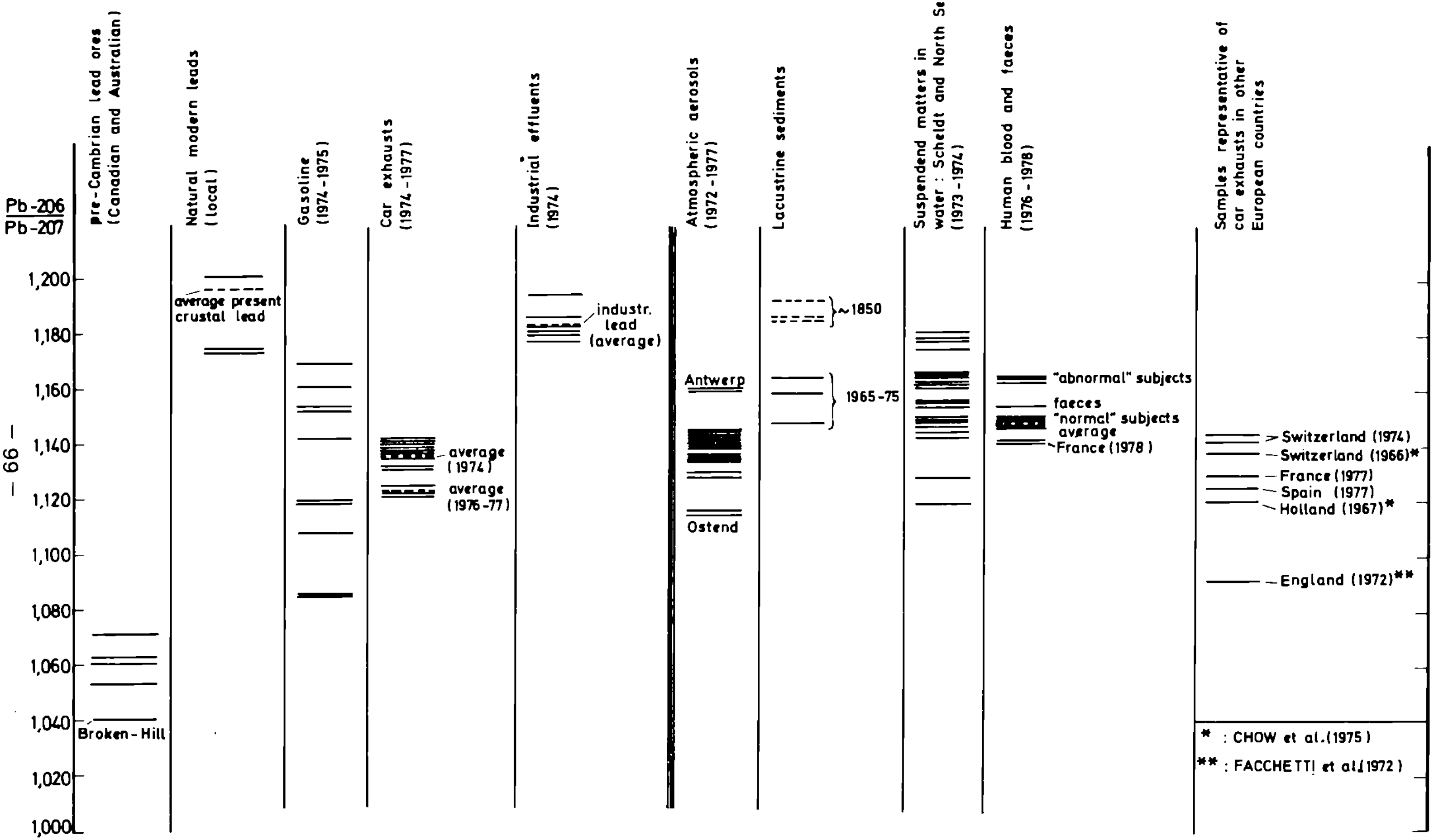

Pb-206/Pb-207 isotope ratio in various samples from Belgium and other European countries.

Contractor : State University of Ghent

Contract n° : 158-77-1 ENVB

Project Leader : Prof. Dr. Ir. A. COTTENIE

Titel of project : Experimental and theoretical study of Distribution Patterns for mineral elements between solid and liquid phases in soils and surface waters

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1. OBJECTIVE OF THE RESEARCH

Fundamental and practical study of the sediment-water distribution of heavy metals and their transport into water (1.1.2.), with regard to the reduction of water pollution (3.1.) and to the consequences of waste disposal for the environment (3.2.).

Physico-chemical parameters, pH and organic matter, govern the release of contaminants from the solid phase to the liquid phase in sediment-river water and soil-water systems. These factors, regulating the mobilization of chemical constituents, determine the environmental impact of dredged material or waste disposal on soil and groundwater. Indeed, total quantities of e.g. heavy metals in soils and sediments are only indicative for potential contamination. This research program is carried out to study the occurrence of mineral elements and heavy metals in river sediments, soils and sewage sludges, as well as the influence of the pH on environmentally compatible disposal alternatives.

2. MATERIALS AND METHODS

Heavy metals in soils, sediments and sludges are analytically separated in free (water soluble), adsorbed, complexed and precipitated fractions. At the same time an analytical procedure for determining mobile and mobilizable amounts is used.

total quantity : -wet destruction with aqua regia or extraction with 5.0 n HNO_3 for a good approximation (0.5 n HNO_3 for normal soils)
free metals : -extracting solution : water
adsorbed metals: -extracting solution : 1 n NH_4Ac (pH 7) gives the fraction which easily can be desorbed
complexed metals : - extracting solution : 0.2 n NaOH (pH 12)

precipitated metals : - substraction of free + adsorbed + complexed fractions from total quantity
metal mobility : - determination of concentrations in the liquid phase after equilibration in water and after stepwise acidification with HNO_3.

Soluble organometals and free metals were separated by ultrafiltration (on Millipore MW 1000 and MW 100000).

3. RESULTS

3.1. Heavy metal content of river sediments, sewage sludges and soils in industrial sites, urban areas, rural regions and adjacent to highways : field observations

Results for urban soils show that unfavourable growth circumstances were rather the result of high total salt content (conductivity), deficient quantities of organic matter or high sulfide concentrations indicating a reduced state, than of heavy metals even though some of the latter can be present in enhanced concentrations.

Contamination along older highways is more pronounced than near new ones. Remarkable enrichment with lead sharply decreases with distance from the road. It is due to the small uptake of lead from the soil that no phytotoxicity is observed. Utilization of highway grass as animal feed however may lead to unacceptable intake of lead, mainly due to external contamination.

Concentrations of heavy metals in sewage sludge are mostly high but depend to a large degree on sampling place as well as on sampling date.

The great variation of heavy metal contents of sewage sludges from different purification stations is the result of discharges of different types of wastewaters (e.g. tannery-Cr into the sewerage). Fe and Zn are frequently present in very high concentrations and Cu, Mn and Pb contents are between 100 and 1000 ppm. Cr concentrations are oftenly 10 to 100 ppm with some samples to about 1000 ; Co contents are less than 100 ppm. Half of the analyses showed Cd contents of 1 to 10 ppm and one fourth of 10 to 100 ppm. Metal contents of river sediments are smaller as it is also mentioned in literature.

3.2. Distribution of heavy metals between different fractions : chemical fractionation

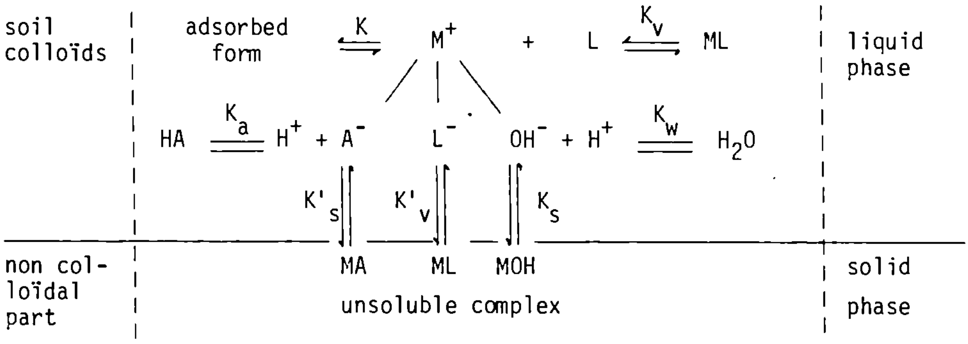
3.2.1. Distribution in soils, sewage sludges and river sediments

A general conclusion is that for all investigated samples a considerable part of the total quantity is precipitated and can only be solubilized in strong acid medium (table 1). Most metals are also distributed over two or more important fractions (table 2). Both remarks are of particular interest with regard to the control of metal availability for plants or transport from the solid to the liquid phase.

Controlling techniques can only be efficient when they affect the total mobile quantities of metals and not only one or some fractions. In fact also the mobilizable metals have to be considered. That are metals which can be suspected to become mobile in future as the result of changing conditions. As illustrated in figure 1, even small pH variations cause important changes of the mobilizable fraction. Also the content and the type of organic matter can be significant.

Low metal concentrations in solution theoretically can be maintained for a long time when they are in an immobile form. Controlling techniques can of course not deplete the total quantity of metals but can only act upon the latter possibility.

Fig. 1. Distribution of metals in soil : equilibrium status



3.2.2. Heavy metals in the liquid phase

Release of heavy metals in the free solution can occur as a consequence of changing conditions (pH, salt content, formation of mineral or organometal soluble complexes), which means that bank- and bottom sediments (precipitated suspended solids) are to be considered as a potential supply.

Table 1. Mobility of heavy metals in a mixed sample of river sediments (mg/kg dry matter)

pH	Zn	Cu	Pb	Ni	Cd	Co	Cr	Fe	Mn
12.80	110	4.4	2.8	1.8	0.2	2.8	1.5	17	0.7
12.60	150	6.5	7.2	2.6	0.4	1.7	1.9	17	1.1
11.80	3.2	2.3	0.7	0.3	0.0	0.0	1.0	17	0.4
11.10	1.6	1.3	0.4	0.2	0.0	0.0	0.2	17	0.4
9.75	1.1	0.5	0.3	0.1	0.0	0.0	0.1	9.9	0.2
8.45	0.5	0.2	0.2	0.0	0.0	0.0	0.0	3.3	0.2
7.80	0.5	0.1	0.1	0.1	0.0	0.0	0.0	<3.3	0.5
7.50*	0.5	0.0	0.0	0.1	0.0	0.0	0.0	<3.3	0.7
7.30	1.6	0.1	0.1	0.1	0.0	0.0	0.0	<3.3	2.2
6.75	11	0.1	0.3	0.1	0.1	0.0	0.0	<3.3	8.6
6.70	30	0.1	0.3	0.4	0.1	0.4	0.0	<3.3	15
4.45	150	0.2	0.8	3.4	0.3	0.9	0.0	26	49
4.10	188	0.3	0.8	2.7	0.3	1.2	0.1	50	58
3.15	325	0.4	2.7	4.6	0.5	1.9	1.1	376	103
2.30	400	0.8	12	4.7	0.8	2.1	2.4	545	109
0.50	525	14	91	6.3	2.3	3.7	28	3300	180

(*) pH mixed sample

Table 2. Total, water soluble, adsorbed, complexed and precipitated metals in a contaminated soil, urban soils and river sediments. Results are expressed on dry matter basis

	Fe	Cu	Zn	Mn	Ni	Cd	Co	Pb	Cr
	contaminated soil (native metals)								
0.5 n HNO_3 extractable	1460	29	79	29	1.4	16	0.7	238	1.1
water soluble	5.8	0	5.5	1.7	0	0.5	0	0.5	0
adsorbed	4.4	0.3	25	6.7	0	9.1	0	48	0.5
complexed	613	23	21	2.0	0.9	2.6	0.1	38	0.5
precipitated	837	5.7	28	19	0.5	3.8	0.6	152	0.1
	urban soil (mean of 20 samples)								
0.5 n HNO_3 extractable	1547	30	124	279	4.0	1.2	2.8	124	5.1
water soluble	22	0.2	0.5	0.3	0.02	0	0	1.4	0.1
adsorbed	0	0.9	2.9	3.9	0	0.2	0.1	1.4	0
	river sediments (mean of 16 samples)								
0.5 n HNO_3 extractable	4610	34	509	205	9.9	2.6	3.8	88	41
water soluble	4.6	0.03	7.6	3.7	0.2	0	0.1	0	0.1
adsorbed	0	0.2	24	30	0.3	0.2	0.1	1.2	5.0
complexed	143	10	22	3.3	1.2	0.1	0.2	2.7	19
precipitated	4462	24	455	168	8.2	2.3	3.4	84	17

The sediment-water interphase where exchange reactions occur is a few cm deep. This is the layer where water organisms spend whole or part of their life so that they are exposed to metal concentrations higher than those at the water surface.

3.3. Changes of this distribution as caused by variations of several parameters

3.3.1. Influence of pH on the mobility of heavy metals

The employed analytical technique for determining the influence of pH on mobile quantities of metals (table 1), permits to draw the following conclusions :

- although all investigated samples showed high total metal contents their mobile fractions were low because the main part is precipitated at the naturally occurring pH values
- the mobile metals are easily mobilized at a more acid pH even small pH decreases had a great impact on metal mobility
- from pH values higher than 7 there is a remarkable increase of mobility and this phenomenon was noticed for all metals at higher pH
- the increase of mobile fractions under alkaline conditions in highway soil, river sediment as well as sewage sludge started for each of the samples at a different pH value
- the enhanced metal solubilities in alkaline medium is the result of solubilization of organo-metal complexes. Each organo-metal complex has a specific solubility as a function of pH and the role of organic matter in maintaining small mobile metal concentrations under alkaline conditions will be topic of b.2.2.

3.3.2. Role of organic matter in maintaining small mobile metal concentrations under alcaline conditions

Ultrafiltration experiments gave following information :

- at pH of about 8 there was a great difference between the metal concentrations in the mobile fractions before and after membrane filtration. This means that under slightly alkaline conditions a great part of the mobile fractions consisted of organo-metal complexes
- at pH of about three the contribution of organo-metal complexes is very small which was shown by the comparable concentrations in membrane filtered and non filtered fractions
- there was only a little difference between the fractions with molecular

weight smaller than 100.000 and 1.000 respectively, which permits to conclude that most of organo-metal complexes had a molecular weight of more than 100.000

- it will be of interest to study such fractionations on more alkaline mobile fractions because of their greater concentrations.

4. GENERAL CONCLUSIONS

The study of the distribution of heavy metals between the solid phase and the liquid phase permits to formulate the following conclusions :

1. The phenomenon of release of heavy metals from solid phase to liquid phase is not in the first instance due to occurrence of high total quantities in the system but of unfavourable circumstances.
2. pH and redox-potential are the most important parameters with regard to mobility of heavy metals in solid/liquid systems.
3. Mobilizable fractions mainly consist of inorganic precipitates (see mobility experiments).
4. Release of metals from solid phase to liquid phase can largely be controlled by an appropriate pH choice.
5. In some cases optimum pH values for immobilizing inorganic metals don't prevent some metals to remain in solution as organo-metal complexes.
6. The solubility of organo-metal complexes as a function of pH is characteristic for each element.
7. Most organo-metal complexes only became soluble under slightly alkaline conditions. This is the main reason for the commonly small release of heavy metals from the solid phase to the liquid phase ; most soil and river sediments being neutral to slightly acid.
8. Some organo-metal complexes are already slightly soluble at about neutral pH and their solubility increases sharply under alkaline conditions so that in certain cases these metals may cause problems.

Publication

COTTENIE A. & DHAESE A.
Content and activity of heavy metals in soils, sediments and water
C.E.C. Freshwater Contact Group, Meeting of Metz : 18-20th October 1978.

Contractor : Université de Liège Belgique
Contract n° 160-77-1-ENV B
Project leader : J. REMACLE, Département de Botanique, Sart Tilman
B-4000 Liège
Title of project : The influence of cadmium on the activities of saprophytic bacterial populations in freshwaters.

OBJECTIVE OF THE RESEARCH

The influence of cadmium upon the saprophytic bacterial communities in freshwaters was analyzed from the following points of view :

- Taxonomical and physiological characteristics of bacteria colonizing aquatic systems contaminated with heavy metals and specially with cadmium.
- Behaviour of bacterial communities contaminated with cadmium in order to elaborate biological models.
- Genetical analysis of wild cadmium resistant strains.

MATERIALS AND METHODS

Bacterial strains were isolated in three aquatic systems : two contaminated sites and one control. The taxonomical and physiological characteristics were examined by factorial analysis. Besides, the sensitivity of the strains to toxicants were checked.

The competitive abilities of some cadmium resistant and cadmium sensitive strains were compared by evaluating their growth parameters in batch cultures.

The influence of cadmium upon bacterial communities was followed in continuous cultures where microorganisms can be grown at steady state in a wide range of submaximal growth rates as it occurs in natural systems. The bacterial inoculum was prepared from samples taken in the river Ourthe.
The analytical determinations (heavy metals, carbon, A.T.P. content) and microbial techniques were made by standard methods.

The genetical analysis were performed by Dr. Mergeay's laboratory by curing wild resistant strains.

RESULTS

- Taxonomical and physiological characteristics of bacteria colonizing aquatic systems contaminated with cadmium.

The ordination was produced by the factorial analysis of correspondence. It shows that two sub-communities could be described. Some strains were able to develop in very high concentrations of cadmium whereas other strains were killed by cadmium.

Besides, the physiological and taxonomical characteristics were different in the resistant strains and in the sensitive ones. By comparing resistant strains of different aquatic systems, it was noted that their characteristics depended on the environment. The correlation between the resistance to heavy metals and to antibiotics was not observed in all cases. The density of resistant strains was roughly related with the level of toxicity in the environment.

- Behaviour of bacterial communities contaminated with cadmium.

Since cadmium resistant strains were isolated in aquatic systems, it was interesting to know whether these isolates could compete successfully in uncontaminated systems.

The growth parameters of the Monod model were determined in the cases of sensitive and resistant strains. It has been noted that all strains were characterized by the same range of values. On the basis of these experimental values, simulations were performed by Dr. Dubois (Inst. de Mathématiques, Univ. de Liège). They showed that, in mixed cultures, resistant strains could compete successfully even in non-poisoned aquatic systems. Experimental cultures are planned now in order to check these theoretical results.

The next step was to study how a bacterial community could develop when it was contaminated with cadmium. Bacterial communities were incubated in continuous cultures characterized by low productivies in order to mimic the environmental conditions. When the cultures had reached a steady state they were contaminated continuously by a cadmium solution (1 ppm Cd) for one month.

Four bacterial cultures set at different growth rates and viabilities (determined by A.T.P. content) were examined by the following criteria :

a) bacterial productivity
b) recovery of Cd-resistant strains
c) cadmium uptake by bacteria
d) cadmium level in the water phase of the cultures.

a) The bacterial productivity was not greatly affected by the cadmium contamination even in cultures with a low viability (0.016 h^{-1}). Therefore, it can be assumed that the biodegradation process was not influenced to a great extent by this contamination.

b) The concentration of cadmium-resistant strains arose quickly in all cultures mainly in the strains resistant to 20 ppm cadmium. Since, the growth characteristics of the resistant strains are similar to those of the sensitive ones, it is predictable that the bacterial productivity remains unchanged after Cd-contamination.

c) In all cultures, cadmium was accumulated to high levels in bacteria. At steady state, the cadmium concentration equalled 673-1083 ppm Cd in the free bacteria, whereas the concentration rose up to 6100 ppm when bacteria were grown on the walls of the culture vessel. The cadmium resistant strains could accumulate the highest concentrations : 11.000 ppm. By considering all cultures (9 experiments), at steady state, the rate of immobilization by bacteria was correlated with bacterial productivity (free bacteria): Rate of immob. (μg Cd $l^{-1}h^{-1}$) = 0,74 + 0,93 Bact. Prod. (mg $l^{-1}h^{-1}$).

d) The removal of cadmium from the water phase was effective just after the start of the contamination. But when the cultures had reached a steady state again, the percentage of removal slowed down except in the culture set at the lowest growth rate (0.016 h^{-1}). In fact, despite increasing cadmium concentration in bacteria during the contamination, their impacts remained little significant owing to their low biomass. At steady state, the cadmium budget was as it follows : water phase 75-96 % cadmium, bacteria 4-14 % cadmium.

- Genetical analysis of wild cadmium resistant strain.

The analysis was achieved by Dr. Mergeay laboratory. The curing of strains has shown that the resistant factor is coded by an extra-chromosomal character (plasmid).

CONCLUSIONS

The analysis of bacterial communities colonizing aquatic systems contaminated with heavy metals and specially cadmium has shown that two sub-communities can be distinguished : a cadmium resistant one and a sensitive one. These sub-communities are characterized by different physiological and taxonomical features.

The growth parameters of resistant strains are similar to those of sensitive isolates and therefore their competitive abilities are similar, too. Microcosm studies of bacterial communities were performed in continuous cultures contaminated with cadmium. It can be concluded that :

- the bacterial productivities and therefore the biodegradation appear undamaged by cadmium doses of 1 ppm.
- the recovery of resistant strains rises quickly in crowded populations.
- the uptake of cadmium by bacteria cells is significant. It has been recorded between 673 and 1083 mg Cd per Kg dry cells. The cadmium resistant strains accumulate the highest concentrations : 11.000 ppm cadmium. It is also true in the case of bacteria growing on the walls of culture vessels (6100 ppm Cadmium).

- the cadmium immobilization by bacteria is correlated with the bacterial productivity.

From these results, it could be expected that in rivers, the resistant and sticked bacteria could accumulate significant amount of cadmium. But their biomass and productivity should be taken into account especially in the case of the sticked bacteria, before drawing up the cadmium budget in aquatic systems.

Besides, it would be necessary to elucidate the balance between the absorption·desorption process in bacteria, the behaviour of bacteria in mixed heavy metals cultures and the transfer along food webs in order to model microbial aquatic systems.

For this purpose, an apparatus was devised to check models *in situ* by monitoring bacterial cultures in the river.

And last, the topics are important because of their potential applications for the removal of heavy metals in waste waters.

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- The composition of the saprophytic bacterial communities in fresh-water systems contaminated with heavy metals (to be published).

- The behaviour of bacterial communities contaminated with cadmium (to be published).

Contractor:	Water Research Centre Stevenage Laboratory Stevenage Hertfordshire SG1 1TH UK
Contract No:	216-77-10 ENV UK
Project Leaders:	Mr J F Solbé, WRC Mr H A Hawkes, University of Aston
Title of Project:	Effects of an oxidised sewage effluent on freshwater fisheries

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Objectives of the research

(a) To investigate the effects, on benthic communities and fish, of the presence in river water of different proportions of a sewage works effluent containing low concentrations of heavy metals, including cadmium, with particular emphasis on survival, growth, and accumulation of metals, in macro-invertebrates and in fish, in relation to water quality.

(b) To contribute to the establishment of an EEC method of biological surveillance of water quality by comparing the community structure developed under the different water-quality regimes.

Materials and methods

The study makes use of artificial streams, a compromise between the controlled, but unnatural, laboratory environment and the natural, but inherently variable, river.

The artificial streams are situated in the grounds of a sewage works which treats industrial and domestic waste containing, among other heavy metals, cadmium. Studies of the river into which the treated waste is discharged indicate that cadmium may be responsible for the absence of brown trout (Salmo trutta) for 9 km downstream of the Works.

The streams, which have been described in detail previously(1), are each 300 m long, 1 m wide, and divided into reaches of 100 m long stony riffles, 0.15 m deep, alternating with 50 m long silty pools, 0.3 m deep.

There are 3 streams, each provided with rotary screens to retain the fish, and with a range of water-quality sampling and monitoring equipment so that almost continuous records of physico-chemical conditions in the streams are maintained. The streams carry waters of different quality at a rate of flow of 4.5×10^3 m^3/d. One always contains the unpolluted river water (Channel A). The others contain mixtures of the river water and the sewage works' effluent; the percentages of effluent were 50 and 75 in Channels B and C in the first series of experiments, and 25 and 50 in the same channels in the second series, now in progress.

In an experiment, after an acclimation period during which the sediment pattern, the algae, and the invertebrates adjust to any change in operating conditions, brown trout are released in the streams at the carrying capacity known to be optimal for growth of brown trout (3.5 kg live fish/stream). Samples of organisms are removed periodically and any responses to, or consequences of, the water quality regime of each stream are examined. For the fish these consequences may include accumulation of heavy metals, change in rate of growth, and mortality. The other organisms may respond by changes in species composition and biomass, as well as accumulation of heavy metals.

Six large tanks (3 of which contain the same waters as the channels) have also been installed on the site. Comparisons of the concentration of metals in fish fed artificial food in the tanks with that of fish feeding 'naturally' in the streams, may indicate the importance of various pathways by which metals enter fish tissue, and perhaps give rise to potential hazards to human health. The tanks will also be used for acclimation of fish before release in the streams, and for preliminary work on the effects of water quality on a non-salmonid fish such as roach (Rutilus rutilus), to be released in the streams at a later stage.

Facilities are also available for hatching fish eggs in different qualities of water and for following the survival and growth of the fry.

The channels have been operating for about 4 years, in the first 3 of which operational problems were identified and resolved and preliminary experiments carried out, and in the last of which work has been the subject of the present EEC contract.

Results

In the first 6 months of the contract (October 1977-March 1978) the channels were prepared for the experimental programme(1). Engineering work, involving an improved river-water intake to the channels and the provision of efficient screens to confine the fish in the channels, was completed. Instruments and pumps were overhauled and the banks of the channel were sealed. (The burrowing of voles had caused leaks from the channel nearest the river.) An alarm system was installed to warn of water-supply failures.

In the second 6 months the first experiment was completed. The results have been fully reported elsewhere(2). About 110 brown trout, comprising a total of 3.5 kg of fish, were released in each of the channels on 23 June 1978 and detailed records were kept of water quality.

The studies on brown trout were complicated by two problems: a) predation of the fish by herons (Ardea cinerea) and b) a spillage of sludge upstream of the river water intake to Channel B on August 19. Although anti-bird netting had been installed 200 mm above the surface of the water the herons were able to land on the banks separating the channels and strike at the fish through the 150-mm mesh. (This netting has now been replaced by 20-mm mesh which extends over the banks so that a complete barrier to the birds is formed.) An accidental spillage of sludge from the sewage-treatment works brought to an end the first experiment by reducing the dissolved-oxygen concentration almost to zero and killing the few fish remaining in Channel B. (Such spillages are no longer possible.)

Data on fish growth and mortality are given in Table 1. Fish condition factors,

$$100[\text{live weight(g)}]/[\text{fork length(cm)}]^3,$$

showed an initial decrease from 1.3 to 1.2 in all channels 15 days after release, but then recovered. Owing to predation, insufficient fish were available for determination of accumulation of metals other than in the unexposed fish, sampled at the start of the experiment.

The sampling of macro-invertebrates also began in June, and changes in the species-composition of the benthos were recorded between channels and with time. Channels B and C resembled one another more closely than they resembled Channel A. In Channel A, Gammarus pulex dominated the fauna numerically with Ancylus fluviatilis and Simulids, but the first two species were rare in Channel B and absent from Channel C. On the other hand Asellus aquaticus was abundant, together with Simulids, in B and C, and rare in A. Chironomus riparius was abundant in C, common in B, and absent from A.

The data on macro-invertebrates are being used to calculate biotic indices. Eventually the role of the invertebrates in the productivity of the channels will be established, together with their effects on the uptake of metals by fish.

Discussion and Conclusions

Table 1 summarises the data on the macro-invertebrate diversity (using the biotic score of Chandler(3)), fish mortality, growth rate, and water quality in the three channels. Mortality due to predation by herons has been excluded.

Table 1. Fish mortality, growth rate, and water quality in the 3 channels measured over a 60-d period

	Channel		
	A	B	C
% effluent	0	50	75
Chandler score†	748	592	255
Fish mortality* (%)	3	17	32
Growth rate (g/d)	0.71	0.42	0.27
Median proportion of 48-h LC50 for rainbow trout(4)	0.03	0.08	0.13
Concentration range of dissolved Cd (μg/l)	1-3	1-11	2-19

† lower riffles, August
* excluding mortality due to heron predation

These data show that the macro-invertebrates and fish responded to the water qualities to which they were exposed, and that the observed water quality in Channel B, which is expressed as a proportion (0.08) of 48-h LC50 for rainbow trout(4), is clearly not good enough for the long-term survival of brown trout.

Despite problems from predation by herons and accidental spillages of sludge, the engineering work described previously(1) has proved effective, the chosen stocking density has been justified, and useful information has already been gained. The present study, which began on 16

October 1978, has made use of the information on (a) the maximum concentration of effluent in river water that should be capable of providing the necessary data from a 90-day test, and (b) the acclimation period required for stabilising the macro-invertebrate communities after a permanent change in water quality.

The study, which is using mixtures of 0, 25, and 50 per cent effluent, will be extended to 161 days, the fish being introduced in February 1979. During the first 11 weeks, samples of water and macro-invertebrates have been taken, so that the succession of the benthic community can be followed.

In the next experiment (Experiment 3) the channels will continue to be run as in Experiment 2, and therefore no acclimation period for the macro-invertebrates will be necessary. Experiment 3 is planned for the period 23 April to 15 July 1979.

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2. SOLBÉ, J. F. DE L. G. *Effects of an Oxidised Sewage Effluent on Freshwater Fisheries*. Report for EEC Freshwater Contact Group Meeting, 18-20 October, 1978, Metz, France.

TOPIC 11 : HEAVY METALS

Health effects

Contractor: Justus Liebig-Universität Giessen
Contract No: 137-76-10-ENVD
Project Leader: Dr. Anna Barbara Fischer, Hygiene-Institut
Title of project: Acute and chronic effects of heavy metals on mammalian cells cultivated in vitro

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Objective of the research

It was the aim of these studies to investigate the effects of heavy metals on cellular functions following acute and chronic exposure. Special consideration was to be given to cadmium and mercury, but other metals of environmental significance should also be included. Different cell types should be used to determine representative cellular reactions. Comparative studies with several heavy metals should lead to a toxicological characterization and classification of these pollutants. Potential synergisms and combination effects of the heavy metals among themselves and with other environmental pollutants, should be investigated later. With chronic exposure heavy metal resistance towards lead, mercury and cadmium was observed, which is based on adaptive processes. The kinetics and conditions of these cellular responses were of special interest.

Material and methods

Most of the materials and methods have been described in detail (Fischer 1975, 1976, 1979). The following cell cultures were employed: (a) Human diploid fibroblasts (strain FH$_{31}$); (b) Human portiocarcinoma line HeLa; (c) Mouse fibroblasts L 929, subline L-A, growing in stationary suspension culture.
Analytical grade solutions of heavy metal salts, mostly chlorides of known molarity were used.

Microscopical examinations of morphology and mitotic rate were carried out with living cells and on preparations stained with hemalum-eosin. Viability was tested by the trypan blue exclusion test. Membrane damage was determined by measuring the release of the enzyme lactic acid dehydrogenase (LDH) into the culture supernatant. As an important parameter of carbohydrate metabolism lactic acid production was monitored. Cell

The uptake of labelled precursors into DNA, RNA and protein was depressed in all the metals studied so far. LDH release was inversely related to lethality indicating that intracellular enzymes are set free concomitant to cell death, whereas gross membrane damage resulting in enzyme leakage preceding death could not be demonstrated. The production of lactic acid was increased after exposure to all metals except nickel. The enhanced glycolysis was interpreted to represent a mechanism to compensate for the impairment of cellular oxidative respiration. It is to date unclear why the cells do not react to nickel in the same way.

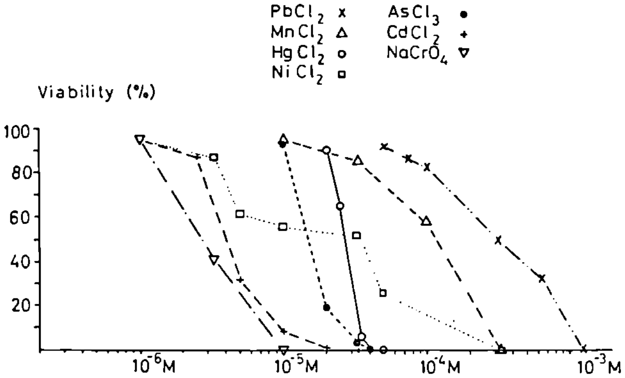

Fig. 1. Effect of heavy metals on viability of L-A cells exposed for 7 days.

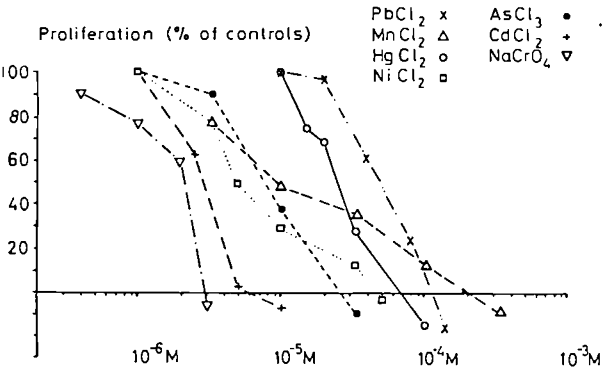

Fig. 2. Effect of heavy metals on proliferation of L-A cells exposed for 7 days.

b) Chronic exposure

In long-term tests cells were passaged for weeks in the presence of gradually increasing heavy metal concentrations. Increasing resistance was observed to lead, mercury and cadmium. The concentrations finally tolerated by L-A cells for periods up to growth and proliferation were followed by cell counting and protein determination. The incorporation of labelled precursors into DNA, RNA and protein was studied by liquid scintillation counting, and ^{3}H-thymidine uptake was also investigated by autoradiography. Cell cycle phases were determined by cytophotometry (collaboration with Dr. F. Otto, Institut für Aerobiologie, Grafschaft, FRG).

Results

a) Short-term tests

Cell cultures were treated with graded concentration of heavy metals for up to 7 days. Figure 1 shows the influence of different metal salts on the viability of L-A cells. The following rank order of toxicity can be established: $Pb < Mn < Hg < Ni < As < Cd < CrVI$. The effects on cell proliferation increase in the following manner: $Pb < Hg < Mn < As < Ni < Cd < CrVI$ (Fig. 2). It is interesting to note that the concentration ranges causing a 50 % depression of proliferation (IC_{50}) and producing lethalit in 50 % of the population (LC_{50}) lie relatively far apart in the metals lead, manganese and arsenic, while they overlap or touch in the metals mercury, chromium VI, cadmium and nickel (Table 1). This means that growth inhibition is accompanied by cell death in the latter group, while this is not the case in the first group.

	IC_{50}	LC_{50}
Cr VI	1,9 - 2,3 . 10^{-6}M	2,4 - 3,2 . 10^{-6}M
Cd	2,5 - 3,3 . 10^{-6}M	3,6 - 4,6 . 10^{-6}M
Ni	4,4 - 5,6 . 10^{-6}M	6 - 39 . 10^{-6}M
As	6,5 - 7,7 . 10^{-6}M	1,4 - 1,6 . 10^{-5}M
Mn	7,7 - 14 . 10^{-6}M	1,2 - 1,6 . 10^{-4}M
Hg	2,0 - 2,9 . 10^{-5}M	2,4 - 3 . 10^{-5}M
Pb	3,7 - 6,2 . 10^{-5}M	2,9 - 3,5 . 10^{-4}M

Table 1. Effect of heav metals on viability (LC_{50}) and proliferatic (IC_{50}) of L-A cells exposed for 7 days.

For most of the metals the parameters mentioned in material and methods were studied only in L-A cells, but for some of them the two human cell lines were also employed, and the results were generally similar.

several months were: Pb - 2 x 10^{-4}M, Hg - 4 x 10^{-5}M, Cd - 3 x 10^{-5}M. These levels approached or even exceeded the respective LCs_{50}. Tolerance to these heavy metals was interpreted to be caused by adaptation rather than by mutation and selection for the following reasons: (1) resistance to lead was again lost after only a few generations in a lead-free environment; (2) in the case of cadmium and mercury a protective effect could be observed after only a few days.

Heavy metal tolerance was demonstrated in L-A mouse fibroblasts as well as in the epithelial-like HeLa cells and in human fetal fibroblasts. As an instance the results of an experiment is shown where diploid human fibroblasts were pretreated with a low dose of $CdCl_2$ for one week and then exposed to an acutely toxic challenge dose (Table 2). It can be seen that not only viability and cell number are improved in the pretreated cells but even the values of LDH and lactic acid have normalized.

Pretreatment	Challenge dose	Cell number (x 10^6)	Viability (%)	LDH (mU/ml)	Lactic acid (mg/10^6 cells)
———	8 x 10^{-6}M $CdCl_2$	2,257 ±0,123	68,7 ± 8,62	135,3 ± 4,04	5,395 ±0,12
4 x 10^{-6}M $CdCl_2$	8 x 10^{-6}M $CdCl_2$	2,623 ±0,125	86,25 ± 1,77	113,25 ± 3,18	3,968 ±0,1

Table 2: Effect of pretreatment with a low dose of $CdCl_2$, applied for one week.

Conclusions

Cell cultures have proved to be a valuable test system for the evaluation of heavy metal effects at the cellular level. With this system a rank order of toxicity as regards lethal and growth inhibiting effects could be established. The sensitivity of all tested cell lines (human - animal; cancerous - noncancerrous; fibroblast - epithelial-like) was similar, and similar biochemical reaction as well as adaptative processes could be demonstrated. Cell cultures seem to be especially suitable for the study of heavy metal uptake, points of attack, and combination effects as well as cellular reactions including mechanism of adaptation (e.g. formation of metal-binding proteins).

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FISCHER, A.B.: Acute and chronic effects of lead on mammalian cells in vitro. In: International experts discussion on lead-occurrence, fate, and pollution in the marine environment, Pergamon Press, Oxford (in press).

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FISCHER, A.B.: Einfluß akuter und chronischer Bleiexposition auf Säugetierzellen in vitro. Umweltkolloquium "Biologie in der Umweltsicherung", January 9, 1978, Giessen.

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FISCHER, A.B.: Akute und chronische Wirkung von Kadmium auf Säugetierzellen in vitro. Deutsche Gesellschaft für Hygiene und Mikrobiologie, Mainz, October 2 - 5, 1978.

Contractor	: Institut für Arbeits- und Sozialmedizin und Poliklinik für Berufskrankheiten der Universität Erlangen-Nürnberg
Contract n°	: 147-77-4 ENV D
Project Leader	: Prof. Dr. med. H. Valentin
Title of project	: External/Internal Dose-Response Relationship for Mercury and Vanadium in Occupationally Exposed and Normal Persons

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1.1 Normal mercury intake by food

Objective of the research:

Monitoring the concentrations of mercury and its compounds in the environment is of great public interest. There is a considerable accumulation of this heavy metal in the food chain in some regions. The possibility of intoxications for human arises due to accumulation in the food chain.The present study was performed to determine the normal load of mercury in the FRG.

Materials and methods:

Nine persons without occupational exposure to mercury were adminstered a uniform diet. Daily measurements of the mercury content in the food, blood, feces and urine of the individuals were performed. For analyzing the specimens a special high-pressure-combustion was applied. The final measurement of the mercury concentrations was performed by theso-called cold vapour AAS.

Results:

There was no statistically significant variation of the mercury concentrations in the specimens of the individuals obtained during the analyzing period of examination. This also applies to the second week, when fish, which is assumed to be especially rich in mercury was served. A negative balance was calculated when analyzing the differences between absorption and elimination of mercury during the period of examination. The period for the calculation of the resorption and retention, resp. in the organism, may have been too short.

Conclusions:

1. The intake of mercury via food is calculated to be about 30 µg for a normaldaily diet in the FRG.
2. The proposed maximal acceptable value of 100 µg Hg/d is not reached.
3. The maximum concentrations of mercury were observed in the meat of fresh-water fish.
4. Unexpectedly high concentrations of mercury were found in sausages containing liver and blood.
5. The concentrations of mercury found in saltwaterfish correspond to these of other foodstuffs.

1.2 The concentration of mercury in the blood of adults in the Federal Republic of Germany

Objective of the research:

1. Determination of the normal concentration of mercury in the blood of adults in the FRG
2. Analysis of potential regional differences in the concentrations of mercury in the blood.
3. Evaluating potential differences between mercury concentrations in blood samples gained from males and females.

Materials and Methods:

Three groups from different regions of Germany with varying levels of industrialization were analyzed. Group I represents an industrial district without important heavy industries. Group II came from a highly industrialized area. Group III represents individuals from a rural district. Mercury was determined by the so-called flameless atomic absorption spectrometry after an oxidative combustion by $KMnO_4$. The concentration of methyl mercury was partly evaluated by gas chromatography.

Results:

The concentration of mercury in the blood show no normal distribution. For this reason medians and the 33.3 and 66.6 percentiles were calculated. The results for the tree collectives are shown in the following table:

			Hg-B (µg/100 ml)
Collective	n	median	0,33/0,66 quantile
I	60	1.02	0,47/2,55
II	50	1.18	0.87/2,12
III	35	0.94	0,77/1,49

The concentration of methyl-mercury in the blood normal persons were as low as 0.5 to 0.8 ng/ml.

Conclusions:

The median concentrations of mercury in the blood show a correlation to the different levels of industrialization of the regions the samples came from. Differences in the evironmental exposure to mercury are reflected in the concentrations of mercury in the blood. These heavy metal concentrations are an indicator of evironmental pollution by mercury. Our results lie within the range of the values determined by other authors. The normal level of mercury in the blood of persons in the FRG is below 2 µg/100 ml. There are no sex-specific differences.

1.3 Examinations in persons occupationally exposed to mercury

Objective of the research:

1. Evaluation of the relationship between the concentration of mercury the ambient air of the working place and in the biological materials of a group of workers
2. Analysis of the variations in the daily renal eliminations
3. Report on minute bio-chemical and neurologic changes in workers and derivation of acceptable limit values

Materials, Methods and Results:

1. Twenty-three workers (16 males and 7 females) of a chemical factory with a long duration of exposure (one to 28 yrs., median 7 yrs.) to mercury, inorganic mercury compounds and instable organic mercury compounds were examined. Stable alkyl-mercury-compounds were not used in the factory. Several random measurements of the concentration of mercury in the air revealed values between 50 and 650 µg/m^3. These results greatly exceed the TLV of 100 µg/m^3 for inorganic and 10 µg/m^3 for organic mercury compounds. The concentration in the ambient air related to individual working places was determined by a mean value of 178 µg/m^3. Correspondingly higher values were found in the urine with mercury concentration varying from 36 to 750 µg Hg/g creatinine (median: 151 µg/g creat.) In addition to this higher concentrations of mercury in the blood were detected. The values varied from 11.6 to 456 µg/L (median: 57 µg/L). There are significant correlations between the concentration of mercury in the air and in the blood and urine (Tab. 1).

The ratio of mercury-concentration in the urine and blood is about 3 : 1. A similiar ratio was calculated for mercury-concentration in the air and in the blood based on the units applied. The ratio of mercury-concentration in the air to that of the urine was determined as 1 : 1 (Tab.1) This relatively low ratio can by explained by the proportion of organic compounds of mercury in the air, which are eliminated via urine after reduction to inorganic mercury-compounds (ratio 2.5:1).

For the analysis of the variations in the concentrations of mercury in the blood and urine of 3 individuals the complete renal excretion was examined daily while the blood samples were examined every 2nd day for a period of two weeks. The renal elimination of mercury shows a great intraindividual variation.This is particularly true when mercury-concentrations are determined on a basis of the volume of the sample alone. More reliable results are obtained when the concentrations are related to the volume of the daily renal excretion and the amount of creatinine, resp. Minimal intraindividual variations of the mercury-concentration are observed in the blood.

To evaluate potential biological effects by occupational exposure to mercury the following parameters were determined

γ-GT alcaline phosphatase cholinesterase α-HBDH	in serum
ß-galactosidase	in urine and serum

All enzyme activities were normal, besides a minute rise of the γ-GT activity in 4 cases. No dependence of these values on the extent of the exposure was found. There was a positive correlation (r = 0.78) between ß-galactosidase-activity in the urine and renal elimination of mercury, but no real dose-response-relationship could be stated. In order to evaluate potential renal damage the following parameters were determined in the urine

total proteine
albumine
acid α_1-glycoproteine
β_2-microglobuline

No significant proteinuria and no indications of renal damage induced by mercury were observed. Positive correlations were calculated between the product of the extent and the duration of the exposure on the one hand and the amount of excretion of acid α_1-glycoproteine and β_2-microglobuline (r = 0.68 and 0.66, resp.) on the other hand. Actual dose-response-relationships could not be evaluated.

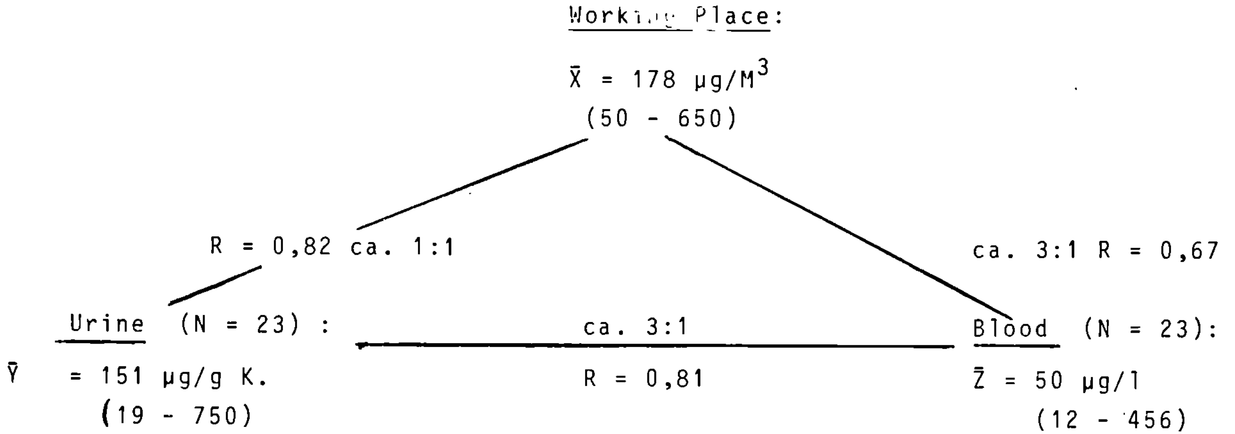
To analyze potential neuro.psychiatric damage due to mercury detailed neurologic and testpsychologic examinations were performed in 23 persons. No indications of neurologic abnormalities and no relationships of functional damage due to the extent or the duration of the exposure to mercury could be stated. There was also no accumulation of effects in certain individuals.

Conclusion:

1. The concentration of mercury in the blood is considered to be the most suitable parameter in occupational medicine for checking the exposure to ihis heavy metal. Analyses of the total daily renal elimination of mercury in relationship to the urine volume or related to creatinine yield similiar varying results.

2. Exposure to organic mercury compounds causes a large increase of the mercury concentration in the blood, compared with the mercury-elimination in the urine.

3. No adverse health effects due to inorganic mercury-compounds are to be expected in persons with a mercury-concentration in urine up to 200 µg/g creatinine, and up to 50 µg/l in the blood resp. and 100 µg/l in the blood of persons with an exposure to organic mercury-compounds. These figures are tolerable limit values. Summarizing experts' statements on occupational diseases due to mercury, a repeated and considerable excess of critical values (500 µg/l urine and 200 µg/l blood, or 400 µg/l in the blood of individuals with exposure to organic Hg-compounds) is necessary to cause adverse health effects.



Tab. 1: Relationships between mercury concentrations in air and in biological material

2. Vanadium

2.1. The normal vanadium intake by food and the corresponding vanadium levels in blood, urine and feces

Objective of the research:

Only little information is found in literature on the daily intake of vanadium via food and drinking water. Occasional statements mention the levels of vanadium in blood, urine, and feces. Given this context, studies on the input and output balances rate special interest. The present study was performed to evaluate the usual load of vanadium for individuals in the FRG.

Materials and methods:

For this purpose a uniform diet was administered for two weeks to 10 persons who had no occupational exposure to vanadium. The concentrations of vanadium were analyzed daily in the several dishes and in the blood, urine, and feces of the individuals.

The specimens were oxidized using HNO_3 and H_2O_2. Interfering substances were eliminated by repeated extractions and vanadium was detected with a very sensitive reaction. In the presence of traces of vanadium, gallic acid is oxidized by an acid persulphate solution. As little as 0.025 µg of vanadium per sample can be detected by this colorimetric reaction. The digestion of the homogenized foodstuffs and feces was carried out in an autoclave under oxygen pressure.

Results:

These analytical procedures revealed a daily intake of vanadium through the food in the range of 8.2 - 13.2 µg. The median was determined to be 10.7 µg. No special foodstuff with an excessive content of vanadium was found.

The concentration of vanadium in the blood of 30 individuals not occupationally exposed ranged below 2.5 µg V/l. This corresponds with our previous results. The daily elimination of vanadium in urine ranged between 0.5 and 4.5 µg/d. Over a period of two weeks the mean concentrations in urine varied from 0.5 up to 2 µg/d for the 10 individuals. The individual variation of daily renal elimination of vanadium was very small during the two-week period. A considerably higher variation was found in the elimination of vanadium in the feces. Depending on the amount of feces, 0 to 69 µg V were eliminated per day. The mean values of fecal elimination for the 10 individuals over a period of two weeks were 15.8 to 33.4 µg V/d. When calculating the difference between intake and elimination of vanadium a positive balance was revealed for the time of examination. The reasons for this result may be - in addition to analytical problems - an additional airborn intake of vanadium by inhalation. Further considerations implicate a shift in time between resorption or retention and elimination of vanadium in the organism.

Conclusions:

The conclusion based on the present results indicates a low individual load of vanadium in the FRG. This is also valid for occupationally exposed individuals. No adverse effects from exposure to vanadium seem to exist for the general population. An evaluation of the maximum acceptable value for the individual daily intake of vanadium is desirable.

2.2. Biological monitoring of occupationally vanadium exposed persons

Materials and methods:

In a metallurgic plant we analysed blood samples, urine and finger-nails of 54 workers exposed to vanadium. On the basis of orientating dust measurements it can be assumed that the results obtained were, as a rule, clearly lower than the MAC value for vanadium pentoxide dust.

Results:

The median vanadium concentration in whole-blood was 2.9 µg/l. This indicates that the exposed persons differ significantly from the control group. The median vanadium concentration measured in urine was 37.8 µg/l. This means that there is a significant difference in comparison with the control group (0.8 µg/l). In order to abtain values of greater reliability, the vanadium concentration resulting from the analysis of spontaneous urine samples was to be referred to the creatinin content. Conversion yields a median vanadium concentration of 33.9 and 0.6 µg/g creatinine for the exposed workers and normal persons respectively.

The cystine content found in the finger-nails of persons occupationally exposed to vanadium was significantly reduced with respect to the comparative group (8.9 against 9.9 mg cystine per 100 mg finger-nails). Within the groups of persons examined no correlation is found to exist between the cystine content of the finger-nails and the age of the persons, nor between the vanadium concentrations in blood and urine.

After a weekend without exposure the vanadium concentrations in blood and urine dropped. In general, this drop was the more pronounced the higher the initial value was. Roughly two to four days after the occupational exposure had ceased, the value dropped to the half of the initial value.

Conclusions:

Based on the test results available, the vanadium concentrations in blood and urine reflect the extent of an occupational vanadium exposure and are suitable indicators for estimating the potential threat caused by this heavy metal.

3. References to publications and oral communications

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   Vortrag auf der Jahrestagung d. Dt. Ges. f. Arbeitsmed. e.V., Frankfurt/Hoechst, 25. - 27. Mai 1978

Contractor : Abteilung Hygiene und Arbeitsmedizin der Rheinisch-Westfälischen Technischen Hochschule Aachen, Federal Republic of Germany

Contract n° : 197-77-4 ENV D

Project leaders : J. Rosmanith, D. Pistorius, D. Prajsnar

Title of project : Lead, cadmium and zinc as composite system in animal experimentation

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METHODS

18 male rabbits were divided up into 6 groups: animals of the first group were injected intra venam 0,1 or 0,4 or 0,8 µg lead as acetate five times the week for four weeks. Animals of the second group got additional 0,1 µg cadmium as acetate and animals of the third got additional to the lead dose 0,1 µg cadmium and 10 µg zinc as acetate. Animals of the fourth group were injected only 0,1 µg cadmium, those of the fifth group were injected intra venam 0,1 µg cadmium together with 10 µg zinc acetate. The rest of the animals was used as a control group.

Three times a week two ml blood was tapped. Additional 1-10 ml urine was taken away by a sterile solitary catheter. The elements lead, cadmium and zinc were determined in blood and urine samples by atom-absorption-spectrophotometry (ref. 2,3). On the first, 12th, 22th, and 29th day of experiment, samples of the back-hair of the animals were withdrawn. On the 29th day of experiment all animals were killed. In the withdrawn organs and tissues (lung, liver, kidney, spleen, sternum, tibia, m. gastrocnaemius, hair) lead, cadmium and zinc were determined by atom-absorption-spectrophotometry after removing the fat and reducing to ash.

RESULTS and COMMENT

The fixed quantities of lead and cadmium in blood show two phases and reached their minima between 12th and 17th day. The depression of the Pb-B begins earlier and increase with the total dose (i.e. Pb+Cd+Zn-dose fig. 1 and 2).

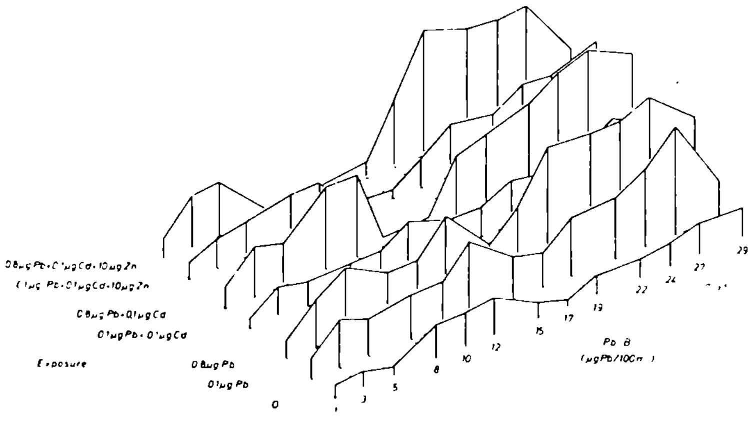

Fig. 1 Course of lead level in blood (Pb-B) according to the different exposure

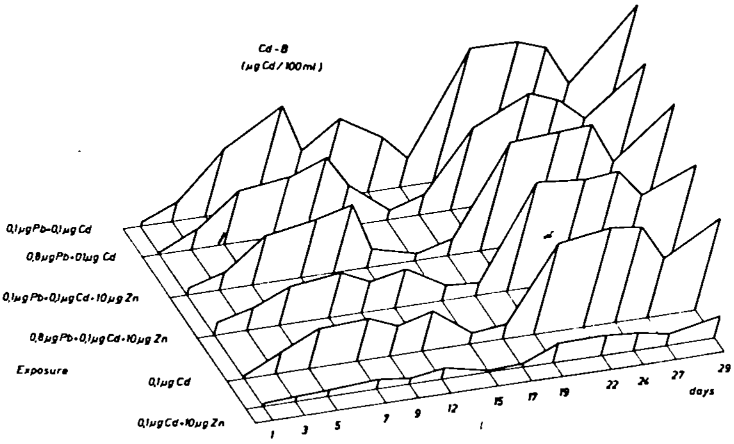

Fig. 2 Course of cadmium level in blood (Cd-B) according to the different exposure

This seems to be a physiological phenomenon as it is even visible in the control group. This biphasic course may be explained by a different half-time of lead (ref. 4) or by the reabsorption of lead from the intestine (ref. 5). This biphasic course reminds of the biphasic elution curve of erythrocytes in vitro after lead exposure (ref. 6).

According to the conditions in children (ref. 1,7) our experiment did not show a steady state for Pb-B and Cd-B.
The Pb-B-values were always dependent on the lead dose: after the same dose of lead, the maxima of Pb-B values have been determined after exclusive supply of lead, the minimal values after simultaneous exposure to lead, cadmium and zinc (tab. 1, fig. 3).

Exposure	Pb-B		Cd-B		Zn-B	
	Mean	SD	Mean	SD	Mean	SD
Control	14,31	8,13			324,21	101,84
Pb	37,87	18,46			328,54	103,55
Pb+Cd+Zn	26,75	18,73	5,12	3,68	374,23	75,02
Cd	12,16	7,91	4,61	3,27	382,04	101,07
Cd+Zn	16,12	9,02	0,95	0,75	352,67	128,18

tab. 1 Blood levels of lead (Pb-B=μgPb/100 ml), cadmium (Cd-B=μgCd/100 ml), and zinc (Zn-B=μgZn/100 ml)

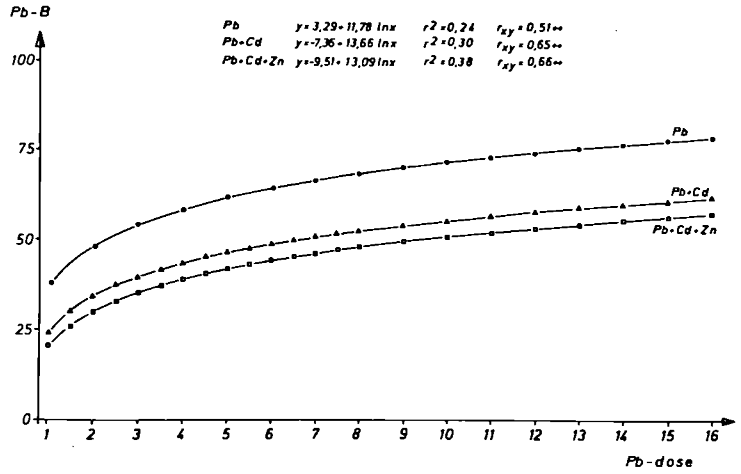

fig. 3 Relationship between Pb-B and lead dose after different exposure (Pb, Pb+Cd, Pb+Cd+Zn) in form of a logarithmic curve

However, the differences of the mean values have not been significant.

The Cd-B-values have been remarkable decreased after simultaneous application of cadmium and zinc, and remarkable increased after simultaneous application of lead and cadmium in relation to those after exclusive cadmium dose (fig. 4).

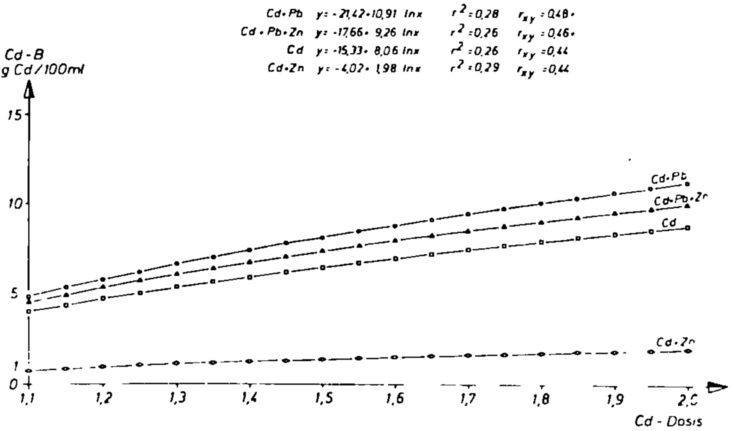

fig. 4 Relationship between Cd-B and cadmium dose after different exposure (Cd, Cd+Pb, Cd+Zn, Cd+Pb+Zn) in form of a logarithmic curve

The Cd-U-values (cadmium excretion in urine) show the opposite trend (tab. 2)

Exposure	Mean	SD	Mean	SD	Mean	SD
Control	31,39	21,43			251,77	131,57
Pb	55,92	61,19			615,45	616,74
Pb+Cd	66,31	50,15	5,20	3,63	384,71	227,96
Pb+Cd+Zn	55,36	36,50	6,24	3,76	310,59	130,95
Cd	35,43	22,44	7,17	11,09	251,30	121,50
Cd+Zn	33,55	17,18	10,68	18,01	378,86	198,28

tab. 2 Urine levels of lead (Pb-U=µgPb/l), cadmium (Cd-U=µgCd/l), and zinc (Zn-U=µgZn/l)

Lead- and zinc-excretion in urine increased if only lead is injected (tab. 2) Increased cadmium concentration in blood was also determined in children with symptomes of lead intoxication (ref. 8).

It was possible to demonstrate clear interactions between lead, cadmium and zinc. These interactions reflect in alteration of absorption, retention and excretion of these metals. In our experiment is demonstrated:

1. A competitive interaction between cadmium and zinc by reduction of cadmium in blood (tab. 1, fig. 4) and by transport of cadmium from liver (tab. 3), spleen (tab. 4) and bones (tab. 5) to kidney (tab. 6).

Exposure	ppm Pb		ppm Cd		ppm Zn	
	Mean	SD	Mean	SD	Mean	SD
Control	2,14	1,26			304,46	72,23
Pb	4,91	1,30			283,70	33,23
Pb+Cd	4,80	3,01	81,74	8,38	428,46	78,15
Pb+Cd+Zn	4,29	1,30	69,57	10,11	346,67	32,61
Cd	1,16	0,26	98,79	9,41	484,23	53,02
Cd+Zn	2,10	1,29	10,04	0,70	339,30	8,59

tab. 3 Liver levels of lead, cadmium, and zinc (µg/g dry weight)

Exposure	ppm Pb	ppm Cd		ppm Zn	
		Mean	SD	Mean	SD
Control	Traces				
Pb	Traces				
Pb+Cd	Traces	10,11	1,14	115,88	12,52
Pb+Cd+Zn	Traces	8,76	1,79	127,80	14,49
Cd	Traces	7,98	0,26	118,89	2,94
Cd+Zn	Traces	Traces		133,77	10,86

tab. 4 Spleen levels of lead, cadmium, and zinc (µg/g dry weight)

Exposure	ppm Pb		ppm Cd		ppm Zn	
	Mean	SD	Mean	SD	Mean	SD
Control	14,43	4,51			190,31	26,95
Pb	29,92	11,38			162,92	31,67
Pb+Cd	32,66	17,39	2,30	0,32	168,56	9,58
Pb+Cd+Zn	28,45	12,13	2,18	0,14	162,75	6,83
Cd	12,46	1,44	2,00	0,05	168,18	2,81
Cd+Zn	11,56	0,11	1,36	0,11	183,96	11,76

tab. 5 Bone (tibia) levels of lead, cadmium, and zinc µg/g dry weight)

Exposure	ppm Pb		ppm Cd		ppm Zn	
	Mean	SD	Mean	SD	Mean	SD
Control	4,40	1,00			135,56	4,03
Pb	5,71	3,24			205,00	104,15
Pb+Cd	10,73	3,45	27,21	5,74	328,16	170,67
Pb+Cd+Zn	7,79	2,06	22,07	5,13	223,51	98,64
Cd			4,42	1,17	280,07	109,17
Cd+Zn	6,20	3,08	23,29	0,09	253,90	81,94

tab. 6 Kidney levels of lead, cadmium, and zinc (µg/g dry weight)

2. A synergistic reaction between cadmium and lead; after combined lead- and cadmium-dose we have found an increasing concentration of cadmium in blood (tab. 1, fig. 4) with a decreasing excretion of cadmium in urine (tab. 2) together with an increasing accumulation of cadmium in liver (tab. 3), spleen (tab. 4), bones (tab. 5) and kidney (tab. 6).

3. Lead disturbed the competetive influence of zinc upon cadmium. Lead exerted the contrary effect on the distribution of cadmium in organism of the animals than zinc did: after simultaneous application of lead, cadmium and zinc, the concentration of cadmium in blood (tab. 1, fig. 4), spleen (tab. 4) and bones (tab. 5) increased. After simultaneous application of cadmium and zinc only, the concentration of cadmium in blood (tab. 1), liver (tab. 3), spleen (tab. 4) and bones (tab. 5) in contrary decreased.

4. Although it was not possible to show a clear competitive interaction between lead and zinc, it cannot be excluded completely. After simultaneous exposition to lead, cadmium and zinc a tendency to decreased concentration of lead in blood (tab. 1, fig. 3) and in urine (tab. 2) was observed along with a tendency to advanced excretion of lead by bile. The concentration of lead in hair, in kidney (tab. 6) and in lungs (tab. 7) of the animals decreased.

Exposure	ppm Pb		ppm Cd		ppm Zn	
	Mean	SD	Mean	SD	Mean	SD
Control	Traces				221,20	79,79
Pb	9,25	1,78			400,44	137,12
Pb+Cd	8,13	2,83	2,62	0,27	298,32	116,82
Pb+Cd+Zn	5,30	2,36	2,14	0,54	177,42	12,16
Cd	Traces		3,79	0,88	308,51	219,61
Cd+Zn	Traces		Traces		167,31	43,48

tab. 7 Lung levels of lead, cadmium, and zinc (µg/g dry weight)

The accumulation of lead in liver (tab. 3) and bones (tab. 5) seems not to be influenced by cadmium or by zinc.

Our research-programme for the next time will demonstrate whether a possible competitive influence of zinc upon lead is dependent on the quantity of injected zinc. The results of other authors (ref. 9 - 12) point to this idea too.
The observed interactions mostly are explained by substitution of metals in the binding on metaloproteins (ref. 13). In our model experiment we just now determine quantities of lead and cadmium which are connected to SH-groups in certain organs (liver, kidney) of the animals.

Our experiment demonstrates, that in lead and cadmium not only the intensities of their absorption, but also the existence of their absorption, but also the existence of their reciprocal interactions are of great importance to the dose-efect, and dose-response-relationship respectively.

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Contractor: Fraunhofer-Gesellschaft, Institut für Aerobiologie
5948 Schmallenberg-Grafschaft
Contract no: 265-77-1 ENVD
Project leader: Dr. G. Oberdörster
Title of project: Toxicological and behavioural studies on rats following chronic inhalation of zinc and cadmium aerosols.
Part I

Determination of lung clearance rates of cadmium and zinc aerosols in rats after acute exposure, alone and in combination. (Oberdörster, Baumert, Hochrainer)

Material and methods:

Rats were exposed to $Cd\,Cl_2$ or Cd O aerosols for 45 min. (concentration 930 μ g/m^3, and 760 μ g/m^3, median aerodynamic diameter 0.38 μ m and 0.46 μ m); another group inhaled Zn O for 17 hours (concentration 15 mg/m^3, aerodynamic diameter 1.4 μ m); the combination group was exposed to Cd O + Zn O for 3 days (20 μ g/m^3 + 2 mg/m^3)
The aerosols were generated by nebulizing the respective acetate solutions and oxidizing these particles by passing them through a heated tube furnace. Animals were serially sacrificed and the metal content in lung, liver and kidney was determined by AAS.

Results:

The half life of the long term lung clearance rates was identical for both compounds, namely 67 days. For Cd O only, an initial faster clearance was observed resulting in a relatively lower Cd burden of the lung as compared to $Cd\,Cl_2$. An increased lung wet weight, a sensitive indicator of Cd toxicity on the lung, was observed only in the $Cd\,Cl_2$ study due to its higher content in the lung. The Zn O particles, on the other hand, were cleared very rapidly in a monoexponential pattern, exhibiting a half-time of 6.3 hours. It is assumed that the protein binding of both oxides in the lung is different and thus may account for the different clearance. The combined exposure of Cd O + Zn O revealed an apparent influence of Zn in the Cd Clearance: The Cd content of the lung remained almost constant for about 50 days, and thereafter declined as expected.

The determination of lung clearance rates of cadmium aerosols after chronic Cd inhalation. (Oberdörster, Hochrainer)

Material and Methods:

Rats were continuously exposed for 7 weeks to Cd O (10 $\mu g/m^3$) in whole body exposure chambers. Thereafter they were exposed to ^{115m} Cd Cl_2 for 1 hour and kept again in the Cd O atmosphere. The decrease of radioactivity in the lung was followed up to 100 days after the ^{115m} Cd exposure. Control animals were exposed for 1 hour only to ^{115m} Cd Cl_2.

Results:

In control animals ^{115m} Cd was cleared from the lungs in a bi-exponential pattern with a clearance half life of the slow phase of 61 days. The Cd O exposed rats showed a monoexponential pattern of the ^{115m} Cd clearance, the half life being 72 days. The deposition of Cd in the respiratory tract was significantly higher in the control animals than in the chronically exposed rats, which points to alterations in breathing mechanics after chronic Cd inhalation. The half life of 72 days found in these experiment is not significantly different from those found after single exposure, i.e. 61 and 67 days, respectively; the duration of Cd exposure does not influence its elimination rate from the lung.

The effect of chronic cadmium and zinc inhalation, alone and in combination, on lung clearance mechanisms.
(Oberdörster, Hochrainer)

Material and Methods:

Rats were continuously exposed for 6 weeks to Cd O (10 $\mu g/m^3$) Zn O (1 mg/m^3) and Cd O + Zn O (10 $\mu g/m^3$ + 250 $\mu g/m^3$). Thereafter the animals were exposed for 1 hour to an inert aerosol of ^{59} Fe_2O_3 and the subsequent clearance of this aerosol was determined up to 70 days after Fe_2O_3 exposure; during this time the animals were kept in the respective Cd and Zn atmospheres. Animals kept in clean air and being exposed to ^{59} Fe_2O_3 alone served as controls.

Results:

Cd O and Zn O aerosols, when inhaled alone at the given concentrations, lead to an impairment of alveolar lung clearance mechanisms: The half life of the clearance of $^{59}Fe_2O_3$ particles was doubled in the Cd and in the Zn exposed animals as compared to controls. The underlying mechanism probably is an adverse effect on alveolar macrophages, as shown in the respective studies (see part 2). Animals, which were exposed to the combination of both oxides, did not show a significant difference to controls in the clearance of Fe_2O_3. Obviously, this is due to a protective action of Zn on the adverse effects of Cd on lung clearance.

Measurement of lung function in rats after Cd aerosol exposure (Oberdörster, Hochrainer)

Material and Methods:

Rats were continuously exposed for 8 weeks to either 50 $\mu g/m^3$ or 10 $\mu g/m^3$ $CdCl_2$. Tidal volume, respiratory rate and heart frequency were measured and respiratory resistance and compliance were determined. To test bronchial sensitivity, Acetylcholinchloride aerosols were given for 30 sec and the respiratory reaction (resistance and compliance) was measured.

Results:

In the 50 $\mu g/m^3$ group, the tidal volume was significantly lower and the respiratory rate was significantly higher. After 4 weeks of recovery in clean air, theses differences could no longer be found. The bronchial sensitivity of both groups was significantly increased, as compared to control. This effect, too, had disappeared after 4 weeks of recovery. The lung weights of the animals of the 50 $\mu g/m^3$ group had increased, whereas no increase in lung weight could be found in the 10 $\mu g/m^3$ group. Apparently, bronchial receptors react to Cd exposure before there are any morphological changes.

Learning behavior and memory of chronically Cd exposed rats and organ distribution of Cd. (Heering, Oberdörster, Hochrainer, Baumert)

Material and Methods:

Rats were exposed continuously to 200 $\mu g/m^3$ $CdCl_2$ (4 months), 20 $\mu g/m^3$ $CdCl_2$ (18 months) and to 50 $\mu g/m^3$ (84 days). Learning and memory were tested in a shuttle box system where the animals were contitioned to an avoidlance reaction and the results were compared to untreated controls.

Results:

The animals exposed to 200 μ g/m^3 showed signs of a highly toxic general effect of Cd. The marked reduction of the conditioned reflexes after 75 days of exposure can be related to the overall toxic effects of Cd. Animals of the 20 μ g/m^3 and 50 μ g/m^3 groups did not show any differences to control animals. Thus, with this method a neurotoxic influence of Cd at these concentrations could not be proven. The organ distribution of Cd after chronic inhalation (84 days) showed that only a small percentage, 0,05 % of Cd deposited in the alveolar area , reaches the brain. It seems that the blood brain barrier plays an important protective role against Cd intoxication of the CNS. If the lung Cd concentration in this group is set to 1, the brain Cd concentration is 0,0009 only. The kidney then has a concentration of 1,08, thus confirming the high accumulation rate of Cd in this organ due to its long biological half life.
Lung histology of the 18 months exposed rats revealed, that all of them had developed adenomatous hyperplasia with 4 adenomas and 1 adenocarcinoma.

Investigations on the effect of cadmium and zinc aerosols alone and in combintation on the metabolism of non-pregnant and pregnant rats and their fetuses. (Weischer, Prigge, Hochrainer, Baumert, Fritsche, Mihm)

Material and Methods:

Pregnant and nonpregnant rats were continuously exposed for 21 days to aerosols of 0.2, 0.4 and 0.6 mg Cd/m^3 as Cd Cl_2 and to 400 μg Cd/m^3 as Cd O, alone and in combination with 10 mg Zn/m^3 as Zn O. Cadmium concentrations in livers and clinico-chemical parameters of adults and fetuses were determined.

Results:

A polycythaemic response of nonpregnant rats was observed. Polycythaemia was less marked in pregnancy. In pregnant rats there was a marked dose dependent decrease of the activity of alkaline phosphatase after cadmium inhalation while there was no effect in exposed nonpregnant rats. The most sensitive parameter after inhalation cadmium exposure proved to be total wet lung weight. Fetal growth rates were reduced at the highest aerosol concentrations. The activity of the fetal alkaline phosphatase increased wit increasing maternal cadmium exposure. Non pregnant animals accumulated more cadmium in their livers than dams and there was nearly no transport of cadmium across the placenta. In pregnant and non-pregnant rats the protective effects of zinc aerosols on Cd-toxicity could be demonstrated on the following parameters: body weight, hemoglobin and hematocrit, wet weight of livers, spleens and lungs, and cadmium concentrations in livers. In non-pregnant rats only: values of erythrocytes, bilirubin in serum and wet weights of spleens; and in pregnant rats only: values of leucocytes and serum urea. In fetuses: leucocytes, serum iron, serum urea and wet weights of placentas.

Investigations with cadmium alone and in combination with carbonmonoxide. (Prigge, Baumert, Hochrainer)

Material and Methods:

Pregnant and non-pregnant wistar-rats were continuously exposed for 21 days to 60, 125, 250, and 500 ppm CO and to 0,2 mg $Cd\ Cl_2/m^3$ + 250 ppm CO and 0,4 mg Cd/m^3 + 250 ppm CO aerosols. At the end of the exposure Cd-Concentrations in livers and blood of pregnant, non-pregnant rats and fetuses were determined and clinico-chemical parameters were measured.

Results:

The exposure to 250 and 500 ppm CO caused a sharp depression in fetal hemoglobin and hematocrit , coincident with a marked reduction of the weight of the fetuses. The concentration of 125 ppm CO did not affect fetal red blood cells wile 60 ppm CO caused a slight increase in hematocrit. The hearts of the fetuses increased in weight, which was even present in the 60 ppm group in contrast to reported data of adult animals. A severe anemia rather than polycythemia occured. It was found that lung wet weights and cadmium concentrations in livers of pregnant and non-pregnant rats of the group exposed to 0,4 mg Cd + 250 ppm CO were significantly more increased than in rats exposed to 0.4 mg Cd/m^3 alone. Also non-pregnant rats exposed to 0.2 mg Cd/m^3 + 250 ppm showed increased lung wet weights in comparison to those exposed to 0.2 mg Cd/m^3 only.

The effect of cadmium and zinc aerosols on the metabolism of rats to evaluate a possible protective effect of Zn on Cd toxicity. (Weischer, Mihm, Hochrainer, Fritsche)

Material and Methods:

Rats were exposed for 3 months to Cd O + Zn O (50 $\mu g/m^3$ + 1,25 mg/m^3) (alone and in combination). At the end of the exposure Cd concentration in lung, liver and kidney were determined and clinico-chemical parameters were measured.

Results:

It could be demonstrated, that Zn has a protective effect on Cd induced alterations. The most striking effects could be seen in lung weight and cadmium levels in organs: Cd concentrations in lung, liver and kidney were significantly lower then the animals were simultaneously exposed to Zn and Cd than to Cd alone. The Cd effects on the lung were less severe than in the group exposed to Cd alone: The increase in lung weight is only 17 % in this study (Cd + Zn) and was 82 % in the study with Cd O alone. The urea cocentrations in urin had increased in the rats which were exposed to Cd alone, whereas in the combination group a decrease was found.

Conclusions:

1. Inhaled Cd is cleared from the lung with a half life of ca. 70 days, independent of its chemical form ($Cd\,Cl_2$ or Cd O) and independent of the duration of exposure. However, when inhaled together with Zinc, Cd clearance from the lung is changed and Cd content in lung, liver and kidney is lower. The critical organ for Cd after inhalational uptake is the kidney due to its long half life in this organ and to its short half life in the lung. A critical concentration in the lung is reached only after unrealistically high exposure levels. However, low concentrations in air affect certain lung functions (see 2 and 4)
2. Zn aerosols act antagonistically on Cd effects on the lung: In studies with combined exposure of Cd + Zn, no adverse effects on clearance mechanisms and lung weight were observed as compared to Cd exposure alone.
3. In non-pregnant and pregnant rats and their fetuses the antagonistic action of Zn could also be confirmed in the following parameters:

bodyweight, weights of liver, spleen and kidney; hemoglobin, hematocrit, number of leucocytes and erythrocytes, serum urea; bilirubin; serum iron; weight of placenta and Cd concentration in liver.

4. Cd aerosols increase bronchial sensitivity in concentrations which are below those for creating morphological changes. This may be of importance for people with hyperreactive airways.
5. Cd may have a carcinogenic potency on the lung when inhaled chronically.
6. Cd accumulation in CNS is very low, possible due to an effective blood brain barrier to the transport form of Cd in blood.
7. The placental tranfer of Cd and accumulation in the fetus is lower than might be expected from blood levels, due to a relatively effective placental barrier.

Future research needs regarding inhalation studies with Cd and Zn:

Resulting from our findings many new questions have been raised which should be solved in further experiments: What is the role of metallothionein in the lung and in lung macrophages when Cd is inhaled? Is there a de novo synthesis of this protein, and is it affected by Zn? How does Zn influence Cd elimination from the lung? What is the transport form of Cd from the lung into blood and other organs? Which is the route of resorption, mainly via lymph or via blood? Is this transport route and form changed by Zn? Also Cd retention in brain should be investigated together with the effectiveness of the blood brain barrier. What is the half life of Cd in brain? Finally the carcinogenic effects of inhaled Cd should be evaluated in a long term study.

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Contractor: Fraunhofer-Gesellschaft, Institut für Aerobiologie
5948 Schmallenberg-Grafschaft

Contract no: 265-77-1 ENVD

Project leader: see text

Title of project: Toxicological and behavioural studies on rats following chronic inhalation of zinc and cadmium aerosols.
Part II

Influence of inhalation of cadmium and zinc in rats on cell number and properties of alveolar macrophages .

(Spiegelberg, Otto, Fingerhut, Hochrainer).

Material and Methods:

Male rats were continously exposed to Cd O- and Cd Cl_2 aerosols up to 7,21,66 and 180 days and to Zn O-aerosols for 7 days. The combination Zn O/Cd O was applied in a concentration ratio of 25:1, which is similar to those found in the environment of factories. The concentrations of cadmium alone where 7;25, and 50 $\mu g/m^3$, of zinc alone 0,3; 0.625;1;3, and 15 mg/m^3; in the combination groups the concentration of zinc were 0.625 mg/m^3, of cadmium 25 $\mu g/m^3$. The median aerodynamic diameter of the aerosol particles was about 0.17μ m (Cd as Cd O), 0.35 μm (Cd as Cd Cl_2), 0.46μ m (Zn), 0.80 m (combination); the geometric standard deviation of the particle was 1.7 (Cd) and 2 (Zn and combination). After exposure the alveolar-macrophages were obtained by standard pulmonary lavage. The number of macrophages was directly determined in the lung washing. The proportion of macrophages, granulocytes and lymphocytes was determined by staining the preparations and counting the cells. Mitosis and diameters of macrophages were evaluated microscopically, phagocytosis was investigated by counting phagocytized fluorochromated E. coli bacteria, which had been added to the cultures of macrophages for 3 hours .

Results:

The following significant changes were observed: An increased number of granulocytes and lymphocytes, increase in size of macrophages, number of mitosis and phagocytosis. These effects were significant at cadmium exposure levels of 7 $\mu g/m^3$ for 66 days, whereas at concentrations of 25 $\mu g/m^3$ and 50 $\mu g/m^3$ these changes occurred already after 7 days. At Cd concentrations of 7 $\mu g/m^3$ for 7 days the only changes found were an increased number of granulocytes and an increase in the size of macrophages (see table 1).

The same effects could be demonstrated following a 7 days exposure to high concentrations of zinc (15 mg - 3 mg - and partly 1 mg/m^3).

The results found in the combination experiments are of special interest (0,625 mg Zn + 25 mg Cd/m^3). Some of the effects, which have been found at concentrations of 25 mg/m^3 cadmium alone (see above), could no longer be observed in both combinedly exposed groups (7 days and 66 days). These results express the antagonistic influence of zinc on the effect of cadmium.

Light- and electronmicroscopical investigations of alveolar macrophages

(Zemanek, Hochrainer)

Material and Methods:

Freshly prepared macrophages from the lung washing were centrifuged and immediately fixed. Two fixation methods were used, one for light-microscopy, the other for electron-microscopy.

Results:

Ligth-microscopical investigations:

Opposite to controls enhanced differences in size of macrophages and in the number and size of vacuoles could be detected at concentrations of 25 μg and 50 $\mu g/m^3$. The higher the concentrations of cadmium the earlier "giant cells" appeared with a diameter of about 20 μm.

Electron-microscopical investigations:

The macrophages of the controls contained three different groups of little granula in their cytoplasm. One group very likely was as phagocytized "tubularmyelin". The big vacuoles in the macrophages of the aerosol-rats could be shown to contain a lot of "myelin-bodies", more seldom structures simular to "tubularmyelin". Often parts of phagocytized cells or sometimes whole cells in the vacuoles could be found.

Measurement of enzymatic activities in alveolar macrophages after inhalation of Cd and Zn in rats.

(Muhle, Hochrainer)

Material and Methods:

Macrophages of exposed animals and controls were obtained as described above. The measurements of enzymatic activities were carried out photometrically; the following wavelengths were chosen: determination of the activity of lactate-dehydrogenase and pyruvate-kinase: 340 nm, acid-phosphatase: 405 nm, and ß-glucuronidase: 540 nm.

Results:

In enzymatic investigations we tried to find a sensitive parameter, which should show changes in the metabolism of the single macrophage. Enzymatic activities were expressed as specific activity in the extract and as activity per cell. The activities of enzymes located in the lysosomes (acid phosphatase, ß-glucuronidase) and of enzymes involved in energy-producing metabolism (e.g. pyruvate-kinase) were measured.

It could be shown that by high concentrations of cadmium (50 μ g and 25 μ g/m^3) after 7 days and low cocentrations (7 μ g/m^3) after 66 days significant differences appeared. Most important, a low concentration (7 μ g/m^3) after a long exposure (180 days) lead to significantly enhanced values in nearly all tested enzyme-activities (exception:ß-glucuronidase).On the other hand, zinc could be shown to have a lower influence on the enzyme-activities; only the highest concentration of zinc (15 mg/m^3) could be shown to cause significantly lowered values in all tested enzymatic-activities.

The result in the combination experiments elucidate the antagonistic influence of zinc. Nearly no significantly enhanced or lowered enzyme activity could be detected. An increase in ß-glucuronidase activity could only be found after 7 days of exposure, whereas after 66 days no significant change was seen. This may point to an adaptive mechanism in the macrophages metabolism, i.e. the acute effect after 7 days is reversible at a more chronic exposure (see table 3 and 4) .

Histochemical proof of enzyme activities (lysosomal enzymes) (Rühl, Hochrainer).

Material and Methods:

Part of the left half of the excised lung was put on a slide, frozen and cut in thin sections. These were air-dried and stained with simultaneous azo-coupling of the substrates.

Results:

The measurement of the lysosomale enzyme-activities was compared with the biochemical method, used by H. Muhle. The results were nearly the same. Both methods proved enhanced activities in the macrophages of rats of short time exposure and high concentrations as well as long-time exposure and low concentrations.

Measurements of the concentrations of cadmium and zinc in lung, liver, and kidney (Mihm, Hochrainer, Spiegelberg).

Material and Methods:

Rats were exposed as reported above. Cd and Zn content in the organs was determined by AAS.

Results:

The lungs of all Cd-exposed animals showed high Cd depositions. After 180 days exposure a saturation in concentration is achieved, whereas the concentration in both liver and kidney is further increasing. This different behaviour is due to the different half-times of cadmium in the organs. The half -time of cadmium in the lung was found to be about 70 days (see Part I). At exposure of long duration there is a balance between deposited and eliminated cadmium. In the kidney and liver, however, the half-time is longer and further accumulation of the metal is found.

Although zinc concentrations in the air were very high, only small amounts could be found in the lung. This is caused by the short half-time of zinc of 6-7 hours. In the combination experiments cadmium deposition in the lung was less than in the group exposed to Cd alone. Only in the 66day experiment a high concentration of cadmium was found in the kidney, too (see table 2).

In vitro studies on the protective effect of Zn Cl_2 in the Cd Cl_2 induced hemolysis. (Weischer)

Material and Methods:

Washed rabbit erythrocytes were incubated for 1 hour at 37° with Zn Cl_2 in increased concentrations and afterwards the samples were incubated with 10^{-3} M Cd Cl_2 for 3 hours. After incubation the presence of hemoglobin in the supernatant of the centrifuged samples was measured photometrically.

Results:

We noticed a 58 % inhibition of the Cd Cl_2 induced hemolysis at a Zn Cl_2 concentration of 10^{-3} M. In the concentration range of 10^{-3} M to 10^{-2} M Zn Cl_2 we found an average inhibition of 63 % of the cadmium induced hemolysis. In lower Zn Cl_2-concentration (lower than 10^{-3} M) the cadmium induced hemolysis was increased by 67 %.

Conclusions:

Zinc is shown to be less toxic than cadmium. Zinc in the combination experiments was shown to moderate the influence of cadmium; but this occurs only in a certain range and ratio of concentrations of both metals. Good correlations could be found between the activity of phagocytosis and size of macrophages. These two parameters are very sensitive for detecting changes in the behaviour of macrophages. In all of these studies, it could be demonstrated, that the alveolar macrophages react very sensitively to indicate toxic influences on the rat lung.

Cadmium as a non essential element is bound in the cells to metallothionin. This may be the reason for its long half-time in the lung of about 70 days; after inhalation significantly increased values are found in all organs. Zinc as an essential element, needed in vital-necessary processes,is probably bound to metallothionin, too, but its translocation in the body is faster. Therefore zinc has a short half-time of 6-7 hours in the lung, and the concentrations in the organs of the exposed animals are in the range of those of the controls. This is elucidated by the results of G. Oberdörster et al. (see part one).

Cadmium could be shown to increase, zinc to lower the measured enzyme activities. Zinc has an antagonistic influence to cadmium as shown in the combination experiments. It would be of interest to test the low concentration of 0.625 mg/m^3 Zn O in a long exposure time.

Table 2

concentration of ZnO/CdO mg/m^3	concentrationes metal in		
	lung	liver	kidney
15 △	↑	↑	—
3 △	↑	—	—
1 △	↑	—	—
0,625 △	↑	—	—
0,3 △	↑	—	—
0,625 + 25 µg △	— Zn ↑ Cd	— Zn ↑ Cd	— Zn ↑ Cd
dito △△△	↑ Zn ↑ Cd	— Zn ↑ Cd	— Zn ↑ Cd
of CdO / CdCl$_2$ µg / m^3			
50 △	↑	↑	↑
25 △	↑	↑	↑
7 △	↑	—	↑
dito △△	↑	↑	↑
dito △△△	↑	↑	↑
7 µg △△ CdCl$_2$ / KCl	↑	↑	↑
dito △△△	↑	↑	↑
dito △△△△	↑	↑	↑

Table 1

concentration of ZnO (mg/m^3)	number of macrophage	macrophages with several nuclei	number of mitosises	phagocytose	macrophages
15 △	↑	↓	○	↑	↑
3 △	↑	—	○	↑	↑
1 △	—	—	↑	↑	↑
0,625 △	—	—	—	↑	—
0,3 △	—	—	—	—	—
0,625 mg ZnO + 25 µg CdO △	—	—	—	↑	—
dito △△△	—	—	—	↑	—
of CdO / CdCl$_2$ µg / m^3					
50 △	↑	↑	↑	↑	↑
25 △	↑	—	↑	↑	↑
7 △	—	↑	↑	—	↑
dito △△	—	—	—	—	↑
dito △△△	↑	—	—	↑	↑
7 µg △△ CdCl$_2$ + KCl	—	—	○	—	—
dito △△△	↑	—	○	↑	↑
dito △△△△	↑	↑	↑	↑	↑

Symbols:

↑ ↓ = Values of the with Zn-or Cd-inhalated animals significantly higher-deeper than controls

— = no significant differences
○ = not determined

△ = Exposition-time: 7 days
△△ = " : 21 "
△△△ = " : 66 "
△△△△ = " : 180 "

Table 3

CdO/CdCl$_2$	fixed grown macrophages	protein	protein macrophages	acid phosphatase	acid phosphatase cell	β-glucuronidase	β-glucuronidase cell	pyruvat-cell	pyruvat-kinase cell	lactat-dehydrogenase	lactat-deydrogenase cell
50 ^	↑	↑	—	↑	—	—	—	↑	↑	↑	—
25 ^	↑	↑	—	↑	↑	↓	↓	—	—	—	—
7 ^	—	—	—	—	—	—	—	—	—	—	—
dito ^^	—	—	—	—	—	—	—	↑	—	.	- -
dito ^^^	↑	—	—	↑	↑	—	—	—	—	—	- -
7 µg ^^ CdCl$_2$	—	—	—	—	—	—	—	—	—	- .	-
dito ^^^	—	—	↑	—	↑	—	↑	- -	—	- -	-
dito ^^^^	↑	↑	—	↑	↑	—	—	↑	↑	↑	↑

Table 4

CdO/CdCl$_2$	fixed grown macrophages	protein	protein macrophages	acid phosphatase	acid phosphatase cell	β-glucuronidase	β-glucuronidase cell	pyruvat-cell	pyruvat-kinase cell	lactat-dehydrogenase	lactat-deydrogenase cell
15 ^	↑	—	↓	↓	↓	↓		○	○	↓	
3 ^	↑	—	—	—	↓	—		○	○	—	
1 ^	↓	—	—	—	—	—		○	○	—	
0,625 ^	—	—	—	—	—	—		○	○	-	
0,3 ^	↓	—	—	↓	—	—		—			
0,625 ZnO 25 g CdO ^	—	—	—	—	—	↑		↑		-	
dito ^^^	—	—	—	—	—	—		↑		-	

Contractor:	Med. Institut für Lufthygiene und Silikoseforschung, Düsseldorf
Contract No.:	289-76-10 ENVD, Project 3
Project leaders:	Prof. Dr. med. H.-W. Schlipköter Prof. Dr. med. J. Bruch
Title of project:	Local effect of lead on lungs after inhalation of lead aerosols a) Inhibition of degradation of carcinogenic polyaromatic compounds b) Deterioration of lung clearance c) Impaired resistance to bacteria

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Two test series were run to investigate the local effect of lead compounds after inhalation. The first test series was to examine the extent to which low concentrations of lead chloride aerosols have an effect on the elimination of bacteria from the lungs. The total duration of these tests was 15 days, the mice inhaled lead chloride during 5 days per week with a concentration of 15 $\mu g/m^3$. Two subgroups were chosen where one part of the animals inhaled the aforesaid lead chloride concentration only for 1 hour a day while another part did so for 3 hours per day. The effects were determined through the count of bacteria retained in the lungs, through the number of alveolar macrophages and the number of macrophages phagocytizing bacteria. The results are shown in figures 1 to 6. The columns indicate that variations of the parameters selected are comparatively high over the test period; merely after a 3-hour exposure to lead chloride of a concentration of 15 $\mu g/m^3$, differences in bacteria retention can be determined for each day. A 1-hour exposure to the aforesaid concentration seems to have no effect on the parameters selected.

The objective of a second test series was to investigate the extent to which additional noxae, such as SO_2 and NO_2, have a synergistic effect with simultaneous exposure to lead chloride. Total duration of this series was 4 weeks, at the end of one week the animal groups were exposed to a bacteria-conteining aerosol, and the lungs evaluated as described above by numbers of bacteria, macrophages and phagocytizing macrophages. Since, in this case, the synergistic effect was the focal point of the evaluation, merely the differences versus the control were used for the graphs in favour of the better comparison of results of the experimental animal groups. Table 1 represents a summary of the findings and results. As the symbols show the results of the second test series fully confirm those of the first one; in addition, an analysis of possible additional effects could be elicited through the simultaneously inhaled noxious gases SO_2 and NO_2. The SO_2 concentration was 0.3 mg/m^3 and that of NO_2 0.3 mg/m^3,too, continuously inhaled over the entire test period. Mean lead concentration was 20 $\mu g/m^3$, exposure to lead for 8 hours daily on 5 days per week. From the comprehensive data material submitted can be seen that the additionally administered noxious substances entail no further deterioration with respect to the parameters selected. Both the number of bacteria retained in the lungs and the alveolar macrophages showed no additional effects with the synergistic action of SO_2 or NO_2 together with $PbCl_2$ as compared to the administration of $PbCl_2$ alone.

The interpretation of the results and findings from the angle of an environmental strain on man through inhaled lead compounds leads to the conclusion that, for the lead compound selected, viz. $PbCl_2$, and for the chosen concentration used in the tests, a lower range of possible effects has been covered. The variations of parameters within the control groups are approximately of the same order of magnitude as the effects of the median differences caused by lead. Future tests and experiments shall clarify the extent to which other lead compounds, especially the PbO aerosols which are hardly soluble in water, have a stronger local toxic effect on lungs.

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Zbl. Bakt. Hyg., I. Abt. Orig.B. 1979 im Druck.

Aerosol tested	1 week			2 weeks			3 weeks			4 weeks		
	Bact.	Macr.⁺	pH⁺⁺	Bact.	Macr.	pH	Bact.	Macr.	pH	Bact.	Macr.	pH
$PbCl_2$	↑	↑	↓↓	—	↓↓↓	↓↓↓	—	—	↓↓↓	↑↑↑	—	↓↓↓
$PbCl_2+SO_2$	↑	—	↓↓↓	—	↓↓	↓↓↓	—	↑	↓↓↓	↑↑	—	↓↓↓
$PbCl_2+NO_2$	↑↑	↑	↓↓↓	—	↓↓↓	↓↓↓	—	—	↓↓↓	↑	—	↓↓↓
NO_2	—	—	↓↓↓	—	↓↓	—	—	—	—	↑	↑↑↑	↓↓↓
SO_2	—	—	↓↓↓	↑↑	—	—	—	—	—	↑↑	—	—
SO_2+NO_2	—	—	↓↓	—	—	—	—	—	↓↓↓	↑↑	—	↓↓
$PbCl_2+$ SO_2+NO_2	↑	—	↓↓↓	—	—	↑	↑	↑↑	↓↓	—	↑	—

Explanation ↑, ↓ ≙ * $p < 0.05$
↑↑; ↓↓ ≙ ** $p < 0.01$
↑↑↑; ↓↓↓ ≙ *** $p < 0.001$

↑ = increase of No bacteria resp. macrophages
↓ = decrease of No bact. resp. macrophages

macr. = macrophages
p.m. = phagocyt. macrophages
bact. = bacteria

Tab. 1: All t-Test experimental- vs control-groupe

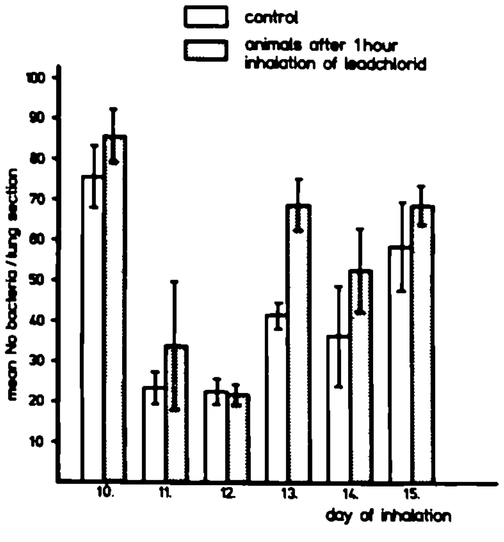


Fig. 1.

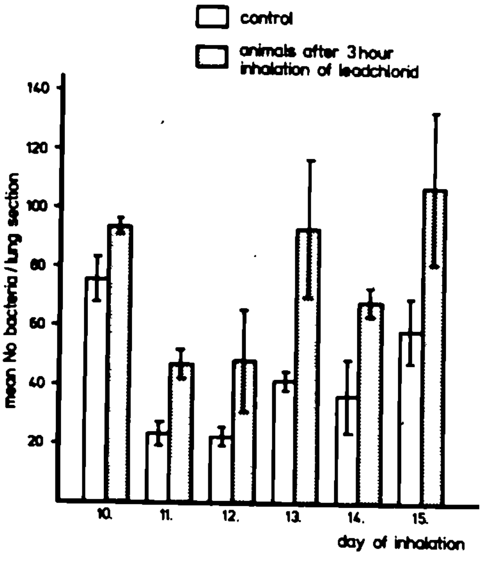


Fig. 2.

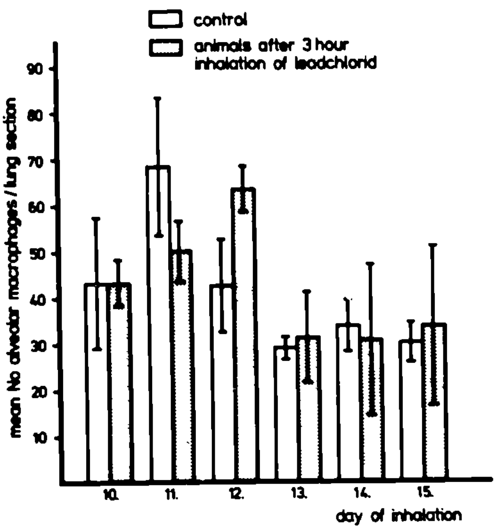


Fig. 3.

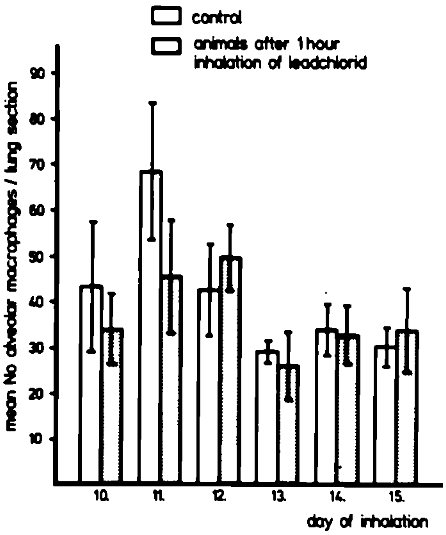


Fig. 4.

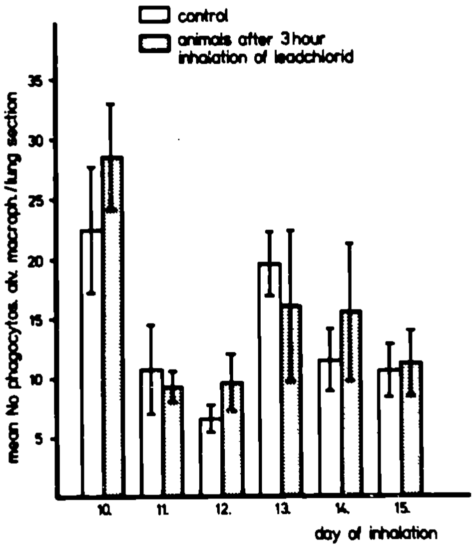


Fig. 5.

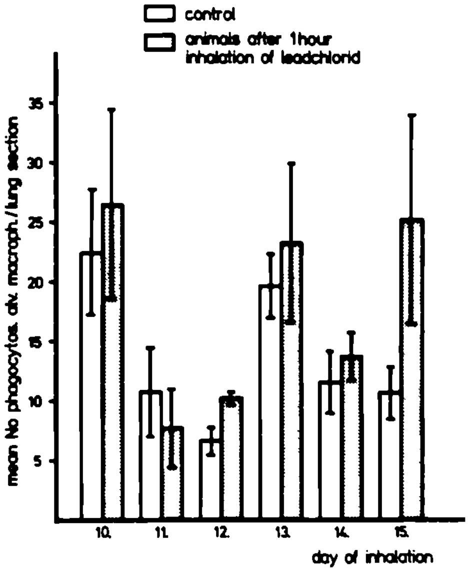


Fig. 6.

Contractor:	Med. Institut für Lufthygiene und Silikoseforschung an der Universität Düsseldorf
Contract no.:	289-76-10 ENVD, Project 4
Project leaders:	Prof. Dr.med. H.-W. Schlipköter Dr. rer.nat. G. Winneke
Title of project:	Behavioral studies on the effects of ingested lead on the developing central nervous system of rats.

Objective of research

Subsequent to earlier findings of Pb-induced impairment of discrimination-learning and of hyperactivity in rats (WINNEKE et al.,1977), the present research deals with two questions:

1. Is lead-induced neurobehavioral deficit reversible, i.e. do the effects disappear at normalized blood-lead-levels?
2. Can a no-effect-level for neurobehavioral deficit be demonstrated at PbB-levels below 25 $/ug/dl?

Mainly for this last topic systematic feeding-studies were run to determine the relationship between Pb in diet (PbD) and Pb in blood (PbB).

Materials and methods

1. Relation between lead in diet (PbD) and lead in blood (PbB)

Lead was given as lead-acetate in a standard laboratory diet (ALTROMIN 1310) to female rats with an initial body weight between 160 and 180 g for a maximum period of 150 d. Blood for PbB-determination was taken retroorbitally and analyzed by flameless atomic absorption photometry (AAS) after pretreatment with strong oxidizing acids and extraction by chelating agents into organic solution.Single PbB-values are based on blood-pools of 3-4 animals each, and two determinations were made in most cases.

2. Reversibility of neurobehavioral Pb-effects

Three experiments were run under this heading, all of which were evaluated in a completely blind manner according to the procedures described in detail elsewhere (WINNEKE et al.,1977). Discrimination-learning was tested in a modified LASHLEY jumping-stand, using a size discrimination-problem, whereas activity and exploratory behavior was assessed by means of an open field-device. Experiment I: Male offspring (n=18) of lead-fed (diet H, see below) dams were fed the same diet until being 120 d old; subsequently lead-feeding was discontinued for 4 months until testing. Experiment II: Three groups (n=10) of male rats were formed as follows: A - Continuous pre- and postnatal lead-exposure (diet F, see below) until testing; B - maternal lead-exposure (diet F) until weaning only; C - Controls. Experiment III: 5 groups were formed from control-dams and Pb-fed dams (diet F). A (n=14) - continuous lead-exposure until testing; B (n=12) - maternal lead-exposure until weaning only; C (n=14) - Controls; D and E - cross-fostering groups, which can be disregarded in this context.

3. No-effect-level for neurobehavioral dysfunction

One experiment was performed under this heading. Behavioral testing was done as indicated above. In addition to the tasks already mentioned a water-maze test for position-learning was introduced. Animals were placed in a water-maze and learned to escape over a ladder by turning to the right(simple position learning). One week later these animals were retested for retrieval and were then tested for habit reversal with the escape-ladder placed left. Three groups (n=10) were formed: Control-group; exposure-group A - continuous exposure (modified diet E; see below); exposure-group B - continuous exposure (diet F).

Results

1. Relation between lead in diet (PbD) and lead in blood(PbB)

Since only two determinations were made in most cases means and ranges are given in table 1. Diets A (0.25 ppm) and B (2,5 ppm) did not result in any detectable PbB-increase above control-levels and are therefore omitted from table 1.

diet	Pb-content (ppm)		days of feeding 30	40	50	80	130	150 d
		mean		5.4	5.6			
C	25		-			discontinued		
		range		4.7-6.0	5.2-6.0			
		mean			8.1		10.7	
D	75		-	-		-		-
		range			7.7-8.5		9.2-12.2	
		mean			15.3		18.3	
E	250		-	-		-		-
		range			14.3-16.3		17.0-19.5	
		mean*)			24.2		31.2	
F	750		-	-		-		-
		range			22.3-26.1		28.8-33.5	
		mean*)	41.3			53.4		82.6
H	2260			-	-			
		range	41.3			51.9-54.8		82.6
		mean*)			3.4		4.7	
CONTROL			-	-		-		-
		range			3.4-3.5		4.1-5.3	

*) data taken from previous feeding-experiments

TABLE 1: Blood-lead-levels (µg/dl), given as means and ranges, resulting from feeding different diets containing between 25 and 2260 ppm lead as Pb-acetate to rats for periods from 30 to 150 days.

Assuming an average daily food-intake of 20 g for adult rats, these diets correspond roughly to the following daily lead-ingestion: 2, 6, 20, 60, 180 mg Pb/kg for diets C, D, E, F, and H, respectively.

2. Reversibility of neurobehavioral Pb-effects

Blood-lead-levels: PbBs in the different experiments were as follows: Experiment I: 39.5 µg/dl at the end of postnatal lead-exposure and 12.0 µg/dl after 4 months of lead-deprivation; PbB in controls was always below 5 µg/dl. Experiment II: Group A - 29.2 at weaning and 27.0 before testing; group B - 29.2

at weaning and 5.2 before testing; C - below 5.0 throughout. Experiment III: Group A - 29.9 at weaning and 30.8 µg/dl before testing; group B - 29.9 at weaning and 1.8 before testing; group C below 5 µg/dl.

Learning-data: As for learning-speed (i.e. mean days-to-criterion) no significant deficit could be shown for the lead-exposed animals as compared to controls, neither for the high exposed animals after PbB-normalization (exp. I) nor for continuously or only maternally exposed rats (exp.II and III). There was, however, a significant increase of error-repetition in the previously Pb-exposed animals of experiment I after PbB-normalization ($p < 0.05$), and the same graded tendency of only borderline significance ($p < 0.10$) for triple-errors in exp. II, with controls being lowest, group B-animals (maternal exposure only) intermediate, and group A-animals (continuous exposure) highest. All control-animals of exp. II did learn the discrimination-task within 27 days of training, whereas only 8(group A) or 7 (group B) of both lead-groups did.

Open-field-data: The lead-exposed animals of exp. I (diet H) did display significant hypoactivity ($p < 0.05$) after PbB-normalization, but no significant changes for rearing or grooming. No significant hyperactivity could be shown for experiments II and III, although, combining results from both these experiments, ambulation-scores (no. of segments crossed) increased from control-level (31.3), over maternally exposed animals (33.2), to continuously lead-exposed rats (34.8).

3. No-effect-level

Blood-lead-levels: PbB at about age 120 d was 3.8 (±2.7) for controls, 17.8 (±1.5) for lead-group A, and 28.6 (±4.3) µg/dl for lead-group B.

Learning-data: Only one control-animal did not learn the task within 27 days of training, whereas 7 out of both lead-groups didn't (fig. 1). Control-animals needed 19.5 days on the average to reach the criterion of successful learning (max. 2 errors on two consecutive days), whereas the lead-fed animals needed 23.6 (A) and 23.4 (B) days, respectively (fig. 1). This deficit of both lead-groups as compared to controls was of only borderline significance ($p < 0.10$), however. Learning-performance in the water-maze-test did not reveal group-differences for initial position-learning or habit-reversal, but for retrieval of the learned habit after one week without training; whereas all controls remained errorfree on retesting, 3 (B) to 4 (A) of the lead-treated animals were unable to retrieve the learned habit without errors (fig. 2).

Open-field-data: For ambulation significant group-differences ($p < 0.05$) indicating hyperactivity, as well as a significant "groups x days"-interaction ($p < 0.01$) occurred; whereas control-animals did adapt to the open-field-situation, both lead-groups did not (fig. 3). For days 4 and 5 clear dose-effect-relations are apparent. No significant group-differences were found for rearing- and grooming-behavior.

Conclusions

As for the relation of lead-intake to blood-lead-concentration in rats it is obvious, that comparatively large amounts of lead are necessary, to arrive at elevated PbB-values by the route of ingestion (table 1). This indicates pronounced differences between this animal model and human conditions for enteral lead-resorption and/or distribution-kinetics between the different body-compartments.
In a qualitative sense our model is, however, well suited to deal with the two questions of this research: The question of recovery of Pb-induced neurobehavioral deficit and the question of the vulnerability of CNS-functions to low-level PbB-elevation. As for the first question no definite conclusions can presently be drawn for learning-speed and hyperactivity. It is clear, however, that learning-performance is still affected in previously Pb-exposed animals after PbB-decrement to about background-levels. In these animals error-repetition is still markedly increased, either significantly so (exp. I) or at least tendentially (exp. II). With respect to hyperactivity as

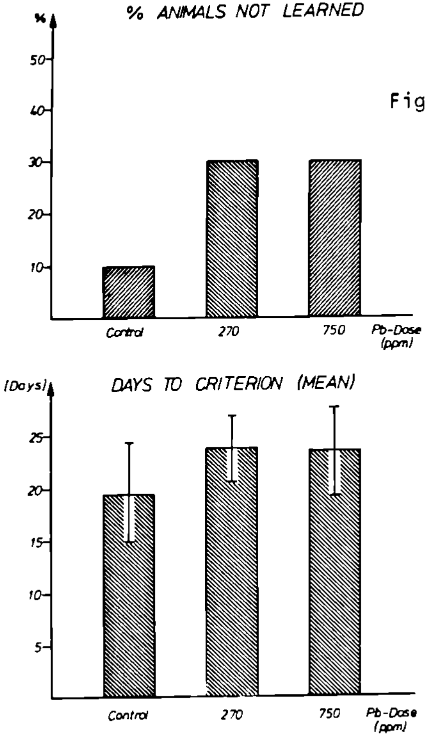
a key-symptom of low-level Pb-exposure no final conclusions can be drawn for the question of symptom-recovery, because it proved difficult to produce this effect in a clearcut manner in these experiments. From the preliminary data of experiments II and III it would seem suggestive, however, that the degree of lead-induced hyperactivity is reduced in maternally exposed animals raised on a lead-free diet later on. Additional evidence is necessary, however.

With respect to the vulnerability of the developing CNS to low-level PbB-elevation it is clear from the data presented here, that 20 μg/dl does not constitute a no-effect-level in rats: Discrimination-learning was retarded, retrieval was disturbed upon retesting in the water-maze, and there was a clear indication of hyperactivity in these animals. Since, however, any straightforward extrapolation of these findings to man would seem premature for the reasons given above, it is necessary, in our opinion, to narrow the gap between rodent models of CNS-dysfunction and human conditions by using subhuman primates as experimental subjects as well.

Publications

Winneke, G., A. Brockhaus, and R. Baltissen: Neurobehavioral and systemic effects of longterm blood-lead-elevation in rats. I. Discrimination-learning and open-field behavior.
Arch. Toxicol., 37 (1977), 247-263

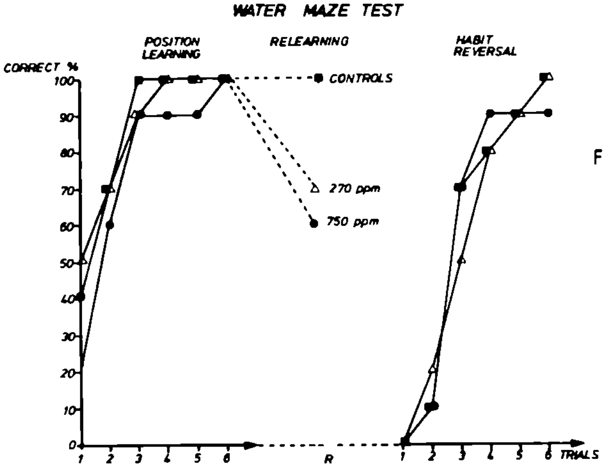
Krass, B., G. Winneke, U. Krämer und N. Grieser: Neuropsychologische und systemische Befunde an früher bleiexponierten Ratten nach Normalisierung ihres Blutb leispiegels.
To be submitted to Zbl. Bakt. Hyg., I. Abt. Orig. B (1979)



% ANIMALS NOT LEARNED

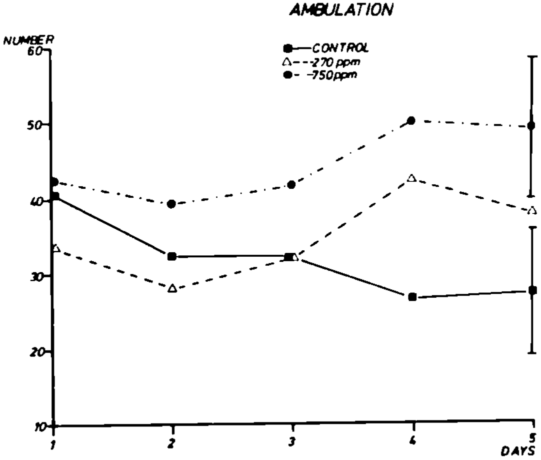
DAYS TO CRITERION (MEAN)

Fig. 1.



WATER MAZE TEST

Fig. 2



AMBULATION

Fig. 3.

Figure 1: Results for discrimination-learning for controls and and two groups of lead-treated rats. The upper part gives the percentage of animals, which did not learn the task within the time-limit of 27 days, the lower gives the average days-to-criterion (means and S.E.M)

Figure 2: Performance in the water t-maze test (% errorfree animals). Initial position-learning (left), retesting after one week's interval (middle), and habit-reversal-learning with the escape-ladder placed opposite are given.

Figure 3: Ambulation-scores (no. of segments entered within 2 min. observation-time) of the open-field-test for 5 consecutive days of testing. Confidence-limits (95%) are given for the 5th day only.

Contractor : Ecole Nationale Vétérinaire, 23, chemin des Capelles,
31076 TOULOUSE CEDEX France.

Contract n° : 148-77-1 E.N.V.F.

Project leader : Professeur RICO André.

Title of project : Medium- and long-term toxicity of the cadmium ion in rats : biochemical and metabolic studies.

Previous studies (Refs. 1 and 2) have shown that in rats slightly contaminated by cadmium (2.5 ppm in drinking water for a year) cold stress could reveal a latent renal dysfunction. The origin of this phenomenon is the subject of the present research; preliminary biochemical results and metabolic data are reported.

I. MATERIAL AND METHODS :

1.1 - Biochemical aspect :

The experiments were carried out on four batches of rats of Cobs-CD (Charles River France) : TT, TF, CdF and CdT strains comprising 20 animals each (10 males, 10 females). For six months batches CdT and CdF received drinking water supplemented with cadmium (2.5 ppm); batches TT and TF were supplied with ordinary drinking water (1 ppb of Cd). At the end of this period batches TF and CdF underwent a cold stress (+ 1°C-48 h), and then were immediately sacrificed and compared with batches TT and CdT kept at normal room temperature; the following biochemical parameters were determined :

-urine : urea, creatinine, sodium, potassium, phosphates, catecholamines.
-blood : sodium, potassium, calcium, chlorides, phosphates, bicarbonates, glucose, urea, proteins, PAL, albumin, renin-angiotensin.

1.2 - Metabolic aspect :

The kinetics of cadmium -109 have been studied in particular by macroscopic autoradiography according to the ULLBERG technique in mice and rats and in the case of the latter, by oral route before and after cold stress. Measurements in liquid scintillation have also been carried out.

II. RESULTS :

2.1 - Biochemical :

2.1.1 - Urinary :

In the two batches TF and CdF, cold gives rise to considerable and comparable polyuria; it is accompanied by an escape of potassium, phosphates, urea and creatinine.

The adrenergic response to the aggression is clearly reflected in the increase of urinary catecholamines.

On the other hand, natruresis remains stable in the TF batch by reason of very significant sodium hypotonia. This does not occur in the animals which received cadmium (CdF).

	TT	TF	P	CdT	CdF	P
Sodium : mEq/l	102.2	56.3	0.1	100.1	81.4	NS
m ± SD (mean ± standard deviation)	45.6	33.5		31.7	37.3	
Sodium : mEq: daily/kg	2.46	3.18	NS	1.76	3.55	1.0
m ± SD	1.15	2.48		1.17	2.26	

NS : not significant.

2.1.2 - Serum *(Table 1)*

Hyperuricemia is observed in all the animals subjected to cold. In the TF batch, hypokalemia and slight acidosis are also noted, the other parameters being stable. In the CdF batch, only hyperphosphoremia and hypoaldosteronemia appear.

These results were obtained with animals whose renal cadmium content (fresh tissue) was 0.44 ± 0.19 ppm in the case of TF and 3.16 ± 2.29 in the case of CdF and which did not show any statistically significant difference at room temperature : under these conditions, therefore, they were perfectly normal.

2.2 - Metabolic :

Distribution : in mice, the target organs are the kidney, the liver, the pancreas and the testicle. The same applies to rats, but by oral route absorption is very limited *(Table 2)*.

III. DISCUSSION :

3.1 - From the biological point of view :

Subject to a more detailed statistical analysis (in this result (Test t)) these experiments show that :

1. Under normal conditions of rearing and in relation to the parameters selected, the contamined animals prove to be similar to the controls.
2. The two batches subjected to cold have a similar polyuria with medullo-adrenal involvement. After six months of contamination, therefore, cadmium does not appear to interfere with the overall response to the stress, in contrast to what we had previously observed after 12 months, when the slightest polyuria in the animals which had received cadmium was quite distinct.
3. In spite of that, there are differecences between the TF and CdF batches : for instance, hypokalemia and urinary sodium hypotonia are absent in the animals which received cadmium. This phenomenon could be due to a difference in hormonal response, mineral or corticosteroid, especially as aldosteronemia is distinctly reduced in the animals which received cadmium. However, this hypothesis cannot be confirmed by the exploration of the renin-angiotensin-aldosterone axis at present being carried out, since the serum determinations were performed at different points of time and therefore do not have the value of a true conspectus; the experiments after 12 months will be chan-

ged accordingly.
It is, moreover, worth while emphasizing that the stability of kaliemia and the hyperphosphatemia, without acidosis, observed in the CdF batch confirm the previous results over 12 months (Ref. 2). The impact of cadmium on the metabolism of phosphorus (and calcium) therefore seems to be confirmed and to be early.

3.2 - From the metabolic point of view :

Distribution in rats shows that intestinal absorption is low : the target organs, although not very marked, are the kidney, the liver and the pancreas. The kinetics do not seem to be changed by the cold stress.

By way of conclusion, these experiments after six months partly confirm the results obtained in a previous study, i.e., the possibility, after regular ingestion of toxic substances in low doses, of revealing, by means of an appropriate stress, underlying anomalies in the functioning of target organs in this case, with cadmium, the kidney. Our results are not fully processed ; in particular the one-year contamination experiment is being continued. In the contest of this experiment in the light of the information obtained, attention will directed to renin-angiotensin-aldosterone and 17-ketosteroids hormonal balances in order to improve our knowledge about the disturbed biochemical mechanisms.
From the metabolic point of view, the studies with cadmium -109 are being continued and here too we are attempting to demonstrate that an influence is exercised by contamination on the observed kinetics of the warm product either without or with stress.

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(Long-term toxicity of cadmium administerd in very low doses in rats : distribution of cadmium, zinc and copper).

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(Long-term toxicity of cadmium administered in very low doses in rats : response to agression by cold).

SERUM FINDINGS

(mean ± standardeviation)

	TT	TF	P	CdT	CdF	P
Sodium mEq/l	137.6 6.7	136.2 7.7	NS	138.1 7.3	141.5 7.0	NS
Potassium mEq/l	4.99 0.78	4.25 0.6	0.1	4.70 0.85	4.31 0.42	NS
Calcium mg/l	91.6 8.5	94.1 7.4	NS	92.6 13.3	97.7 5.7	NS
Chlorides mEq/l	102.7 2.1	104.5 6.2	NS	105.7 3.0	106.9 2.0	NS
Phosphates mg/l	52.1 6.3	53.9 8.4	NS	52.1 6.1	61.4 6.3	0.1
Bicarbonates mEq/l	24.5 3.0	22.2 2.2	2.0	22.5 3.9	23.7 2.1	NS
Glucose g/l	1.27 0.12	1.27 0.20	NS	1.29 0.20	1.31 0.15	NS
Urea g/l	0.29 0.06	0.48 0.11	0.1	0.29 0.09	0.50 0.07	0.1
Proteins g/l	58.68 9.59	61.82 4.48	NS	64.8 8.7	61.6 5.9	NS
PAL U/l	71.1 27.5	80.6 25.5	NS	75.8 22.6	74.6 26.0	NS
Aldostérone ng/100 ml	31.8 18.2	26.0 18.3	NS	39.4 17.6	25.8 13.5	1.0
ARP ng/ml/hour	5.2 2.2	6.1 2.9	NS	7.0 5.4	7.4 4.0	NS
Activity of plasmatic renin)						

n = 20 (10 males - 10 females)

NS = no significant.

Table 1

	2 hours		4 hours		8 hours		24 hours		48 hours		8 days	
	21°C	2°C	21°C	2°C	21°C	2°C	21°C	2°C	21°C	2°C	21°C	2°C
Encephalon	0	0	0	0	0							
Blood			0				0					
Cardiac muscle												
Lungs												
Gastric contents	+++++	+++++	+++++	0	+++++	+	0	0	0	0	0	0
Intestinal contents	+++++	+++++	+++++	+++	+++++	+++	++	++	(+)	(+)	0	0
Cecal contents			++++	+++++	++++	++++	+++	+++	+	+	0	0
Rectal contents			++	+++++	++++	+++++	+++++	++++	+	++	0	0
Gastric mucous membrane	+	+	+	(+)	(+)	(+)	+	(+)	(+)	(+)	(+)	(+)
Intestinal mucous membrane	+	+		+++	++	+++	++	++++	+	+	(+)	(+)
Muscles												
Liver			(+)		(+)	-	(+)	(+)	(+)	(+)	(+)	0
Kidney (parenchyma)	(+)		(+)	(+)	(+)	-	+	+	+	+	(+)	(+)
Suprarenal gland												
Pancreas							(+)	(+)	(+)	(+)	(+)	0

Distribution of cadmium -109 in rats after oral administration without stress and with stress.

Table 2

Contractor : INSTITUT PASTEUR DE LYON ET DU SUD EST
Contract n° 269 77 1 ENV F
Project leader : Professeur Maurice CARRAZ - Pierre TACHON
Title of project : Experimental lead poisoning in the Macacus irus monkey

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I - OBJECTIVE

The aim of the research is to determine the metabolic and histological anomalies caused by a weak dose of lead, administered orally.

II - MATERIALS AND METHODS

II - 1 - Histological anomalies

The work undertaken for the histological examination of different organs continues the work carried out in the contract n° 502 74 1 ENV F.
After poisoning 8 Macacus irus with lead acetate for 12 months, 4 animals were given up for histological examination. The studies carried out by optical microscopy were made on the following organs : liver, spleen, kidneys, testicles, adrenal glands and intestines. The electronic study was only carried out on the kidneys.

II - 2 - Metabolic anomalies

The experiment was based on 15 Macacus irus intoxicated daily with lead acetate, administered via the oesophagus. The animals were divided into three groups: 1 control group; and 2 treated groups (1 mg and 5 mg of Pb ++/24 h.) Among the animals there were on the one hand animals which had undergone 2 successive poisonings (1 year + 1 year) separated by a period of a year's rest, and on the other hand monkeys which were poisoned for the first time.

III - RESULTS

III - 1 - Histological Examination

The experiments were carried out on 4 animals which had been poisoned

with lead (4 and 10 mg Pb^{++}/24 h for one year). Certain organs showed an increase in sensitivity compared to others.
In fact, according to the examination by optical microscopy of the liver the duodenum, the spleen and the testicles, no important lesions were observed. These organs had a practically normal histological appearance. On the other hand, the intoxicated animals showed important lesions affecting the proximal tubes. Detailed analysis revealed that these lesions were produced by intranuclear inclusions larger than the nucleole with a homogeneous appearance and enclosing a certain quantity of lead probably bound up with proteins. The microanalysis showed an emission of X ray characteristic of the L bands of lead.

III - 2 - Metabolic examination

III - 2 - 1 - Weight curve : during the first 9 months of poisoning the animals underwent a weekly weight check. The weight development was shown to be normal throughout the experiment.

III - 2 - 2 - Development of the parameters connected with haem biosynthesis in the animals subjected to two successive intoxications separated by a rest period of one year :

a) UROPORPHYRIN : the levels of uroporphyrin in the control animals remained stable and very weak throughout the three years of observation. Regarding the intoxicated animals, the first poisoning did not provoke any significant increase in the levels of uroporphyrin, while the second seemed to cause more distinct increases. It seems however that in any given animal this elimination is not constant in time and is subject to large variations.

b) COPROPORPHYRINS : the intoxication causes an increase in the elimination of copproporphyrins. During the rest period, the levels returned to normal. It seems that the peak levels of elimination do not form a direct relation with the dose of lead administered.

c) DELTA-AMINOLEVULINIC ACID : the mean level of urinary elimination was 0,2 mg/24 h. in the control monkeys. In the treated animals the first intoxication did not cause a noticeable increase in elimination. However the second intoxication caused important increases in elimination. These observations were made from the fifth month of poisoning onwards.

III - 2 - 3 - Study of the biological parameters in the 15 intoxicated animals :

a) BLOOD LEAD : after the second intoxication, the blood lead level stabilised respectively at : 20 µg/l in the control group, 194 µg/l in the group treated at 1 mg Pb^{++}/24 h. and 201 µg/l in the group treated at 5 mg Pb^{++}/24 h.

b) BLOOD PARAMETERS :

- hematocrit and δ-amino-levulinic acid dehydratase erythrocitic (ALA-D) : no variation has been recorded up until now.

- coproporphyrins : after 5 months of intoxication, the groups treated at 1 mg and 5 mg showed a larger level of coproporphyrins (20.3. µg % and 17.7 µg %) than the average level of the control group (11.5 µg %). These differences tend to lessen after 7 months of treatment.

- Protoporphyrins : the mean level recorded in the intoxicated group is clearly greater than in the control group. However, the wide range of results makes any interpretation hazardous.

- Also throughout the experiment we have carried out an examination of serous proteins using electro and immunoelectrophoretic techniques (in cellulose acetate, in polyacrylamide gel and in agarose), an examination completed by a specific study of certain proteins (Immunoglobulins, Albumin, Transferrin, Retinol Binding Protein and Orosomucoid) according to the principle of immunoprecipitation.

The different traces obtained were shown to be normal. The examination of the serous values of Retinol Binding protein and of Tranferrin shows an overall diminution because of the dose adminstered.

After 4 months of intoxication, the levels of Immunoglobulins G, A and M diminished in the treated groups.

c) URINARY PARAMETERS :

- delta aminolevulinic acid : after 5 months of treatment no great difference was noted. After 7 months of intoxication an obvious increase in the level of urinary delta aminolevulinic acid was noticed in the treatment groups.

- coproporphyrins and uroporphyrins : in the 7 th month of poisoning, we noticed a clear increase in the elimination of coproporphyrins

- aminoaciduria : the excretion of aminoacids in the urine was studied in all the animals after 5 and 8 months of intoxication. When we compared the average amount of aminoacids in the control group and the treated groups we rarely observed a statistically significant difference as the results were frequently dispersed and the number of animals too small. However in these two samples we observed distinct variations in certain amino-acids. Thus when we compared the control group with the group treated with 5 mg Pb^{++}/24 h. the levels of elimination were increased in 13 amino acids out of the 21 studied. Regarding the group treated with 1mg Pb^{++}/24 h. 7 amino acids were eliminated in increased quantities.

- No evident glomerular or tubular trouble was found by electrophoretic techniques.

Equally the renal clearance of certain proteins (Transferrin, Orosomucoide and Retinol Binding Protein) appeared normal throughout the experiment.

III - 3 - Genetic Study

Chromosome investigations have been carried out on each animal, in collaboration with the CEN, Mol, Belgium.

IV - CONCLUSIONS

The results obtained at present, although partial, show the harmful effects of even weak doses of Pb on the renal function.

In fact, although we do not obtain clear indications of glomerular or tubular troubles by electrophoretic techniques, the increased elimination of certain amino acids seems to indicate a renal malfunction.

From the histological point of view, the studies show the appearance of lesions at the level of the proximal tubes of the kidneys. These lesions seem to be reversible.

In fact, the examination of the animals poisoned twice with an interval of one year showed that the different parameters which appeared modified during the first poisoning tended to return to normal after the "rest" period.

Generally, we don't note any important blood modification, but, in the same time, we note same urinary changes.

The subsequent study of the kidneys will allow us to verify the present data.

In addition, histological examinations of the brain, currently in progress will provide indications relating to the action of Pb^{++} on the central nervous system of primates.

Contractor: UNIVERSITA' DEGLI STUDI DI ROMA, ROMA
Contract n° 154 - 77 - 1 ENV I
Project Leaders: Prof. V. Carunchio and Prof. L. Caprino
Title of project: Cadmium metabolism in laboratory animals in relation to the chemically active forms and to some biological and pathological factors

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Objective of the research

The aim of the research is to evaluate cadmium effects on prostacyclin-like activity shown by rat and rabbit arterial tissue on platelet aggregation "in vitro" and "ex vivo" and cadmium effects on platelet sensitivity to some aggregating agents; and also to evaluate the results obtained in relation to cadmium distribution in tissues and its metabolism as well as to its chemically active forms and to some biological and pathological factors.
This research was undertaken in order to contribute to the explanation of the thrombosis of glomerular and peritubular capillaries of the selective damage to the capillary and venular endothelium, and of the arterial hypertension.

Materials and methods

A) Evaluation "in vitro" of prostacyclin-like material

Male Wistar rats were anesthetized with pentobarbital. Abdominal aorta were rapidly removed and processed.
About 20 mg of aortic rings were incubated for 5 min at 37°C in 200 μl Tris buffer solution (0.05 M, pH 7,4) containing $CdCl_2$ at different concentrations (1.1, 4.4, or 17.6×10^{-4}M). Fifty microliters of the supernatant was added to 1 ml of rabbit platelet-rich plasma (PRP) 1 min before addition of sodium arachidonate (AA, 150 μM) as platelet aggregating agent.
In other experiments the effect of $CdCl_2$ on the antiaggregating activity of rat aortic rings was assessed. Aortic rings (about 10 mg) pretreated with $CdCl_2$ (3, 6, or 12×10^{-4} M) for 5 min at 37°C were transferred to rabbit PRP (1 ml) and stirred at 37°C for 10 min. Sodium arachidonate (150 μM) and adenosine-5'-diphosphate (ADP, 6 μM) were employed to induce platelet aggregation. Platelet aggregation was monitored by continuous recording of light transmission in Born aggregometer. Cadmium activity on AA-induced platelet aggregation was also tested at doses of 0.135, 0.27, and 2.7×10^{-3}M. Aspirin was used as a reference drug.

B) Evaluation "in vitro" of prostacyclin-like material after "in vitro" dosing ("Ex vivo" tests).

In these tests, 120 male adult rats of the Wistar strain and 20 male New-Zealand rabbits were treated according to the following dosing schedule:
Rats

a) single subcutaneous $CdCl_2$ injection (10 and 35 mg/kg) and sacrifice 8 and 24 hours and 7 days later
b) single intra-arterial $CdCl_2$ injection (0.5 and 1 mg/kg) and sacrifice 15 and 30 mins later
c) single intravenous $CdCl_2$ injection (2 and 4 mg/kg) and sacrifice 15 and 30 mins later
d) single 0,1 mg/kg intravenous injection of $CdCl_2$ (sacrifice 30 mins and 3 days later)

e) 2 mg/kg intraperitoneal injection of Cd^{++} (as acetate) followed, 21 days later, by a second injection of 1 mg/kg of Cd^{++} (sacrifice 3, 10 and 15 days after the last administration)
f) intraperitoneal injection 1,5 mg/kg/day repeated for 5 days of Cd^{++} (as chloride). The animals were sacrificed at the end of the treatment and 1 and 3 days later
g) prolonged administration for two months of cadmium acetate, at the doses of 2, 20 and 200 ppm, mixed into the drinking water (deionized). The sacrifice of the animals was performed at the half and at the end of the treatment period.

Rabbits

a) single 5 mg/kg intra-arterial injection of $CdCl_2$ (sacrifice 15 and 30 mins later)
b) single 5 mg/kg intravenous injection of $CdCl_2$ (sacrifice 15 and 30 mins later)
c) $CdCl_2$ intravenous repeated for 3 days at the doses of 200 and 1000 µg/kg/day (sacrifice at the end of the treatment).
d) $CdCl_2$ injection by intravenous and subcutaneous route (2 mg/kg each) simultaneously, repeated for three days.

At different time interval from the end of the treatment, the animals were sacrificed and the abdominal aorta and femoral veins were drawn, following the methods of Moncada.
The prostacyclin-synthesizing and releasing activity of the vessel-wall was assayed by the methods described in the previous paragraph and evaluated as inhibition ratio of the aggregating activity induced by arachidonic acid on rabbit' plateled rich plasma.
At the end of the treatment, from some rats of each experimental group the following organs: lungs, liver, kidney, spleen, heart, testicle and arteries were removed, and fixed in formol. The cadmium dosage is in progress.

C) Plateled aggregating sensitivity to AA, collagen or ADP

1) "In vitro" studies

Citrated blood samples were obtained by cardiac puncture conscious rabbits and collected in a plastic centrifuge tube. Platelet-rich plasma (PRP) was prepared at room temperature by centrifugation at 150 g for 20 min.
Cadmium chloride (dissolved in saline) was added to 1 ml of PRP at concentrations of 2,7, 1,3, 0.52, 0.27, 0.05 mM.
Sodium arachidonate (AA), collagen and adenosine-5'-diphosphate (ADP) were employed as plateled aggregating agents at the doses: for AA of 150, 110, 74, 60, 49, 32, 24 µM; for collagen of 3 µg and 12 µg/ml of PRP; for ADP of 0.7, 2 and 6 µM.

2) "Ex vivo" studies
("in vitro" after "in vivo" treatment)

The experiments were performed, according to the method previously described for "in vitro" studies, on PRP samples obtained from rabbits which underwent the treatment described under "d" ($CdCl_2$ injection by intravenous and subcutaneous route- 2 mg/kg each-simultaneously, repeated for three days). Only AA as aggregating agent was used.

Results

A) Evaluation "in vitro" of prostacyclin-like material

Untreated vascular rings incubated for 5 min in Tris buffer released prostacyclin-like activity, as indicated by inhibition of platelet aggregation when 50 µl of supernatant was added to PRP. No prostacyclin-like was revealed when $CdCl_2$ was added to buffer solution at a dose of 17.6×10^{-4}M; slight activity was shown at a dose of 4.4×10^{-4}M. Rings pretreated with $CdCl_2$ (12×10^{-4}M) for 5 min at 37°C prior to incubation in PRP did not show any anti-aggregating activity (absence of prostacyclin activity); cadmium pretreatment at a dose of 6×10^{-4}M yielded a decrease in the prostacyclin-like activity for both aggregating agents; for ADP this inhibition was linear with the $CdCl_2$ doses. Repeated washings of the cadmium-pretreated rings did not restore prostacyclin activity; similar results were obtained with aspirin-pretreated rings but not with those pretreated with indomethacin at a dose of 7×10^{-5}M.

B) Evaluation "in vitro" of prostacyclin-like material after "in vivo" treatment.

The prostacyclin synthesizing and releasing activity of the vessel wall was not blocked.

C) Platelet aggregating sensitivity to AA, collagen or ADP

Cadmium addition to PRP increased the platelet responsiveness to aggregating action of AA and collagen but not of ADP.
The platelet responsiveness was evaluated as lowering the minimum dose of AA or collagen required to trigger platelet aggregation in comparison with the controls (no $CdCl_2$ added).
At the concentration of $CdCl_2$ of 2.7, 1.3, 0.27 mM the threshold doses were for AA, respectively 24, 49, 60 µM. For collagen, aggregation inducing dose was 3 ug/ml of PRP. The threshold dose of AA in the controls was 110 µM and of collagen 12 µg/ml of PRP.
A reduction of the threshold dose of AA was also obtained on samples of PRP from treated rabbits (threshold dose 110 µM for controls, 60 µM for treated).

Conclusion

These results show a new aspect of cadmium toxicity on the cardiovascular system. The "in vitro" Cd activity on the synthesis and release of prostacyclin could explain, at least partially, the hypertensive action of the metal.
This activity in addition to the greater platelets sensitivity induced by cadmium "in vitro" as well as "ex vivo" to aggregating action of AA and collagen, could also explain the formation of vascular thrombi described by several authors.
$CdCl_2$ dose which decreased the mean platelet aggregation threshold was ten fold lesser than the needed one for inhibiting the prostacyclin-like material release. This result is in agreement with those obtained by other authors who found a different sensitivity of platelet prostaglandin system compared to that of the vessel wall, to some anti-inflammatory drugs. The anti-prostacyclin activity shown "in vitro" was not confirmed "in vivo". This may be likely attributed, for rat, to their greatly different sensibility (due to strain, sex, etc) to cadmium, particularly to its hypertensive action and, for rabbit, to the short $CdCl_2$ treatment period used.
Cadmium determination in the above mentioned organs, including arteries, removed from rats used in the experiments, is now in progress and could explain the "in vivo" lack of the activity which was found "in vitro". Preliminary results, concerning the organs from animals which received the metal added

to diet at the dose of 200 ppm, showed in the arteries Cd levels of 12.2 µg/g Further experiments are in progress in order to evaluate cadmium levels in the arteries, PRP and PPP and to determine the interaction between platelets and prostacyclin in animals treated for longer periods: this could ascertain a) the eventual relation to some biological and pathological factors such as sex, strain, experimental atherosclerosis, etc. and b) the effect due to the chemical form of cadmium.

Pubblications and oral communications

Caprino, L., Togna, G., Togna, A.R., Inhibition of prostacyclin formation by cadmium. Oral communication to XIX Congress of the Italian Pharmacological Society, Ancona, Settembre 1978.

Caprino, L., Togna, G., Inhibition of prostacyclin-like material formation by cadmium. Toxicology and applied Pharmacology, 47 (3), 1979, in press.

Caprino, L., Togna, G., Platelets hypersensitivity to aggregating agents induced by cadmium (under preparation).

Contractor: National Institute of Public Health, Bilthoven, NL
Contract no: 215-77-1 ENV N
Project leader: G.J.van Esch, M.J.van Logten, F.X.R.van Leeuwen
Title of project: Toxicity of lithium

Objective of the research

The objective of this study is to establish the "no toxic effect level" of lithium, to calculate the acceptable daily intake (A.D.I.) for human beings, and to determine the influence of sodium dietary restriction on lithium toxicity.

In order to carry out a 90-day experiment with rats to establish this "no toxic effect level", the most sensitive criteria with respect to the effect of lithium on carbohydrate metabolism and thyroid functioning will be determined in subacute (6 weeks) experiments.

Materials and methods

1. SPF-Wistar female rats (120-156 g) were housed in wire cages under conventional conditions. Drinking water and semi-purified diet, Muracon SSP-Tox (Trouw Ltd., Putten, The Netherlands) were provided ad libitum. To 4 groups of 6 animals each lithium chloride was fed in concentrations of 0, 100, 400 and 1600 mg/kg diet, respectively. Body weight gain, food and drinking water intake were measured throughout the experiment. After 6 weeks the animals were sacrificed and blood was collected from abdominal aorta for biochemical determinations. Heart, brain, liver, kidneys, thymus, adrenals and pituitary were weighed. Parts of liver and musculus quadriceps were immediately frozen in liquid nitrogen and stored at -70°C until biochemical analysis. Liver and musculus quadriceps were studied histopathologically, with special attention to glycogen content.
2. SPF-Wistar male rats with a body weight of 106-136 g were used in the standard diet study, and rats with a body weight of 117-152 g were used in the low salt study. The rats were housed in wire

cages and drinking water and diet were provided ad libitum. The standard diet contained 3.4 g sodium/kg and 7.4 g chloride/kg; the low salt diet contained 1.0 g sodium/kg and 1.5 g chloride/kg. The rats were divided into 5 groups of 12 animals each, receiving 0, 100, 400, 1600 and 3200 mg LiCl/kg diet for 6 weeks. Body weight gain and food and drinking water intake were measured throughout the experiment. Alternating from half of the number of the animals of the various dose groups, blood was collected via orbita puncture weekly. In serum thyroxin (T_4), T_4-N and thyroid stimulating hormone (TSH) were measured. At the end of the experiment the rats were sacrified and heart, brain, liver, thymus, kidneys, adrenals, thyroid and pituitary were weighed. Half of the number of the animals of the various dosage groups were perfused with buffered formaline (10 %) in order to obtain optimal fixation. Histological examination was carried out on heart, brain, pituitary, thyroid, thymus, liver, kidneys and adrenals.

Results

1. Mean organ weight/body weight ratios, determined at the end of the experiment, were significantly increased for heart, thymus, adrenals and thyroid at 1600 mg/kg. Biochemical observations showed a significant decrease in liver glycogen at 100 and 400 mg/kg and an increase in phosphorylase activity at 400 and 1600 mg/kg. Histopathologically no significant alterations in liver and musculus quadriceps were observed.

2. LiCl caused a decrease of serum thyroxin in the two highest dose groups. This effect was more pronounced with the restricted sodium diet than with the standard diet. Serum lithium concentration and lithium content in the thyroid was only effected by sodium restriction in the highest dose group. Histopathologically no alterations of the thyroid gland were observed at 1600 mg LiCl/kg standard diet, whereas inactive epithelial cells and empty follicles were found under restricted sodium conditions. Furthermore, with the restricted sodium diet, degenerative alterations

of the proximal and distal tubules of the kidneys were observed, due to a disturbance of the water and salt balance by lithium chloride.

Conclusion

Lithium toxicity is only enhanced by dietary sodium restriction at critical serum lithium concentrations.

References to contract research

Van Leeuwen, F.X.R., Vos, J.G., Van den Eshof, A.J. and Van Logten, M.J.
Subacute oral toxicity of lithium chloride in rats.
I. Effect on the carbohydrate metabolism.
National Institute of Public Health, report 204/78

Van Leeuwen, F.X.R., Berkvens, J.M., Van den Eshof, A.J., Vos, J.G. and Van Logten, M.J.
Subacute oral toxicity of lithium chloride in rats.
II. Effect of lithium on thyroid functioning.
National Institute of Public Health, report in preparation

Contractor : C.E.N.-S.C.K., Department of Radiobiology, B-2400 Mol, Belgium.

Contract n° : 140-76-12 ENV B

Project leaders : H. REYNERS, E.G. de REYNERS, J.R. MAISIN

Title of project : Study of the effects of heavy metals on the central nervous system (CNS). (Project 1).

The toxic effect of lead on the central nervous system has been evidenced since long and commonly attributed to the onset of blood vessel lesions (hemorrhagy, edema, occlusions) in the brain. Nevertheless, this view appears questionable due to the very high toxic levels commonly used in this field of current neuropathological research. In the present work, although electron microscopic observations revealed evident vascular changes (mainly capillary density), these appeared to be mostly consequent to intrinsic variations concerning the nerve tissue itself and particularly the glial elements supporting the neurons in the cerebral cortex. The 3 types of glial cells were found to be differentially involved by lead poisoning and part of their reaction can still be detected at a dose of 100 ppm of Pb^{++} in food which provides to the rat a daily intake rather comparable to what is ingested by man in polluted industrial environments.

MATERIALS AND METHODS.

Groups of 5 male and female albino rats were submitted (from 8 days before birth) to 0, 100, 1000, 5000 and 10000 ppm of Pb^{++} (lead acetate) in food and sacrificed at 3 month old by formaline perfusion of the brain via the aorta. The changes in the different cellular compartments of the neural tissue (nerve cells, glia and vascular supply) were evaluated by means of morphological and morphometric analysis. In this report, the density of capillary vessels and glial cells are studied. The affinity of glial cells for neurons (satellitism) and for other glial cells (dyadism) was also quantified. Data on the influence of age on lead encephalopathy are still incomplete due to the relatively long lifespan of the rat and to the highly time consuming aspect of this mode of approach.

RESULTS AND DISCUSSION.

a) The influence of low dose treatment on the capillary density in the cerebral cortex was found to be opposite to the effects observed in our preceding report on this subject at 10 000 ppm. Low dose treatment appears indeed to be associated with a brain capillary population more sparsely distributed than normal (at 100 ppm). Such situation is believed to be consequent to the larger thickness of the cerebral cortex as measured in this material (see full report). Capillary density appears thus to be correlated passively to cortex volume changes directly induced by lead action.

b) Brain parenchyma is a complex network of nerve cells and processes wrapped by supporting glial cells. Our preceding work had revealed the occurence of very faint modifications at the level of the nerve cell endoplasmic reticulum after 10000 ppm during 3 months in female rats. Oppositely, glial cells display more evident lesions at this dose : astrocytes show prominent intracellular gliofibrillogenesis, microglia appears often necrotic, or with enhanced lysosomal activity sometimes accompanied by overt neuronophagy. These obvious symptoms of lead intoxication can only be found at high lead levels (10000 ppm and 5000 ppm). It was nevertheless concluded that glial cells are real target cells in low level lead poisoning and supposed that at still lower doses, the more elusive traces of their impairment could only be found by large number statistical approach of quantitative parameters (cell densities, cell affinities, etc.). This approach was realized for 100 and 1000 ppm and recently extended to male animals. At 3 month old, lead acts differentially on the 3 glial populations (Chart I), the affinity of these cells for neurons is also dose dependent (principally for female microglia) (see full report). On the other hand, dyadism is never modified. Glial response to lead is also found to be influenced by sex (Chart I). The desequilibrium introduced by lead at the level of the neuron supporting system appears to be eventually reversible with the elapsed time for a dose as large as 5000 ppm (female). Nevertheless, glial density appears more elevated than normal in the latter material, a symptom which could indicate the presence of precocious aging processes.

CONCLUSIONS

Although the dynamic of the above mentioned changes still remains obscure for the time being, the glial cells appear as a main target of lead intoxication. They react differentially to the presence of lead and their behaviour is also sex dependent. The present data suggest the primacy of parenchymal lesions in lead encephalopathy contrarely to the current theory of vascular injury still widely adopted.

LISTE DES PUBLICATIONS récentes du chef de projet et de ses collaborateurs.

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H. Reyners, E.G. de Reyners, J. Van der Parren, J.R. Maisin, Etude morphométrique des citernes submembranaires des neurones et de leurs relations spatiales avec les processus astrocytaires dans le cortex cérébral du rat. Biologie Cellulaire 30 (1977) 265-278.

B.P. Eyden, J.R. Maisin, G. Mattelin, Long-term effects of dietary lead acetate on survival, body weight and seminal cytology in mice. Bull. Environm. Contamin. & Toxicol., p. 266-272 (1978).

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H. Reyners, E.G. de Reyners, J. Van der Parren, J.R. Maisin, Evolution de l'équilibre des populations gliales dans le cortex cérébral du rat intoxiqué au plomb. C.R. Soc. Biol. (1978) sous presse.

H. Reyners, E.G. de Reyners, J.R. Maisin, Altérations du cortex cérébral du rat causées par certains facteurs de l'environnement (RX, Pb). Ann. Soc. Roy. Zool. Biol. (1978) sous presse.

J.R. Maisin, M. Lambiet-Collier, L. de Saint-Georges, Toxicité du plomb pour les embryons de la souris. C.R. Soc. Biol. (1978) sous presse.

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1. Effets de l'irradiation et du saturnisme sur l'ultrastructure du cortex cérébral.
   Société Royale de Zoologie de Belgique, 14.5.1977, Mol.
2. Ultrastructural differentiation between nerve and glial cell membranes by means of a new ultra-low viscosity resin.
   Société Belge de Microscopie Electronique, 14.4.1978, Gand.
3. Effets des métaux lourds sur le système nerveux central.
   C.E.C., 25.4.1978, Louvain-la-Neuve (Faculté de Médicine).
4. Transformations des populations gliales causées par le plomb.
   Société Belge de Biologie, 29.4.1978, Institut Bordet, Bruxelles.
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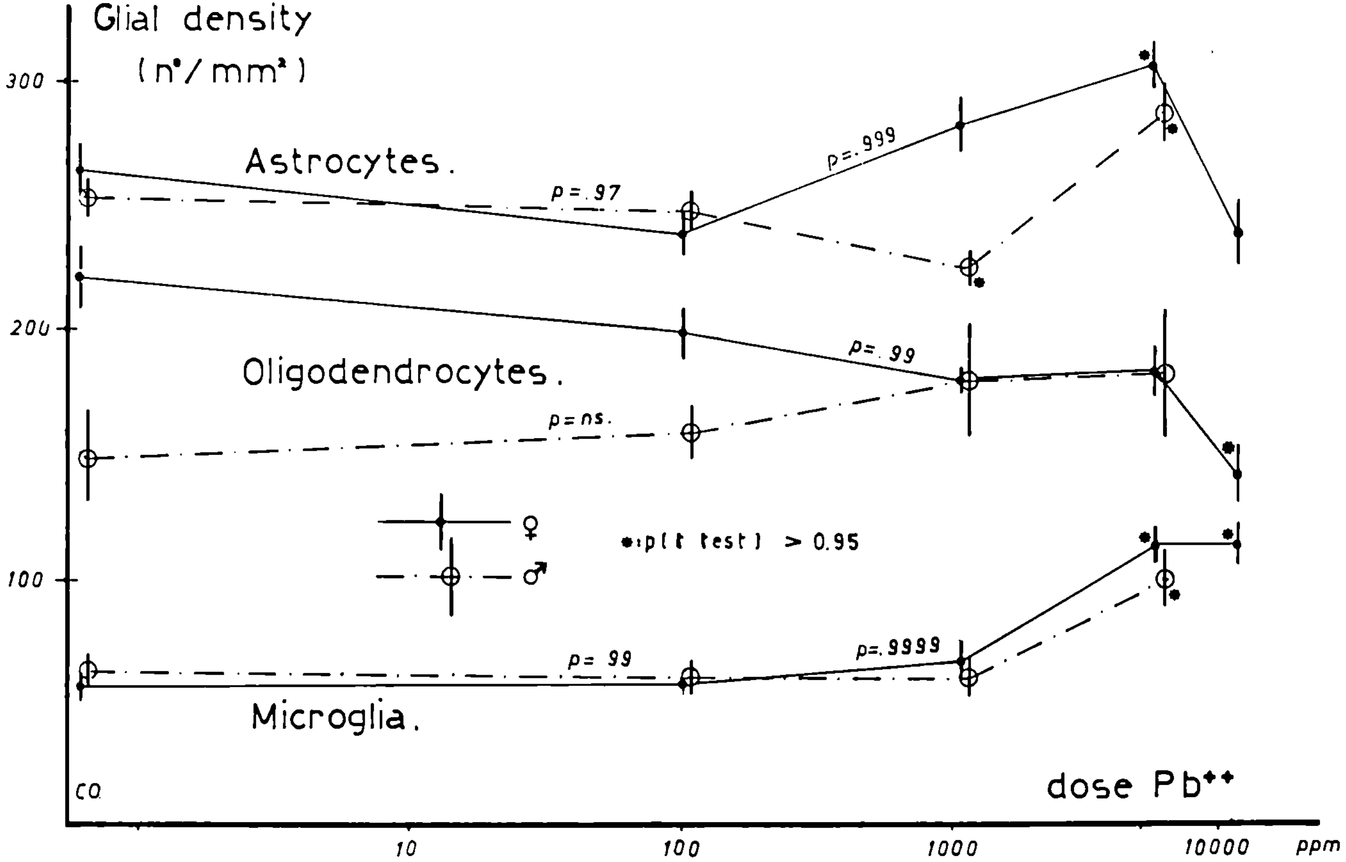
   Radiobiologie, 30.11.1978, Séminaire, Mol.
6. Altérations du cortex cérébral du rat causées par certains facteurs de l'environnement (RX, Pb).
   Société Royale de Zoologie de Belgique, 16.12.1978, Bruxelles.
7. Influences de l'environnement sur le cerveau.
   Département de Morphologie Animale (Institut Zoologie), 19.12.1978, Séminaire, Louvain-la-Neuve.
8. Effets du plomb sur la glie.
   C.E.C., 28.2.1979, Bruxelles.

CHART I



Glial density
(n°/mm²)
300
200
100
Astrocytes.
Oligodendrocytes.
Microglia.
p=.97
p=.999
p=.99
p=ns.
p=.99
p=.9999
♀
♂
*:p(t test) > 0.95
CO.
dose Pb++
10
100
1000
10000
ppm

Contractor : C.E.N.-S.C.K., Department of Radiobiology, Mol, Belgium

Contract n° : 140-76-12 ENV B

Project leaders : A. Léonard and G.B. Gerber Scientist : P. Jacquet

Title of project : Studies into the toxic action of lead in biochemistry of the developing organism and on the cytogenetics of the post meiotic germ cells. (Project 2).

Objective of the research :

A first aspect of our studies is concerned with the mechanisms of action of lead on the developing organism. These experiments are dealing with the organism before implantation and in utero after implantation as well as with the newborn organism. A second part of our experiments is devoted to the potentiality of lead to cause chromosome aberrations in somatic and in post meiotic germ cells.

Materials and Methods :

Effects prior implantation : In order to elucidate the reduction of pregnancies observed in mice after lead treatment, the development of the embryo until presumed implantation and the reaction of the mother were studied in histological sections of the uterus, oviduct and ovary of mice treated with lead from the day of fertilization. In addition, different hormones were measured in the mothers during this period, by radioimmunoassay.

Effects after implantation : Different experiments were undertaken to explain the retarded growth as well as the important mortality observed in mouse fetuses exposed to lead via their mother. Biochemical parameters were measured to study hem synthesis. Availability of nutrients was studied by measuring the placental blood flow, by means of radioactive microspheres, and by studying the uptake of an unmetabolisable amino acid, α-AIB (α-amino isobutyric acid).
The development of the skeleton and the presence of skeletal abnormalities were followed in fetuses after injection of lead at different moments of fetal organogenesis (days 8 to 12). Influence of a lack of dietary calcium on the incidence of skeletal abnormalities caused by lead was also tested. For this study,

skeletons were prepared and stained with red alizarin S, and examined under the stereomicroscope.

Effects on the newborn organism : In order to better understand the mechanisms of lead encephalopathy, experiments were dealing with the biochemistry of the brain in mice and rats after lead treatment from birth to 12 months of age. Many parameters were measured, such as DNA, proteins, lipids and lysosomal enzymes. Blood flow and α-AIB uptake were also studied in this organ.

Genetic effects : Extensive investigations were carried out in man, monkey and mouse exposed in vivo to lead, zinc or cadmium as well as in human lymphocytes cultured in vitro in the presence of these heavy metals.

Results :

Effects prior implantation : Lead causes a delay in early divisions of the embryo. Blastocyst formation is not impaired, but blastocysts tend to remain smaller. Differentiation of giant cells in the trophoblast and of deciduous cells in the uterus is however inhibited, and the corpora lutea remain underdeveloped. Results of radioimmunoassays show that in control mice, implantation is preceded by a rise in estradiol and a fall in prostaglandins, whereas progesterone increases as implantation takes place. Lead treatment apparently does not act on the first phase but interferes with the increase of progesterone, a fact which correlates well with the underdeveloped corpora lutea found in these mice. Furthermore, injections of progesterone and estradiol from days 4 to 7 or 5 to 8 restore the implantation in the lead-treated females.

Effects after implantation : Biochemical studies after treatment of pregnant females with dietary lead show a decrease of hem concentration and of δ-ALA-D activity as well as an increase of porphyrin concentration in the lead-exposed fetuses. Impairment of fetal hem synthesis is confirmed by the abolition of Fe incorporation normally occuring at days 16-18 of pregnancy. On the other hand, placental blood flow is diminished, whereas uptake of α-amino isobutyric acid is paradoxically enhanced in the exposed fetuses. Injection of lead to pregnant females given a normal diet increases the postimplantation mortality and the rate of skeletal anomalies among the fetuses, especially when lead is injected at days 8 or 9 of pregnancy. The anomalies are restricted to the anterior part of the axial skeleton and consist essentially in the fusion of 2 or more cervical vertebrae. In addition, injection of lead diminishes the calcium blood levels in the pregnant females. When lead is injected to pregnant animals given a low-calcium diet, all these effects are considerably increased and fetuses suffer a loss of weight and retardation in the ossification.

Effects on the new born organism : The most important findings in brain biochemistry of infant mice were, a retardation in development in DNA, a decrease in ALA-D and sialic acid and a decrease in AIB uptake in this organ. In rats, cathepsin was enhanced at almost all times, OH-proline increased from 4 months, peroxides and peroxidation increased in many samples. A raise in serotonin and in noradrenaline was also noted. AIB uptake by brain was decreased whereas brain blood flow increased. These observations suggest an important role of proteolytic processes and biogenic amines in lead encephalopathy.

Genetic effects : Our observations on men from zinc industry, exposed simultaneously to zinc, lead and cadmium, established the presence of severe chromosome aberrations in the lymphocytes of such people. In vitro exposure of human lymphocytes to these heavy metals added separately results mainly in the occurence of chromosome fragments. In these conditions, severe aberrations seems to occur only in the presence of low concentration of zinc chloride. However, as the doses used in the experiments were far above those to which people of zinc industry are submitted, it is impossible to decide if the anomalies found in these people were provoked by zinc. The problem is more complex, and one may not exclude the possibility of an interaction between heavy metals and other factors, such as, for instance, dietary calcium. Indeed, examination of bone-marrow cells of mice maintained on a normal diet and exposed to dietary zinc for one month showed severe aberrations in the chromosomes, but their number was considerably increased if zinc was given in combination with a low calcium diet. On the other hand, a few severe aberrations were also detected in the chromosomes from monkeys submitted for long periods to low doses of lead in their drinking water, but their incidence was again considerably higher when the lead-treated animals were given simultaneously a low-calcium diet. In summary, these extensive investigations suggest that zinc and, in a lower exten , lead, may be capable to provoke severe chromosome abnormalities, and that the clastogenic potency of these two heavy metals is enhanced by a deficiency in dietary calcium.

Conclusion :

Many effects of lead on the pre- and post-implantation embryo were found, with different doses of lead administered at short term. Important effects were also found on the mother. With respect to the preimplantation embryo, in vitro studies will now be performed, using very low doses of lead, and different parameters of the kinetics of embryonic development will be followed. On the other hand, studies are now in course, concerning the genetic and teratogenic effects of very low doses of lead administered for long periods in the diet. Influence of a lack of dietary calcium on these effects will be tested in these conditions, since a real synergism between lack of calcium and lead appears from experiments reported here.

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Contractor : Université Catholique de Louvain
Contract n° 229-76-12 ENV B
Project Leader : Professeur R. Lauwerys
Title of project : Renal toxicity of three heavy metals (Pb, Hg, Cd) in man and study of the critical burden of cadmium in the human organism.

Objectives of the research

1. The main objective of the first research project is to compare the nephrotoxic action of three heavy metals (lead, mercury and cadmium) in man in order to propose specific methods for the early detection of kidney damage induced by these metals and to evaluate the level of internal exposure which causes renal dysfunction.
2. A second project was carried out in collaboration with researchers from the University of Birmingham (UK) to evaluate the critical level of cadmium in the human organism.

Materials and methods

The first project was carried out on four groups of workers : control workers (n = 88); workers exposed to lead (n = 25); workers exposed to inorganic mercury (n = 63); workers exposed to cadmium (n = 148). A subject was considered as control if he was not occupationally exposed to any heavy metal and excreted less than 2,5 and 50 µg/g creatinine of Cd, Hg and Pb and had a plombemia lower than 35 µg/100ml. The workers in the other groups showed either an increased concentration of lead in blood or in urine or an increased urinary concentration of mercury or cadmium. The following biological variables were determined on each person = in blood and in urine : Cd, Hg, Pb, creatinine, several specific proteins (β_2-microglobulin, orosomucoïd, albumin, transferrin, IgG); in urine only : δaminolevulinic acid, aminoacids, total protein, several enzymes (βgalactosidase, lactic dehydrogenase, alkaline phosphatase, total and tartrate resistant acid phosphatase, catalase), electrophoresis of urinary proteins; in blood only : hematocrit and hemoglobin. The parameters which in the control group were found to be correlated with age were standardized to an age of 40 years before comparing the results between the different groups.

The second project was carried out on 309 male workers in two Belgian cadmium-producing plant. Liver cadmium concentration and total amount of cadmium in the left kidney were measured with a portable neutron-activation analysis apparatus. The cadmium concentration in blood and in urine and the urinary concentration of total protein, albumin and β_2-microglobulin were also determined.

Results

First project

A moderate exposure to lead (plombemia below 62 µg/100 ml and plomburia below 300 µg/g creatinine) does not increase the prevalence of signs of renal dysfunction.

In the group of workers exposed to inorganic mercury, the prevalence of increased excretion of some enzymes and high molecular weight proteins as albumin, orosomucoïd, IgG was significantly greater than in the control group. The measurement of these specific proteins allows an earlier detection of kidney dysfunction than does the determination of total proteinuria. The excretion of β_2-microglobulin, a low molecular weight protein, remains unchanged. The prevalence of kidney impairment is not related to total mercury level in blood. This is not unexpected since it is known that mercury in blood is not a good indicator of exposure to inorganic mercury. On the other hand, the prevalence of abnormal urinary excretion of specific proteins increased with mercury level in urine. Our data suggest a critical level of 50 µg Hg/g creatinine.

Exposure to cadmium causes an increased prevalence of total proteinuria, an increased excretion of high molecular weight proteins (albumin, transferrin, orosomucoïd and IgG) and also an increased urinary excretion of β_2-microglobulin and alkaline phosphatase. The renal dysfunction induced by cadmium appears to be tubular as well as glomerular. The prevalences of abnormal results is correlated to cadmium level in blood and in urine. They start increasing in the subgroups with a cadmium concentration in blood between 1 and 2 μg/100 ml and a cadmium level in urine between 10 and 20 μg/g creatinine.

Second project

Among the 309 male workers examined, 78 had evidence of renal dysfunction (proteinuria > 250 mg/g creatinine and/or β_2-microglobulin > 0.2 mg/g creatinine and/or albuminuria > 12 mg/g creatinine). In workers with normal renal function the 50th, 90th, and 95th percentiles of renal cortical cadmium (p.p.m.) were 87, 200, and 248, respectively, with corresponding liver cadmium levels (p.p.m.) of 17, 39, and 48, and Cd-U levels (μg/g creatinine) of 3.5, 16.1, and 23.4. In these 231 men there was a good correlation between liver cadmium and renal cortical cadmium ($r = 0.48$, $p < 0.001$) and between log Cd-U and renal cortical cadmium ($r = 0.54$, $p < 0.001$). In the 78 workers with renal dysfunction there was a significantly higher liver cadmium level than in those with normal renal function (χ^2 on cumulative frequency distributions : $p < 0.001$) though the kidney cadmium concentration was not different. This latter finding is, at first sight, surprising and suggests either that renal dysfunction is not related to absolute cadmium concentration in the kidney or that renal cortical cadmium decreases progressively after the onset of kidney damage. Two arguments strongly support the latter second explanation. First, urinary cadmium excretion was significantly greater in the workers with increased β_2-microglobulinuria ($n = 46$): their median and mean urinary cadmium levels (μg/g creatinine) are 8.8 and 13.9, respectively, against 3.5 and 6.3 for the workers with normal kidney function. Secondly, the slope of the regression line between renal cortical cadmium (ordinate) and liver cadmium is 2.1 times lower in the workers with renal damage.

This implies that, as renal cortical cadmium rises towards the highest levels observed in the workers with normal kidney function whom we studied, renal dysfunction may develop. This "critical" or "effect" level of cadmium in the renal cortex might be defined as the value found in those men who have the highest tissue levels but without overt signs of renal damage. This level would thus appear to lie between 200 and 250 p.p.m. (the 90th and 95th percentiles). Since renal cortical cadmium and urinary cadmium are well correlated in undamaged renal function we can estimate from the curvilinear regression that the critical level of urinary cadmium is between 10 and 15 µg/g creatinine.

This result accords well with the indirect estimate made on the basis of the results of the cross-sectional study described above (see first project).

If the critical level of cadmium in the kidney cortex is independent of the route of absorption our results suggest that for a sizable proportion (up to 5 %) of the middle-aged population in Europe, the margin of safety afforded by the current level of cadmium in kidney cortex is below 5.

Conclusions

Moderate occupational exposure to lead does not lead to renal dysfunction. Exposure to mercury exposure causes an increased urinary excretion of specific proteins mainly when mercury level in urine exceeds 50 µg/g creatinine. Signs of renal disturbance are mainly found in workers whose cadmium concentration in urine exceeds 10µg/g creatinine. The critical level of cadmium in human renal cortex lies between 200 and 250 ppm. Hence for a sizable proportion (up to 5 %) of the middle-aged general population in Europe, the margin of safety afforded by the current level of cadmium in kidney cortex is below 5.

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Contractor :	The Victoria University of Manchester, Department of Occupational Health.
Contract No :	143 - 77 - 7 ENV UK
Project Leader:	Professor W.R. Lee
Title of Project :	Relationship between nerve conduction velocity and biochemical studies in lead poisoning.

Objectives:

To search for a relationship between the nerve conduction velocity and corresponding changes in the biochemical indices.

To understand the effect of lead on nerve activity and explore association with structural changes in the nervous system.

To establish and to clarify the mechanism of the development of these nervous system changes in increased lead absorption.

Methods:

A longitudinal study has been set up using an experimental group of 30 rats which have had lead introduced into their diet in the form of lead chloride solution (initially at 100p.p.m. of lead) as drinking water.
Prior to the introduction of lead the experimental animals were submitted to the tests listed below, in order to establish base-line levels, and after lead has been added, they undergo these measurements on a regular basis, once every 14 days:-

Biochemical Tests

Blood lead (PbB)
Erythrocyte Aminolaevulinic acid dehydrase (A.LA.D)
Erythrocyte Protoporphyrin (ZnP.P)
Haemoglobin (Hb)
Packed Cell Volume (P.C.V)
Urine lead (Pb-U)
Urinary Aminolaevulinic acid (ALA-U)

Neurophysiological Tests

Maximum Motor Conduction Velocity (M.M.C.V.)		Median Nerve
Slow Fibre Conduction Velocity (S.F.C.V.)	of	Ulnar Nerve
Distal Latency (D.L.)		Sciatic Nerve

By monitoring any changes in this close manner and comparing the findings with those gathered from the same measurements carried out on a control group (30 rats, matched for age and sex to the lead group, on the same diet but with no lead added) it is hoped that correlations will become evident and that an indication of the underlying mechanism will occur.

Individuals from both groups will be sacrificed so that nerves can be excised from control and lead fed rats and compared by light microscopy and electron microscopy. This technique will also be used regularly, but on a less frequent basis than the tests listed above, ie. once every 8 weeks rather than once every 14 days.

Neurophysiological Methods

All the neurophysiological tests were carried out using a Medelec Electrophysiological Recording System in a warm laboratory. To prevent heat loss during the experiment the animals were examined on a thick wad of cotton wool, whilst under anaesthesia (Halothane, Nitrous Oxide, Oxygen mixture). Heat was also supplied from an overhead lamp which was focussed onto the animal. Neurophysiological measurements were only made if the skin temperature of the proximal part of the limb was above 33°C and at the distal part of the limb above 31°C.

Subcutaneous needle electrodes were used for both recording and stimulating purposes. The maximum motor conduction velocity was found by applying a supramaximal stimulus to a proximal and then to a distal stimulating site along a limb over the nerve whose conduction velocity was to be found. The action potentials produced by the stimuli were recorded by an active electrode under the skin over the belly of a muscle that the particular nerve to be examined supplies and an indifferent electrode under the skin over the tendon of that muscle.

The muscle action potentials were recorded using a fibre optic system on to light sensitive paper. A time scale was recorded at the same time so that the time from the stimulus artifact to the muscle action potential gave the latency for the particular stimulating site. The difference between the proximal latency and the distal latency gave the time taken for the action potential to travel between the two stimulating sites. The distance between the proximal and distal stimulating sites was measured over the skin using calipers.

For the median nerve the proximal stimulating site was at the elbow and the distal stimulating site was at the wrist. The muscle action potentials were recorded from the flexor pollicis brevis muscle.

For the ulnar nerve the proximal stimulating site was at the elbow and the distal stimulating site was at the wrist. The muscle action potentials were recorded from the abductor digiti quinti muscle.

For the sciatic nerve and its posterior tibial branch the proximal stimulating site was in the upper thigh and the distal stimulating site was at the medial side of the ankle. For recording muscle action potentials stainless steel needles were used, potentials being recorded between an active electrode over the bellies of the interesseous muscles in the plantar surface of the foot and a remole electrode over the lateral toe.

Comparison of the temporal dispersions of proximally and distally produced muscle action potentials gives a method of measuring slow fibre conduction velocity, or to be more precise conduction velocity in fibres other than the fastest fibres. Amplitude decreases proportionately with temproal dispersion. As amplitude can be measured more easily and accurately, comparison of amplitudes is more often used than temporal dispersion. Comparison of the proximally and distally produced amplitudes was affected by expressing the amplitude of the proximally produced muscle action potential as a percentage of the distally produced muscle action potential.

To determine the overall experimental error of the neurophysiological methods, repeatibility was studied (Table 1). One subject was tested on ten separate occasions. Tests were carried out on different days and at different times of day so in fact intra-individual variation was included in the estimates of overall error. The repeatability results indicate that the methods used have a good degree of consistency.

Results

A table of the neurophysiological parameters measured for the serial recordings (Day 0 and Day 14) is presented (Table 2). Using a grouped 't' test to compare the results of the lead group and the control group, no significant differences were found in any of the parameters on the two serial recording days.

A table of the biochemical parameters measured for the same serial recordings is also presented (Table 3). There is a significant difference in one of the biochemical parameters between the lead group and the control group on day 14. There is a significant decrease ($P < 0.01$) in the activity of the enzyme ALA-D in both the male and female lead group when compared to their control groups on a paired 't' test. There is no significant differences in any of the other biochemical parameters.

Discussion

These preliminary results confirm that the enzyme ALA-D is very sensitive to lead. This parameter is the only one that has been affected within the first 14 days at a level of lead in male and female rats of 23-24 µg/100ml. There has been no effect on any other measured parameter as yet. After the next serial recording the lead intake will be increased by increasing the strength of the lead solution given as drinking water from 100ppm lead to 500ppm of lead. The effect of this increase on the various parameters that are measured will be investigated regularly as before.

Table 1:

Repeatability Study

	Highest Recorded	Lowest Recorded	Mean	S.D.
MMCV metres/sec	52.8	49.3	51.3	0.95
Sciatic/Tibial Branch				
% Amplitude	78.3	70	74	2.8
MMCV metres/sec	57.8	53.9	55.9	1.38
Ulnar Nerve				
% Amplitude	98	89.5	92	3.06
MMCV metres/sec	60.5	57.2	58.7	1.13
Median Nerve				
% Amplitude	81	72	77.7	2.95

Table 2:

NEUROPHYSIOLOGICAL RESULTS

The figures in each column represents the mean of the results of 15 animals and their standard deviation.

		MALES					FEMALES				
	Test	Control Group Mean	Control Group S.D.	Lead Group Mean	Lead Group S.D.	t test	Control Group Mean	Control Group S.D.	Lead Group Mean	Lead Group S.D.	t test
DAY 0	M.N.C.V. Ulnar Nerve	59.25	4.98	58.9	4.97	N.S.	58.3	2.78	59.3	3.45	N.S.
	% Amplitude ulnar	96.7	7.62	98.9	6.83	N.S.	98.8	6.77	96.6	6.84	N.S.
	M.N.C.V. Median Nerve	59.1	1.76	59.5	1.99	N.S.	58.5	1.77	53.8	2.84	N.S.
	% Amplitude Median	98.8	7.70	95.9	5.97	N.S.	97.7	3.43	96.8	5.99	N.S.
	M.N.C.V. Sciatic Nerve	49.9	2.95	50.6	1.77	N.S.	51.3	3.72	51.4	2.91	N.S.
	% Amplitude Sciatic	79.8	3.66	78.9	3.88	N.S.	78.5	3.42	78.0	4.00	N.S.
DAY 14	M.N.C.V. Ulnar Nerve	58.7	3.79	59.1	4.2	N.S.	57.9	2.99	59.1	3.43	N.S.
	% Amplitude Ulnar	95.9	7.71	97.2	8.1	N.S.	94.8	6.91	95.7	7.62	N.S.
	M.N.C.V. Median Nerve	58.9	2.83	59.3	2.93	N.S.	57.9	3.04	59.2	2.79	N.S.
	% Amplitude Median	97.7	9.10	96.1	7.7	N.S.	98.1	9.10	97.9	7.10	N.S.
	M.N.C.V. Sciatic Nerve	50.3	2.73	49.8	2.91	N.S.	49.9	3.10	50.1	2.81	N.S.
	% Amplitude Sciatic	79.9	3.27	77.7	3.83	N.S.	78.9	4.91	79.1	4.07	N.S.

Table 3:

BIOCHEMICAL PARAMETERS

The figures in each column represents the mean of the results of 15 animals and their standard deviations

Recording	Test	MALES				Paired t test	FEMALES				Paired t test
		Control Mean	Group S.D.	Lead Mean	Group S.D.		Control Mean	Group S.D.	Lead Mean	Group S.D.	
DAY 0	Pb-B	4		4		N.S.	4		4		N.S.
	ALA-D	5.53	1.41	5.62	1.67	N.S.	5.41	1.35	5.00	1.78	N.S.
	ZnPP	1353.7	361.3	1376.1	341.3	N.S.	1281.6	305.1	1304.8	352.1	N.S.
	Hb	13.5	1.81	13.7	1.47	N.S.	13.8	1.72	13.7	1.56	N.S.
	Htc	47.1	1.91	46.6	1.82	N.S.	46.8	1.77	46.7	1.76	N.S.
	ALA-U	0.302	0.131	0.277	0.126	N.S.	0.293	0.116	0.240	0.122	N.S.
DAY 14	Pb-B	4		24	2.32		4		23	2.11	
	ALA-D	5.67	1.06	2.96	1.01	$P<0.01$	5.41	1.66	2.70	0.78	$P<0.01$
	ZnPP	1313.7	320.3	1299.3	297.4	N.S.	1221.8	201.7	1183.1	217.8	N.S.
	Hb	13.6	1.79	13.8	1.91	N.S.	13.9	1.54	13.7	1.81	N.S.
	Htc	46.9	1.82	47.2	1.87	N.S.	47.3	1.44	46.6	1.84	N.S.
	ALA-U	0.297	0.110	0.289	0.140	N.S.	0.272	0.121	0.251	0.131	N.S.

Units		
	ALA-D	umoles PBG formed per hour per litre of red cells
	PbB	ug per 100 ml
	ZnPP	umoles per litre red blood cells
	Hb	gms per 100 ml
	ALA-U	umoles per 24 hours.

Contractor : University of Glasgow, Department of Materia Medica.

Contract No 166-77-1 ENV UK

Project Leader : Professor A Goldberg, Dr M R Moore.

Title of Project : The valuation of certain aspects of lead and cadmium exposure in terms of biochemical effects and consideration of bio-analytical indices of exposure.

Original objectives

1 Mental retardation and neurophysiological studies
A larger scale study of mental retardation will be undertaken throughout Scotland, with especial consideration of the neonatal period. The effects of lead on peripheral nerve conduction velocities will be investigated in a group of workers industrially exposed to lead. In view of the probable association between the neurological effects of lead exposure and the presence of δ-aminolaevulinic acid, this work is being extended into an examination of the effects of δ-aminolaevulinic acid in animals and in man.

2 Hypertension and renal effects
More extensive studies will be undertaken of hypertension in continuing association with the M.R.C. Blood Pressure Unit, Western Infirmary, Glasgow. Measurements will be made of urinary lead excretion in patients with renal failure and normal subjects. A study of hyperuricaemia will be carried out in association with the Center for Rheumatic Diseases, Baird Street,Glasgow. Evidence of exposure of environmental lead will be sought in subjects with proven gout.

3 Indicators of Exposure
Studies into the "in vitro" and "in vivo" effects of zinc and aluminium on the activity of erythrocyte ALA dehydratase will be continued together with studies on other bioanalytical measures of lead exposure. A study to assess the hypothesis that in children dentine lead levels may reflect the exposure to lead in early life has been instigated in conjunction with the chief Dental Officer of Glasgow Corporation Health Department and Glasgow Dental Hospital.

4 Lead Kinetics
The study of the kinetics of gastrointestinal absorption of lead will involve measurements of retention of radioactive ^{203}Pb in man and animals. The absorption of ^{203}Pb from oral and intravenous doses will be measured in normal volunteers by whole body radiation monitoring at the Southern General Hospital, Glasgow. This study will be paralleled by work with laboratory animals administered ^{203}Pb orally and intraperitoneally. The percutaneous absorption of lead, that is the absorption of lead through the skin, will be investigated in normal volunteers.

Methods

1 Subjects

The subject in each of the studies using humans is defined in the terms of the type of population required to be studied. In the mental retardation studies, these will be a random selection of mothers at delivery and their children, in the Glasgow area. In studies on renal insufficiency, it will be those people presenting to the Reanl Unit of Stobhill Hospital, combined with an age and sex-matched control group taken from the general hospital population. A similar choice will be made of patients with gout and equivalent sex and age matched controls. For each study involving industrial lead exposure, subjects will be chosen from specific lead-using industries in the Glasgow area. This choice will be assisted by association with the Employment Medical Advisory Service. In each study using normal volunteers and patients, the processes of the study have been vetted by the Research and Ethical Committee of Stobhill Hospital and given clearance for the studies to proceed.

2 Delta-aminolaevulinic acid dehydratase

This is measured by the Commission of European Communities Standardised method (Berlin and Schaller, 1974).

3 Metals

Concentrations of the metals examined (lead, cadmium, zinc and aluminium) are measured by flameless atomic absorption spectrophotometry by the methods of Meredith et al (1977). For more difficult biological matrices such as teeth or faeces, the sample is initially ashed in a muffle furnace or wet-ashed using acid. All studies are checked and calibration maintained by participation in three quality control schemes. Storage was covered by the techniques of Moore and Meredith (1977).

4 Porphyrins

These are measured by the method of Rimington (1971) and porphyrin precursors by the method of Mauzerall and Granick (1956). High pressure liquid chromatography is also used to give qualitative and quantitative porphyrin analysis.

5 Gamma counting

Whole body monitoring is being carried out on the Southern General Hospital's whole body monitor in Glasgow. Other gamma counting procedures use the Wallac gamma counter in Stobhill Hospital, together with the Selo monitor in the Department of Nuclear Medicine.

6 Behavioural Studies

Ethological studies of animals are being carried out by the methods of Grant and Mackintosh (1963) together with standard organ-bath techniques.

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Results

1 Mental retardation and neurophysiological studies

Blood lead concentrations were measured retrospectively in the blood contained on cards used for testing for phenylketonuria in the first two weeks of life. Cards belonging to 80 of a group of 77 children with mental retardation of unknown aetiology and 77 controls were identified. Of 77 usable cards, 41 were from mentally retarded children and 36 were from controls. Twenty-four mental retardation/control pairs were found. There was a highly significant trend towards higher blood lead concentrations in the mentally retarded children. Water lead concentrations in the maternal home during pregnancy correlated with blood lead concentrations in the mentally retarded children. These results, reinforce the probable association between lead exposure during pregnancy and the development of mental retardation of otherwise unknown aetiology(17)In further studies, 232 mothers have been examined at delivery of their children and samples obtained of maternal blood, cord blood and water supply of the maternal home. These studies have shown that there is a cube root regression of blood lead on water lead similar to that found in a study of the general population. (11, 14, 16,) Following on this study, 119 children at the age of 6 weeks have been examined for blood lead status as associated with water lead concentrations in the home. Of this cohort 7 children have been identified to date in whom lead concentration exceeded 1.5 µmoles/litre. At this stage of the study when the children in question are fifteen months old, no behavioural or developmental deficit has been observed. Nerve conduction velocities have now been measured in a group of six industrial workers identified during a demolition job in Glasgow (2) and in four children excessively exposed to lead and identified as mentally retarded from previous studies. The industrial workers have shown lowered peripheral nerve conduction velocities although no specific nervous deficit could be identified in the children.

The association between the neurological effects of increased lead exposure and over-production of δ-aminolaevulinic acid have been investigated further. Work has shown an association between lead exposure and acute porphyria in increases in the activity of δ-aminolaevulinic acid synthase and over-production of δ-aminolaevulinic acid. This may be coupled with the similarity of clinical presentation. (4) The mathematical relationship in lead exposure in humans between elevation of ALA synthase and depression of ALA dehydratase with consequent over-production of ALA has been elucidated. (10) Ethological analysis has been used to study the behavioural effects following injection of ALA in mice. All mice so treated showed increasing immobility, decrease in exploration and investigation and abnormal gait and movement. In frog nerve-muscle preparations (sciatic-gastrocnemius) levels of ALA that did not interfere with nerve conduction, inhibited the muscle's response to nerve stimulation for 50 to 120 minutes. The amounts of ALA in the muscle were declining 2 h after topical administration. (8) These studies are continuing.

2 Hypertension and renal effects

In work following on our previously published results upon the effect of lead on hypertension, we examined 70 hypertensive patients and 70 controls matched for age and sex. These were investigated for a possible relationship between blood cadmium and hypertension. No significant differences between the two groups were detected, although the blood cadmium level was significantly higher in smokers as compared to non-smokers. The results obtained to date do not support the hypothesis that cadmium is involved in the development of hypertension in man. (1, 13) Previous studies have shown that chronic sub-clinical lead exposure may be associated with hypertension, which may be associated with damage to renal tubules and cortex. Plasma renin concentrations in response to frusemide have been determined in eleven subjects with industrial lead exposure. No significant differences in plasma renin concentration were found in this lead poisoned group compared with seven control subjects. This work suggests that renin release is not suppressed in lead poisoning. (7) Water lead concentrations were measured in 970 households throughout Scotland. Blood lead concentrations were measured in 283 people living in houses with water lead levels of over 0.48 µmol/litre. A highly significant correlation was found between lead concentrations in water and blood. Raised blood lead concentrations were associated with renal insufficiency reflected in raised serum urea concentrations and with hyperuricaemia although there was no evidence of clinical disease in any of the affected people. (3) The association between blood lead concentrations in an adult population and the concentrations of lead in first flush and running water were investigated further. It was found that blood lead varied as the cube root of water lead in a sample population of 949 persons. (11, 16) Further studies have shown in a group of 12 patients, 4 with normal renal function, 4 with moderate renal impairment and 4 with severe renal failure, that urinary lead output was of the same order irrespective of renal function. These findings indicated that renal impairment is not a significant cause of elevated blood lead concentrations and indeed it is more probable that excessive water lead concentrations are one factor in the development of renal disease and hypertension in some subjects. (5) This work is continuing in a larger group of subjects. We have studied 32 patients with documented primary gout without known lead exposure, and 32 age and sex matched controls. Most of the gouty patients had normal serum urea and creatinine. Sixteen of the gouty patients but only 4 controls had blood lead concentrations greater than 1.5 µmol/litre. This difference is highly significant ($p<0.001$). Gout and elevated blood lead concentrations are therefore associated and it seems likely that sub-clinical lead exposure may be one factor in the aetiology of some cases of gout. (19)

3 Indicators of exposure

Tooth lead levels have been measured in 109 children living in Glasgow. A significant association has been demonstrated between molar tooth lead concentration and domestic drinking water lead concentrations. Tooth lead concentrations were found to be greater the longer a child had lived in older housing with lead plumbing during foetal life and following birth. Tooth lead concentrations were also found to be age-related although no difference with respect to social class could be found. (12)

The effects of aluminium, lead and zinc on ALA dehydratase were examined "in vitro" and "in vivo". Aluminium alone added to the erythrocyte enzyme assay caused a highly significant increase in enzyme activity. A similar result was found with zinc alone. When lead and aluminium were added together the inhibition found with lead alone was depressed in an additive manner by aluminium. Zinc and aluminium together showed an activation that was stoichiometrially additive. Studies in vivo using rats injected with aluminium and lead showed similar results for hepatic ALA dehydratase as those found in vitro. These studies are at present being extended to the effects of these metals on ALA dehydratase activity in man. Other factors may influence the activity of ALA dehydratase. Studies on the effects of carbon monoxide have now been completed. ALA dehydratase activity was measured in the presence of varying concentrations of carboxyhaemoglobin. Both "in vivo" and "in vitro" carbon monoxide causes a small but consistently significant diminution of the activity. At concentrations of carboxyhaemoglobin likely to be found "in vivo", it is unlikely to significantly influence the use of this enzymė as a measure of lead exposure. (15)

4 Lead kinetics

The absorption of lead from the gastrointestinal tract as ^{203}PbCl has been studied in 11 volunteer subjects. Retention after 9 days ranged from 12.5% of the dose to 56.7% of the dose. This work is continuing with further volunteers, under varying dietary conditions. In a further group of 6 volunteer subjects, 20 µCi of ^{203}Pb was administered intravenously and the distribution of lead followed for up to one month using a whole body monitor. The whole body retention of lead was found to have a half-life of between 60 and 85 days. Twenty-four hours after administration, 8% of the administered dose had been excreted in the urine, and 40% to 50% of the residual dose was found in the blood. Urinary lead excretion in each subject was found to be about ten times greater than the faecal lead excretion. A very rapid equilibration of the distribution of lead between plasma and red cells was observed, which reached plateau levels after about half an hour. These studies are being continued in a further group of subjects. In initial studies on 10 subjects in whom the percutaneous absorption of lead acetate solutions from the forehead were studied, no absorption of lead was observed at a sensitivity level of 0.1% of the applied dose on the basis of change in cold blood lead determination, blood ALA dehydratase activity, urinary ^{203}Pb excretion or accumulation of ^{203}Pb in the blood. This work has now been extended to increase the sensitivity to less than 0.01% of the applied dose using whole body counting techniques in a group of 8 volunteers, using forehead applications of lead acetate solutions containing ^{203}Pb acetate. Initial results show a mean whole body uptake of 0.058% of the applied dose.

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The Royal Society

Biochemical basis of lead toxicity. Moore, M.R.

Environmental lead poisoning : Clinical effects at the lower limit. Goldberg, A.

Contractor: University of Aston & City of Birmingham.

Contract n° 167-77-1 ENV UK

Project leader: Professor J.A.Blair

Title of project: The intestinal absorption of lead.

Objective of the research

(i) To determine in detail the mechanisms whereby inorganic lead compounds are transported from the gut lumen into the body cavity.

(ii) To define dietary states, metabolic interactions and disease states in which the entry of inorganic lead into the body would be increased or decreased.

(iii) To establish the existence of any toxic effects or inorganic lead upon gastrointestinal processes.

Materials and Methods

The everted sac technique and the radio-isotope ^{203}Pb were used to study the transport of the lead cation across the rat small intestine. Investigations were carried out at concentrations of lead similar to the normal dietary intake, and the data reported in detail in a number of publications(1-4)

Results

Unchelated lead cations were transported at a similar rate across all sites in the rat small intestine. The rate of absorption was slow and (a) varied linearly with the concentration of lead in the mucosal soluation from 10^{-7}M to 10^{-5}M, (b) was independent of energy source and little affected by temperature, (c) paralleled that of water movement. In the absence of calcium or under very acidic conditions (pH 2.4) the rate of lead transport increased significantly. The bile salt sodium deoxycholate did not affect lead transport at low concentrations but significantly reduced it when present in higher concentrations (10^{-3}M). When the lead cation was chelated with a suitable organic ligand the rate of lead transport across the epithelial sheet was significantly increased.

The slow movement of lead across the epithelium occurred at the same time as a rapid and massive uptake of lead onto the tissue surface. The amount of lead taken up by the intestinal tissue was proportional to the concentration of lead in the lumen and was little affected by changes in calcium concentration, temperature or anoxic conditions. Uptake was increased in the presence of low concentrations of sodium deoxycholate, and decreased in the absence of glucose, in acidic media and high concentrations of sodium deoxycholate (10^{-3}M).

No effect of lead has been observed on the intestinal acid microclimate nor on the mucosal surface enzymes maltase, lactase or trehalase. However, significantly larger amounts of 5-methyltetrahydrofolate were bound to the mucosal surface in the presence of 10^{-5}M lead. Glucose transport across the intestine was also significantly reduced at lead concentrations of 10^{-5}M.

Conclusions

These data demonstrate that a large proportion, if not all of the free lead cations diffuse across the intestinal epithelium via an extracellular route located at the tight junctions, in a manner which is related to water movement. Calcium deficiency and increased acidity change the dimensions and charge of the tight junctions thereby causing an increase in the amount of lead crossing the intestinal barrier. Chelated lead, on the other hand, because of its size and lipid solubility is more likely to move across the intestinal epithelial sheet via an intracellular route. Hence chelated lead compounds are potentially more toxic to the epithelial cells than the free lead cation.

It is probable that the large uptake of lead by the intestinal tissue is due to its interaction with $H_2PO_4^-$, present in the glycocalyx from enxymic hydrolysis of ATP, and the formation of $Pb(H_2PO_4)_2$. The decrease of lead transport in the presence of high concentrations of bile salts is probably due to the partial loss of glycocalyx by micellar solubilisation.

Tissue uptake of lead appears to be an adsorption process, hence the proportion of lead bound to the tissue increases as the fluid to tissue ratio decreases. In man the fluid to tissue ratio, under normal physiological conditions is approximately 0.3/1 and the amount of lead bound to the tissue in the presence of low concentrations of bile salts is approximately 90%. The binding mechanism of lead to intestinal tissue plays an important role in excluding dietary lead from the body, as tissue bound lead is not available for transport, and is probably lost as lead phosphate in the faeces.

Using the figure of 90% for the amount of lead adsorbed and the lead transport model developed from our studies, it can be demonstrated that a dietary lead intake of approximately 200µg per day (an average U.K. figure) should give a blood lead level of approximately 20µg/100ml (a typical U.K. value). Hence the amount of lead in blood appears to be controlled by the concentration of lead in the gut lumen and will alter as the luminal concentration of lead changes. Blood lead levels therefore are only a measure of present and recent past exposure to lead, and not necessarily a reliable indicator of total body burden.

Conditions favouring increased gastrointestinal transport of lead compounds

Any reduction in the binding of lead to the intestinal tissue will increase the amount of lead available for transport. Thus, for example, a decrease of 50% in the binding (as is seen for bile salts) will increase the lead available for absorption from 10% to 55% of the total ingested. Increased lead uptake into the body will also be found under conditions in which the tight junctions become larger, e.g. when there is increased acidity or a severe deficit of calcium in the gut lumen. This may also explain why neonates absorb more lead.

No studies have yet been performed on human subjects in our laboratory but it is possible to predict situations in which more lead will be taken up into the body due to a diminished interaction with the intestinal tissue. Any state in which the glycocalyx is lost or diminished, or the supply of adenosine triphosphate is reduced or its activity decreased will cause a decrease in lead binding and subsequently an increase in the amount of lead entering the body.

Such conditions in man could be due to a

(1) Lack of glucose, e.g. in fasting

(2) Lack of adenosine triphosphate, e.g. in anaemia

(3) Dietary deficiency of magnesium and/or zinc

(4) Total lack of sodium deoxycholate in bile

(5) High concentration of sodium deoxycholate in bile

(6) Partial or complete loss of glycocalyx, e.g. in coeliac or Crohn's disease.

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Contractor: Institute of Hygiene, University of Copenhagen,
2loo Copenhagen, Denmark

Contract No.: 17o-77-7 ENV DK

Project leader: Philippe Grandjean, M.D.

Title of project: Psychological effects of exposure to inorganic lead or mercury

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Both lead and mercury are known to be toxic to the nervous system. Severe cases of poisoning are associated with serious neurological symptoms. Comparable results have been obtained from experimental studies on animals. Recent investigations have indicated that some symptoms of brain dysfunction may occur with increased frequency in people with occupational exposures to lead or mercury. Thus, severe damage to the nervous system may not occur suddenly when a threshold is exceeded. Rather, the degree of damage may increase with exposure, and slight dysfunction may occur at low levels of exposure.

We have examined this possibility by means of psychological tests. Such tests are used on a routine basis in psychiatric departments for the determination of IQ and the degree of dementia or organic brain damage. Reference materials, i.e., "normal levels", are available, and two experienced clinical psychologists from the National Hospital in Copenhagen, Eva Arnvig and Jørn Beckmann, participated in this project.

The exposure levels were determined by means of blood, hair and urine analyses for lead and mercury. Further, zinc protoporphyrin in blood was measured as an indicator of long-term lead exposure. Air measurements were made available to us in a few cases, but they were too few to allow any general conclusions. From the biological analyses, however, it appears that the lead exposure of the individuals examined ranged from about "normal" to about the limit value for occupational lead exposure. The mercury-exposed subjects had significantly increased mercury levels in the body, though in most cases much below permissible limits. The exposure levels were assessed with the excellent cooperation of Anette Christensen, Jens C.Hansen, Erik Fjerdingstad and the Danish Labor Inspectorate.

In our first attempt to utilize psychological tests, we examined nine males with long-term heavy lead exposure at a battery plant. These individuals were selected by the shop steward as the workers with the highest exposures in the plant. A tenth individual had to be excluded because of a recent cerebral haemorrhage. The nine men were 28-65 years old and had been exposed to lead from 6.5 to 4o years. The blood lead levels were about 8o µg/1oo ml, and urine tests confirmed that the exposure was about the permissible limit. The primary intelligence of these individuals was within the normal range, and cognitive functions seemed to be preserved. The results of the 24 psychological tests performed showed, however, that memory, concentration, visual and visuospatial abstraction, and psychomotor functions were significantly impaired. The clinical impression was in accordance with the test results. Thus, several subjects bursted into tears or felt severely insufficient, most of them had difficulties in concentrating, and the tests lasted up to 3 hours compared to a normal 1 - 1.5 hours. It was concluded, therefore, that these nine individuals suffered from light degrees of dementia, probably chronic lead encephalopathy. Lead is apparantly not very selective in its toxic effects on the brain. A broad battery of tests is, therefore, necessary to evaluate the total damage. Since lead workers may be a selected group with intellectual characteristics which differ from the average of the general population, it is necessary to include an assessment of the primary intelligence by means of tests which are relatively insensitive to organic brain damage. In the following studies, the neuropsychological test battery was changed slightly, and all subtests from the Wechsler Adult Intelligence Scale (WAIS) were included.

In order to study the dose-response relationship, we examined a total of 66 males from four different industrial facilities with lead exposures ranging from slight to heavy. The majority were under 5o years of age, and aging processes would therefore not interfere with the psychological test results. At the same time, a control group was examined. These controls were blue-collar workers processing fatty acids and soap, and none of them had had any known exposure to lead, mercury or other neurotoxins. All subjects had gone to school for 5-7 years only, alcohol consumption was not extreme, and few had experienced a light concussion. The control group exhibited basically normal results in the tests. Their IQ as determined from the complete WAIS was, however, above the normal average. This average was assessed more than 2o years ago, and due to increased availability of public education through television, other mass media and improved school systems, the "normal IQ" has increased. Significant differences were found between the controls and the lead-exposed group, especially concerning long-term memory, verbal and visuospatial abstraction, and psychomotor speed. This difference was also reflected in the significant correlations between impaired test results and increased lead exposure. Such significant correlations were even found in several tests as determined for the lead-exposed group alone. Blood lead and zinc protoporphyrin correlated better with the test results than did hair lead. Age and exposure time were not found to be significant confounding factors. Since the blood lead levels varied from 12 to 85 µg/loo ml with a median of 45 µg/loo ml, it appears that only slight increases of lead exposures above the normal level may be associated with brain dysfunction.

In order to evaluate further the dose-response relationship, we have re-examined several subjects after about 1.5 years. Unfortunately, many had left their jobs recently. We plan to examine these individuals later in order to see if their psychological performance improves when lead exposure has ceased. The study was further complicated by the fact that some subjects rejected a second examination. This was especially true for those individuals who had high lead exposures and severe problems in completing the tests during the first examination. So far we have been able to re-examine 14 men under 45 years of age, about 1.5 years after the first test. During this period most of them had experienced increases in blood lead and zinc protoporphyrin. Most blood lead levels were between 4o and 6o µg/loo ml. The test battery was limited to those tests which can be repeated without any memory effect. The results of this limited study shows that the cognitive functions are virtually unchanged, but memory and psychomotor performance are further impaired. These dysfunctions may be due to both the increased blood lead concentrations and the increased exposure time. Though precise dose-response relationships cannot be defined at the present time, the evidence obtained so far indicates that brain damage may be caused by increased blood lead levels at about 4o-6o µg/loo ml. Cross-sectional studies may, however, underestimate the effects, since demented individuals may have left their jobs or may be unwilling to participate. Moreover, the exposure levels measured in connection with the psychological testing may not be identical to the ones which caused the impairment. Cross-sectional studies carried out at the same time in Finland and USA have shown almost the same results as obtained by us in Denmark. Thus, there can be little doubt about the neurotoxic effects of lead at blood lead levels above 4o µg/loo ml, but prospective investigations, or follow-up studies, must be carried out to obtain more precise data.

Mercury has traditionally been associated with tremor and the "mad hatter" of Alice in Wonderland. Since projective psychological changes might be expected, we added Rorschack's inkblot test to our test battery for mercury-exposed workers and the controls. Mercury occurs in two major chemical forms in the environment, organic compounds, e.g., in fish, and inorganic mercury, e.g., in air pollution from a chlor-alkaline plant in central Copenhagen. We have examined 19 young males from this industrial facility in order to assess the possible presence and extent of central nervous system damage. The cognitive functions of this group were mostly within normal ranges, and only few WAIS subtests showed significant impairments as compared to the results of the unexposed workers. More significant changes were found in the memory and psychomotor tests. The Rorschack results showed some surprising results. "Versagen' only occured in the mercury-exposed group, and the low number of answers in this group, the high proportion of trivial answers and low number of answers involving human beings in movement indicate that dementia, i.e. brain damage on an organic basis, rather than toxic psychosis, is associated with the mercury exposure. Psychomotor performance and several memory tests indicated that the lead-exposed and mercury-exposed groups suffered the same degree of impairment. In most other tests, however, the mercury workers were somewhat better than the lead workers. Thus, the brain damage caused by long-term exposures to these two neurotoxins may differ from each other.

Average performance of lead-exposed (N=66), mercury-exposed (N=19) and unexposed (N=22) males in selected psychological tests. Statistically significant impaired performance, as compared to the performance of the unexposed controls, are indicated with an x.

TEST	LEAD	MERCURY	CONTROL
Visual gestalts (memory after 1 hour)	8.4^x	7.9^x	1.9
Word pairs (memory after 1 hour)	6.3	6.1	5.4
Digit learning	10^x	8^x	4
Story recall	121	117^x	132
Verbal IQ	110	108	113
Performance IQ	110^x	111^x	117
Total IQ	110	110	115
Finger tapping (preferred hand)	50	51	54
Finger tapping (non-preferred hand)	44^x	45^x	50

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Contractor:	University of Aarhus, Denmark.
Contract n°:	171-77-1 ENV DK
Project leaders:	J.C. Hansen and G.J. Bonde.
Title of project:	Comparative Toxicology of Heavy Metals: Cellular Distribution and Interaction of Mercury and Cadmium in Relation to Selenium Intake.

Original objectives: To clarify the antagonistic effects of Se with regard to Hg and Cd in target organs.

The original design was based upon experiments using rats with chemical determination of metals, but as equipment for isotope studies was made available in the beginning of the contract period, it was decided to replace chemical analysis by isotope technique since this would expand the analytical capacity considerably. It was furthermore necessary to use mice instead of rats to perform wholebody countings, as a wholebody scanner suited for counting of rats would be extremely expensive. Only for two initial non-isotopic technique studies rats were used.
The study of cadmium mentioned in the objectives has not been carried out so far. It was considered necessary to perform an extensive study of selenium kinetics before the metal interaction studies in order to create sufficient basic information, as limited information on this topic was found in the literature.

Considering the delayed start of the project (July 1, 1977), and the changes in the objectives mentioned, progress has been made according to schedule.

Methods:

The studies were carried out on female mice to which ^{75}Se labelled Selenite (SeO_3^{--}) and ^{2o3}Hg labelled inorganic mercury ($Hg\ Cl_2$) were administered in various molar ratios (< 1, = 1, > 1), dosage levels (tracerdoses and subtoxic), and routes of administration (I/P, oral, continuous through drinking water).

Determination of selenium and mercury was carried out by means of Searle-Nuclear Solid Scientillation Spectrometer (2 channels) connected with a 2" x 2" Na (TI)-crystal.

To separate simultaneous countings from the two isotopes a technique involving "channel ratio" was used. ^{2o3}Hg emits γ-radiation at o.28 MeV. ^{75}Se has three marked γ-peaks at o.14, o.27, and o.4o MeV. The o.28 MeV ^{2o3}Hg energy cannot be resolved from the o.27 MeV in the ^{75}Se spectrum. The o.4o MeV ^{75}Se peak is, however, without influence from ^{2o3}Hg, and was thus used as reference. All countings were corrected for background and radioactive decay by reference to ^{75}Se and ^{2o3}Hg standards counted simultaneously.
A special programme has been developed for data treatment at 'The Regional EDP-Center of the University of Aarhus'.

For histological examinations the Timm Staining technique has been used. This technique makes intra-cellular visualization of mercury possible.

Results:

1) Effect of inorganic mercury on the nervous system has been studied in the brain, spinal marrow, and spinal ganglia of rats treated with mercury chloride via drinking water. Tissues have been analysed for mercury content and examined histologically. The concentrations of Hg were low, but mercury ions were found to be taken up selectively by certain types of cells. The localisation of mercury in these particular cells of the nervous system may be related to neurological disturbances observed in inorganic mercurialism. (Data not yet published).

2) Mercury-selenium interactions of selenium and mercury vapours in concentration levels as prevalent in nature has been studied in a preliminary study of rats. It was found that dietary selenium influences the distribution of mercury in tissues, including blood of rats exposed to metallic mercury in the air. This study is the first on interaction with metallic mercury vapours. The results indicate the necessity of getting knowledge of the geographical distribution of dietary selenium before evaluating environmental and occupational exposure to mercury on the basis of blood concentration. (Data published, conf. ref. No 1).

3) A kinetic study of selenium has been carried out on 54 female mice to whom ^{75}Se was administered as a single dose, I/P or orally through drinking water. Different dosage levels were used, ranging from tracer dose to subtoxic doses. Wholebody countings revealed a biphasic

elimination. The first rapid phase was strongly dose-dependent, while the slower second phase of elimination appeared independent of dose as well as of mode of administration with a biological half-life of 14.4 ± 3.2 days. Liver, blood, kidneys, and lungs were found to contain most of the selenium, and also here a two-phased elimination was observed. The biological half-lives for organs calculated during the second phase gave a mean of 1o.1 days with a range of 8.7 to 12.5 days. With continuous administration through drinking water a steady state was obtained after 3oo-4oo hrs. of administration. According to concentration a linearity between amount retained at steady state and log-concentration in drinking water is indicated.

This study has provided a better basis for understanding the distribution and elimination of selenium in the body, and is valuable for understanding metal interaction with selenium as well as the need of warmblooded animals for selenium to maintain a physiological homeostasis. (Data accepted for publication, ref. No 2).

4) A study of wholebody elimination of selenium and inorganic mercury administered separately and simultaneously has been carried out by injecting ^{2o3}Hg ($Hg\ Cl_2$) and $^{75}(SeO_3^{--})$ separately or simultaneously in mice. The results have indicated the existence of a homeostatic mechanism for selenium, but not for mercury. Increased retention is observed for both elements when administered simultaneously. Maximum retention of one element is observed when the other is given in excess or equimolar ratio to the other. Excess mercury pro-

vokes a pattern of selenium retention similar to that found in Se deficit animals.
The wholebody ratio(Hg/Se) tends by time towards 1, when this happens mercury as well as selenium are eliminated at a rate different from what is seen when the elements are administered separately. Selenium therefore seems to play a direct role in metabolism of inorganic mercury by forming a complex which is excreeted much slower than mercury given alone. This means an increased tendency to accumulate mercury, and makes the beneficial influence of selenium at chronic exposure to inorganic mercuric compounds questionable. (Data accepted for publication, ref. No 3).

Besides the above mentioned fully evaluated data, the following experiments have been carried out, but data are still under evaluation.

1) Distribution and elimination of inorganic Hg and Se in organs after simultaneous administration.
2) Excreetion of Hg and Se after simultaneous adminstration.
3) Sex-differences in distribution and elimination of Hg and Se after simultaneous administration.
4) Influence of experimental conditions on distribution and elimination of Hg and Se.
5) Molar ratios of Hg and Se in various organs and excreta after simultaneous administration.
6) Distribution of inorganic mercury in the rat's nervous system.

Conclusion:

The results of the work carried out during the contract period have shown that both selenium- and mercury-metabolism is highly influenced by simultaneous administration of the other element, since both retention levels and elimination rates are increased. The fact, that selenium and mercury are excreeted with a similar rate when administered simultaneously and different from the elimination rate seen after separate administration of the two elements, indicates the formation of a Hg-Se complex. This, on one hand, raises the question of bio-availability of the essential selenium when exposure to mercury takes place, and, on the other hand, the question of the beneficial effect of selenium in case of chronic mercury exposure because selenium administration leads to longer biological halflife of mercury which means an increased tendency to accumulation in the organs.

References to Publications on Contract Research:

1. Mercury-Selenium Interaction at Concentrations of Selenium and of Mercury Vapours as Prevalent in Nature. Søren Peder Nygaard and Jens Carl Hansen. Bull. Environment. Contam. Toxicol. 2o, 23 (1978).

2. The Kinetics of ^{75}Se-Selenium in Relation to Dose and Mode of Administration to Mice. Jens Carl Hansen and Preben Kristensen. The Journal of Nutrition (to be published).

3. Wholebody Elimination of $^{75}Se_3^{--}$ and $^{2o3}Hg\ Cl_2$ administered Separately and Simultaneously to Mice. Preben Kristensen and Jens Carl Hansen. Toxicology (to be published).

TOPICS 12 & 14 : ORGANIC MICROPOLLUTANTS & NEW CHEMICALS

Ecology

Contractor: Institut für Biochemie des Bodens, Bundesforschungsanstalt für
Landwirtschaft, 3300 Braunschweig, Federal Republic of Germany

Contract No 174-77-4 ENV D

Project Leader: K. Haider

Titel of Project: Degradation of chlorinated aliphatic and aromatic compound
by aerobic and anaerobic soil microorganisms

Objective of research: Chlorinated cyclic hydrocarbons such as isomers of hexachlorocyclohexane, hexa- or pentachlorobenzenes and several aliphatic chlorinated hydrocarbons are frequently observed as environmental pollutants. The compounds are partly used as pesticides, are possible metabolites from pesticides or are released during industrial processes. Chlorinated aliphatic hydrocarbons were furthermore found to originate from organic residues during the chlorination of drinking-water.

After their release into the environment the compounds come into contact with microorganisms of soil and water under aerobic or anaerobic conditions. Several mixed or pure cultures of aerobic and anaerobic bacteria were therefore examined for their ability to transform or dechlorinate these compounds. Since the potentials of anaerobic microorganisms are not yet well known, this part of the investigation was more emphasized. To follow degradation and dechlorination during incubation, methods were developed for labelling the compounds by ^{14}C and/or ^{36}Cl.

Materials and methods: Hexachlorocyclohexane isomers were prepared by chlorination of benzene at -35 C with $^{36}Cl_2$ under UV-light (Haider and Jagnow, 1975). The mixture was separated by column and preparative thin layer chromatography to obtain α-, β-, γ- and δ-HCH in pure form. By chlorination of ^{14}C- or ^{3}H-labelled benzene the isomers were labelled with ^{14}C or ^{3}H. ^{36}Cl penta- and hexachlorobenzene was synthesized by chlorination of monochlorobenzene with $^{36}Cl_2$ in the presence of $FeCl_3$ at low temperature followed by further chlorination at elevated temperature. The resulting mixture of tetrapenta- and hexachlorobenzenes was separated by fractionated vacuum sublimation and preparative gas and thin layer chromatography.

^{36}Cl-labelled $CHCl_3$ was prepared by Dams and Lieser (1976) by isotopic exchange of $Al^{36}Cl_3$ with unlabelled $CHCl_3$. The $Al^{36}Cl_3$ was obtained by reaction of $Ag^{36}Cl$ with aluminium at 500 C. This exchange method can also be used to label other chlorinated aliphatic compounds. However, yields are sometimes low and depend on temperature and reaction time. Since unreacted $AlCl_3$ can be recovered again as AgCl, the losses of ^{36}Cl can be minimized. The method of isotopic exchange was used to label tri- and tetrachloromethane, 1,2-dichloro-, 1,1,2-trichloro- and 1,1,2,2-tetrachloroethane, and 1,2-di-, tri- and tetrachloroethene.

The indicated compounds were incubated with mixed microbial cultures obtained aerobically or anaerobically from arable soil or sewage sludge. Furthermore pure cultures were isolated or obtained from different sources.

Results:

1. Degradation experiments with isomers of hexachlorocyclohexane

Former experiments (Haider and Jagnow, 1975) with anaerobic mixed cultures from arable soils showed a 90 % degradation of γ-HCH within 4 - 5 days. This degradation resulted in a rapid release of chloride and in the formation of partly volatile chlorine free metabolites. CO_2 was not formed during this incubation. Soil incubation studies with γ-HCH also showed a much more rapid degradation under submerged conditions than under upland conditions. However, under both conditions CO_2 was formed from γ-HCH (Kohnen et al., 1975). Similar experiments with rumen-fluid or rumen organisms also showed a rapid dechlorination of γ-HCH during anaerobic incubation (Haider et al., 1976).

During the EC-Contract screening experiments were conducted to isolate organisms which are active degraders of hexachlorocyclohexane isomers. The experiments should show whether only strict or also facultative anaerobic bacteria are able to degrade those isomers. It was found that beside Clostridia also several facultative anaerobic bacteria quickly dechlorinated γ-HCH and its isomers. These organisms belonged to the Enterobacteriaceae and Bacillaceae. Only those organisms were active in γ-HCH degradation which are known to evolve hydrogen during fermentation. The aerotolerant anaerobes such as Lactobacilli, Leuconostoc or Propionibacteria were inactive. The extent of dechlorination was dependant upon the presence of an energy supporting substrate and upon the structure of the isomer. γ- and α-HCH were more readily dechlorinated than β- and especially δ-HCH (Table 1).

Table 1: Dechlorination of ^{36}Cl α-, β-, γ- or δ-HCH by an anaerobic mixed soil flora, by Citrobacter freundii, Clostridium butyricum and C. pasteurianum during incubation in a complex glucose medium with 10 ppm of the respective HCH-isomer. Incubation time 6 days at 25 C.

Isomer	Mixed soil flora	C. freundii	C. butyricum	C. pasteurianum
	% of organic ^{36}Cl released as ^{36}Cl$^-$			
α-HCH	6.5	13.9	97.4	53.2
β-HCH	7.4	15.3	23.8	10.1
γ-HCH	78.0	91.5	98.3	91.0
δ-HCH	1.6	2.8	38.5	5.0

In arable soils the growth conditions of strict anaerobes are limited, therefore the observation of a rapid dechlorination by facultative anaerobes is of ecological importance. These bacteria are widely distributed in the ecosystem and propagate under aerobic or anaerobic conditions. It was shown that they degrade HCH isomers under anaerobic conditions even if they were grown before aerobically.

The main intermediate metabolite isolated during anaerobic degradation of γ-HCH was γ-tetrachlorocyclohexene (γ-TCCH). This compound, however, was readily further dechlorinated (Jagnow et al., 1977). Heritage and Mac Rae (1977) recently confirmed the formation of γ-TCCH from γ-HCH during anaerobic incubation and detected furthermore δ-TCCH as a metabolite from α-HCH. Small amounts of γ-TCCH were also found during aerobic incubation of γ-HCH (Engst et al., 1977). However, the more prominent intermediate metabolite was γ-pentachlorocyclohexene (γ-PCH), which was slowly transformed into chlorinated benzenes and phenols. These originate from γ-PCH by further elimination of HCl and addition of water. During anaerobic incubation γ-PCH could be not detected and chlorinated benzenes and phenols were only minor metabolites. With ^{3}H- and ^{36}Cl double labelled γ-HCH it could be shown that γ-TCCH was formed by the loss of two chlorine atoms only and not by HCl elimination followed by a replacement of the original hydrogens against substrate hydrogens. The further degradation occurred nearly quantitatively through elimination of chlorine and not of hydrogen. Therefore the formation of highly chlorinated aromatic compounds can be fairly excluded under the conditions of anaerobic incubation.

2. Experiments about isomerization of γ-HCH during microbial incubation

Several authors (e.g. Benezet and Matsumura, 1973) indicated a isomerization of γ-HCH into α-HCH during incubation with several microbes. Since this isomer is more stable against microbial degradation and frequently found as an environmental contaminant the possibilities of the isomerization of γ-HCH during anaerobic or aerobic incubation was investigated. For this reason ^{14}C-, ^{3}H- and ^{36}Cl triple labelled γ-HCH was supplied with small amounts of unlabelled γ-HCH and incubated with cultures of C. pasteurianum, C. butyricum or C. freundii under anaerobic, with Serratia marcescens, Escherichia coli with and Klebsiella exytoca under semiaerobic, and with Pseudomonas putida or P. testosteroni under aerobic conditions. These bacteria were selected since several of them were reported to cause isomerization. At distinct time intervals the culture solutions were extracted and γ- and α-HCH were separated and measured for radioactivity and their isotopic ratio. However, with none of the organisms a transfer of activity from γ- into α-HCH could be found and also the isotopic ratio of γ-HCH remained constant. Therefore it can be excluded that any of the organisms cause isomerization which is worthwhile to mention and to explain the considerable occurrence of α-HCH in the environment. The limits of error of the experiment were smaller than 0.1 % related to γ-HCH.

3. Degradation of hexa- and pentachlorobenzenes

These compounds belong to the most stable compounds with respect to microbial degradation and are frequently observed as environmental pollutants. Incubation studies of the ^{36}Cl labelled compounds with several aerobic and anaerobic mixed or pure bacterial cultures indicated some dechlorination (3 - 4 %) during anaerobic incubation and Clostridia belonged to the most active species. With aerobic Pseudomonas spp. dechlorination was less but the GC-analysis of benzene extracts indicated some formation of pentachlorophenol and of trace amounts of tetrachloro-p-hydroquinone.

4. Incubation studies with chlorinated methanes, ethanes and ethenes

These experiments were only yet made with Clostridia and some facultative anaerobes which were actively dechlorinating hexachlorocyclohexane. The bacteria were incubated with $CHCl_3$, CCl_4, 1,2-dichloro- and 1,1,2,2-tetrachloroethane as well as with tri- and tetrachloroethene in concentrations up to

100 ppm. The cultures were incubated anaerobically for 1 - 2 weeks. Concentrations of 50 - 100 ppm of the chlorinated methanes reduced the growth of the bacteria in the first few days, but after 3 - 5 days they propagated rapidly. It was found that most of the tested bacteria released 50 - 70 % of the organic bound chlorine as chloride during incubation. $CHCl_3$ was more stable than the other compounds but also nearly 50 % of its chloron substituents were released as chloride. During incubation appreciable amounts of the compounds or their derivatives were volatilized as gaseous products along with the gas evolved by the microorganisms. These volatilized products could be yet only partly trapped and analyzed. Further experiments under progress deal with the analysis of the metabolites and the volatile compounds released during incubation.

Conclusions: The experiments conducted during this contract indicate that the compounds investigated are more or sometimes much more readily transformed and degraded under anaerobic than under aerobic conditions. Expecially the chlorinated cyclic or aliphatic hydrocarbons are rapidly dechlorinated while the chlorinated benzenes are stable under both, aerobic and anaerobic conditions.

Anaerobiosis is widely distributed in the environment and occurs in microareas of arable soils, in waterlogged soils, sludges or sediments. Also nutrients in the rumen are anaerobically fermented. The microflora of anaerobic environments contains organisms which are active in the dehalogenation of most of the investigated compounds. It is interesting to note that not only several strict but also some facultative anaerobic bacteria degrade those compounds.

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Contractor: Gesellschaft für Strahlen- und Umweltforschung mbH München
Contract No.: 187-77-1 ENV D
Project leader: Prof. Dr. F. Korte
Title of project: Behaviour and Ecological Effects of 14-C-Labelled Organochlorine Compounds in a Small Pond

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1. Objective of the Research

Extensive studies by different groups have dealt with the occurrence of organochlorine compounds in the aquatic environment, with the fate of these compounds in isolated aquatic organisms or in simulations of aquatic systems. In order to obtain data on the fate and to establish a balance sheet of a chemical in the natural environment, the chemical has to be isotopically labelled. Therefore only one study on the fate of DDT-14-C in a Finnish lake has been published so far. (ref. 1)

The objective of project reported here has been the elucidation of the behaviour, dispersion, accumulation, and metabolism of chlorinated organic chemicals in small ponds. Hexachlorobenzene (HCB), pentachloronitrobenzene (PCNB), and pentachlorophenol (PCP) have been used as models for highly chlorinated chemicals, 4-chloroaniline (PCA) as a lower chlorinated one. For comparison, similar work with a biodegradable substance namely the detergent dodecylbenzenesulfonate (DBS) has been initiated.

2. Materials and Methods

2.1 Chemicals and Applications

For the implementation of the program 6 small ponds with a volume of 6-8m^3 each were digged early in 1976 in a marsh-meadow nearby a larger natural pond. One experimental pond each was treated with 14-C-traced HCB, PCNB (Aug.-Sept. 1976) and 14-C-PCP (April-May 1977) respectively. In summer 1978 the detergent (DBS) and the 4-chloroaniline were applied.

The concentration of each chemical was maintained at about a 50 ppb level over a period of 4-6 weeks. The total of the chemicals applied during the dosing-period, calculated as concentrations, was 250 ppb HCB, 250 ppb PCNB, 310 ppb PCP, 475 ppb PCA and 350 ppb DBS respectively.

Following the last application of the chemicals in the respective ponds, the radioactivity-traces were measured in the aquatic phase in constant time-intervals until it fell below the detection limit (~2 ppb). There were no significant differences in the number of heterotrophic microorganisms in the experimental and the control ponds.

2.2 Quantitative Analyses

2.2.1 Water

Quantitative determination of 14-C in water and in organic extracts were made by low-level liquid scintillation counting. The corrections of the values for quenching etc. were obtained by internal standardization of the samples.

2.2.2 Flora and Fauna

The amount of 14-C in the available biomass in different seasons of the years was estimated by combustion to 14-CO_2 in an automatic oxidizer.

2.2.3 Sediment

Likewise samples of the sediment collected in 5 cm layers were combusted to 14-CO_2 and quantitatively measured by liquid scintillation counters. The values of 14-C have been calculated on dry weight basis of sediment and for the molecular weight of the parent chemical used.

2.3 Qualitative Analyses

Attempts to identify or characterize the chemical nature of residues were made for the sediment only. Different extraction and clean-up methods like continuous cold extraction, hot extraction, hydrolysis and derivatisation of samples were used for the isolation and identification of parent compounds and metabolic products. Some of the substances were identified by GLC and GLC-MS analysis.

3. Results

The major results of these experiments are summarized in the tables (1-4) and graphs (1+2). 28-30 weeks after the last application plants in the ponds treated with HCB and PCNB respectively contained no more any residues at a detection limit of ~2 ppb. On the contrary residues were found in those plants collected in the ponds treated with PCP, PCA, and DBS. Some fauna species from all treated ponds contained residues after the time interval of 28-30 weeks. Samples of soil-sediments collected as layers of 0-5, 5-10, 10-15 and 15-20 cm both horizontally from the walls and vertically from the base of the ponds were analysed after different time intervals for total residues. About 95% of the accountable residual radioactivity was found in the 0-10 cm soil layer. Due to a high percentage of unextractable residues and/or low rate of metabolism in the sediment of the different experimental ponds no metabolites could be identified except pentachloroaniline and pentachlorothioanisole in the pond which had been treated with PCNB. The metabolic conversion of PCNB was relatively high as compared to the other model substances.

4. Conclusions

The analytical data of the abiotic compartments as well as of flora and fauna samples suggest the following conclusions:

- The decrease of the concentrations of the chemicals in the water phase follows a simple exponential function.
- In the sediment there is a build-up phase of residues over many weeks (see graph 2), followed by a very slow disappearance.
- All five chemicals, including the polar PCP, do not significantly leach vertically or horizontally into the surrounding soil.
- Among the analyzed flora species, the water plants contained the highest residues.
- Because of the complex nature (saisonal variations, size, age and position of the individuals in the food-chain) of the ecosystem investigated, the comprehensive interpretation of the results in fauna is not yet possible. However, the results of the PCP-pond summarized in graph 2 reveal some of the correlations for a few factors in this aquatic system.

5. Reference

1. L. Salonen and H.A. Vaajakorpi, IAEA-SM-175/ 39, 201 - 211

weeks after appl.	sediment	notonecta glauca	bulimus tentaculatus	dytiscus marginalis	libellula quadri-maculata (larvae)	anax imperator (larvae)	colymbetes fuscus	zooplankton	limnophilus rhombicus (larvae)	triturus vulgaris
8	0,85	3,5	-	-	-	-	-	0,75	-	-
18	1,98	3,7	-	-	-	-	-	-	-	-
24	1,80	3,6	-	-	-	-	-	-	-	-
28	1,79	4,3	-	-	4,7	-	-	-	-	-
32	1,51	5,3	-	-	2,8	-	-	-	-	-
36	1,40	6,5	0,56	1,37	2,3	-	-	-	-	-
40	1,38	<0,002$^+$	0,98	1,98	1,97	-	-	-	-	2,67
44	0,60	0,64	0,70	-	-	-	0,79	-	-	2,38
48	0,23	0,90	-	-	-	-	-	-	-	-
52	0,22	0,96	0,43	-	0,47	-	-	-	-	-
58	0,19	0,81	0,28	-	0,09	0,18	-	-	-	-
62	0,21	0,51	-	0,052$^+$	0,039	0,027	0,85	-	-	-
66	0,20	-	-	-	-	0,014	-	-	-	-
72	0,19	-	0,088	-	-	-	0,16	-	0,18	-
76	0,098	0,034$^+$	0,052	-	0,012	-	-	-	0,130	-
84	0,10	0,29	0,049	-	0,026	0,053	-	-	0,046	-
88	0,11	0,74	0,060	0,17	0,12	0,11	0,078	-	0,063	-
92	0,10	0,12	0,120	-	0,13	0,20	0,24	0,007	0,13	0,042$^+$
96	0,11	0,28	0,170	-	0,08	0,32	0,26	0,004	0,11	-
100	0,096	0,17	0,062	-	0,092	-	0,21	<0,002	0,13	0,051$^+$
104	0,12	0,12	0,060	-	0,069	-	0,24	<0,002	0,083	0,083$^+$
108	0,118	0,206	0,059	-	0,074	-	0,22	<0,002	0,12	-
112	0,14	0,33	0,089	0,11	0,093	-	0,29	<0,002	0,23	0,067
116	0,13	0,27	0,072	-	0,15	-	0,26	-	0,21	-

$^+$= juv. animals

Tab. 1 Residues (in ppm on the basis of fresh weight of fauna and dry weight of sediment) in the HCB - Biotope

weeks after appl.	sediment	notonecta glauca	bulimus tentaculatus	dystiscus marginalis	libellula quadri-maculata (larvae)	anax imperator (larvae)	colymbetes fuscus	zooplankton	limnophilus rhombicus (larvae)	triturus vulgaris
8	1,32	2,63	-	-	-	-	-	2,07	-	-
18	3,0	2,68	-	-	-	-	-	-	-	-
24	2,8	2,86	-	-	-	-	-	-	-	-
28	2,48	3,98	-	-	2,25	-	-	-	-	-
32	1,95	1,23	-	-	2,14	-	-	-	-	-
36	1,83	0,57	1,84	0,89	0,69	0,25	-	-	-	-
40	1,29	<0,002$^+$	-	1,23	0,014	0,42	-	-	-	2,06
44	0,90	0,19	-	-	-	0,43	-	-	-	1,70
48	0,12	0,18	-	-	-	0,062	-	-	-	-
52	0,13	0,29	-	-	0,014	0,059	-	-	0,089	-
58	0,09	0,22	-	-	0,075	0,049	-	-	0,21	-
62	0,12	0,074	-	-	0,082	-	0,027	-	-	-
66	0,13	0,13	-	-	-	0,092	-	-	-	-
72	0,083	0,16	-	-	-	-	0,033	-	-	-
76	0,066	0,07	0,05	-	0,083	0,10	0,043	-	0,16	-
84	0,073	-	0,075	-	0,080	0,097	-	0,008	0,074	-
88	0,068	0,094	0,12	-	0,094	0,14	-	0,006	0,085	0,044$^+$
92	0,072	0,087	0,083	-	-	0,19	0,097	-	0,15	-
96	0,070	0,092	0,045	-	0,076	-	0,13	0,007	0,16	-
100	0,066	0,093	0,042	-	0,080	-	0,14	0,005	0,11	0,022$^+$
104	0,069	0,130	0,11	-	0,065	-	0,14	< 0,002	0,12	0,085$^+$
108	0,094	0,22	0,14	-	0,11	-	0,13	0,008	. 0,20	0,13
112	0,10	0,25	0,14	-	0,16	-	0,12	-	0,19	0,15
116	0,13	0,24	0,23	-	0,25	-	0,20	-	0,27	0,19

$^{+)}$ = juv. animals

Tab. 2 Residues (in ppm on the basis of fresh weight of fauna and dry weight of sediment) in the PCNB - Biotope

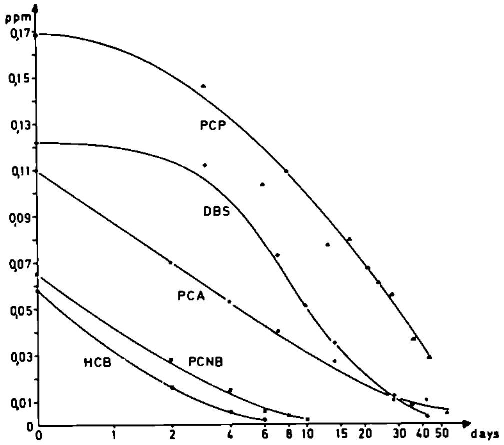
weeks after application	notonecta	libellula larvae	daphnia spec.	sediment	date
8	-	-	-	0.410	July 77
12	6.190	4.390	-	0.505	Aug.
14	3.120	4.470	1.440	0.700	
16	-	3.820	0.970	0.690	
18	5.000	3.295	-	0.765	Sept.
20	3.920	3.520	-	0.515	
22	5.160	3.015	0.530	0.875	Okt.
24	4.805	2.825	-	0.770	
26	-	2.610	-	0.665	Nov.
28	-	2.695	-	0.660	
30	-	2.210	-	0.650	Dec.
32	5.640	2.430	-	0.685	
35	-	2.390	0.970	0.710	Jan. 78
36	-	-	1.320	-	
39	6.840	3.125	-	0.745	Febr.
46	-	3.010	-	0.645	March
50	4.880	3.270	-	0.570	April
55	-	3.670	0.570	-	May
63	0.885	2.215	0.190	0.500	July
69	1.090	0.640^{+}	-	0.705	Sept.
73	1.070	0.480^{+}	-	0.600	Oct.
77	1.183	0.650^{+}	0.380	0.754	Nov.
81	1.188	0.651^{+}	-	0.610	Dec.

$^{+)}$ = juv. animals

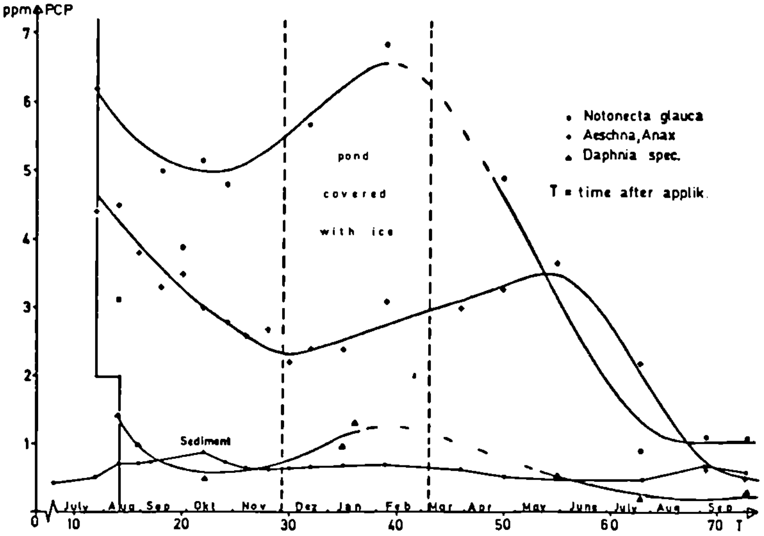
Tab. 3 Residues (in ppm on the basis of fresh weight of fauna and dry weight of sediment)in the PCP - Biotope

time after application	water	sediment	Notonecta glauca	Libellula quadr. (larvae)	Colymbetes fusc.	Limnophilus spec.	Gerris najas
10 min.	0.11	2.76	3.20	1.80	-	4.8	2.8
2 days	0.07	2.62	4.10	1.79	-	5.9	4.0
4 "	0.053	2.59	5.70	-	1.39	8.8	3.5
1 week	0.04	2.56	1.38	2.90	2.40	6.4	2.98
2 weeks	0.027	2.40	1.97	4.30	2.20	-	2.79
4 "	0.011	2.80	3.00	3.80	-	6.1	2.2
6 "	0.010	3.10	2.65	3.00	2.60	4.7	2.38
8 "	0.004	2.90	2.40	3.20	1.50	5.1	3.6
10 "	0.005	1.49	3.60	3.70	2.08	2.88	1.7
12 "	0.006	1.43	3.90	3.90	2.20	3.2	1.68
14 "	0.004	1.95	3.89	4.70	2.28	3.4	1.8
16 "	0.0035	2.14	3.76	4.52	2.60	4.02	-
18 "	0.002	2.08	1.87	4.36	2.48	6.5	-

Tab. 4 Residues (in ppm on the basis of fresh weight of fauna and dry weight of sediment) in the PCA - Biotope



Graph 1. Concentration of the chemicals in the water after terminating the application



Graph 2. Concentration in the sediment and some fauna species in the PCP - Biotope

Contractor : Inst. f. Ingenieurbiologie, Universität Karlsruhe

Contract n° : 244-77-7 ENV D

Project leader : Prof. Dr. L. Hartmann

Title of project : Development of a method to assess the noxiousness of environmental chemicals on bacteria

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The research was designed to develop a test method that enables a description of the impacts of environmental chemicals on microbial biocommunities. Such investigations should be related to that ecosystem which will be most probably affected by the test substance according to its probable distribution in nature. Of special importance are ecosystems typical for

- running waters with different degrees of pollution
- biological sewage treatment plants.

Those systems are characterized by steady state conditions resulting from the interactions between the biocommunity and the environmental factors. Therefore, to obtain valid results it is absolutely necessary that the biosystem will not change during the test except those changes resulting directly from the substance to be tested.Such changes may be short term effects as well as long term adaptations.

The impacts of the test substance are evaluated on the basis of its influence on microbial metabolistic activity. A substance may be tolerated if it is usable for metabolistic purposes (assimilation or gain of energy) and has no inhibitory influence on other metabolistic reactions.
Therefore, aspects as substrate elimination, degradation, assimilation, inhibition of metabolistic reactions resp. toxicity must be an integral part of the test.

To avoid the imponderables connected with open air investigations and thus obtain reproducible results a representative lab-copy of the chosen ecosystem is used to grow the biocommunities that serve as biological test material.

For the investigation of the effects of dissolved organics a model self-purification stretch consisting of 27o vessels and continuously supplied with synthetic sewage of constant composition is used. This model allows to copy the self-purification processes typical for natural rivers.

For the single investigations biomass from different zones of saprobity was harvested. In batch experiments metabolistic activity was measured as reaction velocity after dosing different combinations of test substance and additionals.

The effects of the following chemicals were tested:
- Phenol
- 2-Cl-Phenol
- 4-Cl-Phenol
- Diethylene glycol

Warburg experiments were conducted to observe the substrate respiration. Concomitantly the elimination of the test substance was measured as decrease of DOC. A combination of Na-acetate and NH_4Cl served as reference substance for dissolved organics.

The evaluation of the results was based on reaction kinetics. As shown in former investigations (Hartmann, Wilderer etc.) the kinetics of bacterial metabolism may be mathematically described using the standard formulations of enzyme kinetics as a model conception. Such an interpretation yields the parameters V^*max, Km and Ki.

To study the long term influence of Diethylene glycol on the chosen ecosystem, i.e. the adaptation process and its consequences for the composition of the biocommunities, the test substance was continuously dosed to the model river in a concentration of loo mg/l. During the time of adaptation the changes in the composition and the activity towards the test substance of the biocommunities were closely observed.

After a new steady state was reached the metabolistic activity of chosen biocommunities was measured again in batch experiments The kinetic parameters resulting from those investigations are characteristical values for the adapted biological system.

The investigations yielded the following results:

1. A degradation of Phenol, 2-Cl-Phenol and 4-Cl-Phenol could neither be found by measurements of substrate respiration in the Warburg apparatus nor by short term elimination tests measuring DOC. Those three substances cause an inhibition of the acetate respiration that may be mathematically described according to the equation valid for noncompetitive enzymatic inhibition

$$\overset{*}{V}max_I = \overset{*}{V}max \frac{Ki}{Ki+I}$$

   This type of inhibition occured as well with polysaprobic biocommunities as with biocommunities from the transitory zone and purely α-mesosaprobic biocommunities.

2. For the investigation of the effects of Diethylene glycol three special biocommunities were chosen originating from
   - the polysaprobic zone
   - the transitory zone polysaprobic/α-mesosaprobic
   - the advanced α-mesosaprobic zone.

   Previous to the main test the chosen biocommunities were closely analyzed with regard to their composition and the kinetic parameters of Na-acetate degradation.
   Short term experiments revealed no degradation of Diethylene glycol by the chosen biocommunities; acetate respiration was not influenced by the test substance.

   In the subsequent adaptation test an elimination of Diethylene glycol in the course of the self-purification stretch could be observed after 8 days of continuous loading; the steady state was reached after 14 days. Differences between the adapted and the nonadapted system occured especially with the biocommunity of the advanced α-mesosaprobic zone. Those biocommunities exhibited changes in composition and in reaction kinetic parameters.

   Detailed analysis shows that the degradation of Diethylene glycol mainly takes place in the advanced α-mesosaprobic zone, whereas it proves to be persistent in the polysaprobic zone where the supply of easly degradable substances is high.

A stop in the continuous dosing of the test substance after 12 h already led to a decrease of the degradation velocity; after 8 days no remainder of metabolistic activity towards Diethylene glycol could be perceived.

The conclusion may be drawn that Diethylene glycol can only be tolerated in the environment if it is continuously dosed to the α-mesosaprobic reach of a river resp. to low load treatment plant. If a polysaprobic system is charged with Diethylene glycol no bioelimination will take place even if Diethylene glycol is added continuously.
In polysaprobic systems, therefore, Diethylene glycol is inadmissable.

Definition of Symbols

$\overset{*}{V}max$	mg O_2/g N,min	Maximum reaction related to biomass concentration
$\overset{*}{V}max_I$	mg O_2/g N,min	maximum reaction velocity related to biomass concentration in presence of inhibitor
Km	mg.../l	reaction constant (Michaelis-Menten-theory)
Ki	mg/l	inhibitor constant; concentration of inhibitor that yields half maximum inhibition
I	mg/l	inhibitor concentration = test substance concentration

Contractor : Laboratoire de Physiologie de la Nutrition-TALENCE

Contract n°·151-77-1 ENV F

Project leader : J. BLAIZOT and J.F. NARBONNE

Title of project : Effects of polychlorinated biphenyls in fish and rats.

OBJECTIVE OF THE RESEARCH

Polychlorinated biphenyls (PCBs) have been used extensively for diverse industrial purposes during the past 40 years and a number of accidental exposure of workers involved in PCB production have occured. In recognition of their toxic potential Monsanto Company, the sole US producer, in 1971, voluntarily restricted its sales to only "closed" systems in transformers and capacitors. In France this restriction occured in 1975. This has been a significant factor in restricting the accessibility of PCBs to the environment. However, because of the extreme persistence of PCBs in the environment, human exposure via air, water, and food will continue to be a serious environmental health problem for years to come. Many studies show that PCBs occur in fish wildlife and human foods in Europ. Thus in order to gain better insight into the possibility of PCB accumulation and into the toxic nature of these products a representative commercial product (Phenoclor DP6) was administred to two organisms of the food chain : rat and an estuarine fish (Chelon labrosus). Emphasis was placed upon hepatic microsomal enzyme induction. Moreover we studied the role of some exogenous and endogenous factors, such as age and dietary variations, on DP6 effects.

MATERIALS AND METHODS

Animals:

- Male rats of the Sprague-Dawley strain were housed in individual cages under conditions of controlled temperature (20°C) and fed a control diet (15 % casein) for a 8 days period of acclimatization before the experiments.
- Fish (Chelon labrosus) were netted in the bassin d'Arcachon and then were kept in 20 l tanks containing seawater at 11-12°C renewed every 48 hrs. Fish were fed a dry diet "Aqualim" mullet chow. All fish were acclimated to this environment prior the experimental exposures.

Chemicals : The Phenoclor DP6 (contains 60 % chlorine) used in these experiments was supplied by Prodelec Co (France). This compound was dissolved in peanut oil and mixed with the experimental diets. The labeled compounds were manufactured by the CEA-FRANCE.

PCB extraction and analysis : Tissues samples were extracted according with ERNEY (1974) and the clean up was performed according with MURPHY (1972). The samples were analyzed by electron capture gas-liquid chromatography using a INTERSMAT IGC 16 gas chromatograph. The multiple-peaked Phenoclor DP6 was quantitated by averaging the heights of six major peaks.

Biological parameters of proteic, lipidic and glucidic metabolism were estimated by using an automated analyser (TECHNICON).

Cell-fractionation were carried out by centrifugation procedure as described by NARBONNE (1979).

Enzyme activities were essayed according to NARBONNE and BOURDICHON (1978).

RESULTS

I. EFFECTS OF PHENOCLOR DP6 IN RATS

1°) DP6 accumulation and elimination

In DP6 fed rats we found a very high absorption level of this PCB. The low excretion and metabolism rates of DP6 resulted in a low disappearance rate from the whole body after removal of DP6 diet. The dietary variations (either low protein - 3.5 % casein, or high lipid - 20 %, or vitamin A deficient) did not significantly affect the rate of PCB storage in the liver or the carcass.

2°) Metabolic alterations

The study of metabolic parameters in rats fed a DP6 diet show an enlarged liver, an increase of the protein and lipid content of the liver, and hyperlipemia. The metabolic alterations are established within 8 days. When DP6 was removed from the experimental diet, the metabolic alterations disappear whithin 52 days, although the PCB tissue levels remain elevated. When we investigated more profoundly the liver injuries, we found that the increase of liver weight was due to cellular hypertrophy (DNA remained unaffected), with an increase in lipid storage (triglycerides) and in lipids associated with the microsomal membranes (phospholipids and cholesterol). Liver ATP, GTP and vitamin A contents were not modified by DP6 ingestion. On the other hand, the parameters or urinary excretion were less modified. However, the urinary reducers and the fecal lipids were increased. The phagocytosis in the reticulo endothelial system and the activity of liver drug metabolism were both enhanced by DP6 ingestion.

The study of effects of DP6 ingestion on some liver enzymes show a large increase in activity of drug metabolizing enzymes and in cytochrome b_5 and P_{450} liver contents. The total liver activities of ATPases and G6Pases remain little modified by the DP6 treatment. The spectra given by DP6 binding cytochrome P_{450} is a typical type I. The DP6 ingestion increases the protein synthesis in microsomal membrane fraction from rat liver. The nature of the relation between DP6 and RNA metabolism is still not clear in our experimental procedures. However, the increased incorporation of ^{3}H-orotic and into the soluble RNA from DP6 fed rats suggests that this fraction participates in the stimulatory effect of DP6 ingestion. The measurement of the time course of induction of mircosomal enzymes shows that the induction pattern of these enzymes can be divided into two different characteristic groups :

- the monooxygenases for which the maximal activities were observed

3-5 days following the beginning of DP6 ingestion and then remained significantly higher than control values.

- The cytochrome for which their microsomal levels continued to increase the entire 8 days of treatment and the time profile of the incorporation of labeled amino-acids was similar with those of monooxygenase enzymes.

The study of the average turn over of proteins in subcellular fractions of the liver from DP6 treated rats shows that the half-lives of total liver proteins are significantly shorter than in the control group. The difference is due to the rate of breakdown of the microsomal proteins. In this fraction only the membrane proteins are affected by DP6 treatment. The results suggest that microsomal membrane proteins metabolism (synthesis and breakdown) is enhanced by DP6 ingestion.

The study of in vitro effects of DP6 on mixed function oxydase (MFO) activities shows that the increased activity of MFO in DP6 fed rats is not due to a direct action of the chlorinated compounds on microsomal enzymes. The term "enzyme induction" can be used in these processes which increase the rate of the microsomal synthesis.

After having studied the relationship between DP6 and induction of MFO activity we studied the role of some exogenous and endogenous factors :

- Female rats are less affected by DP6 treatment than male rats;
- Age has little effect on liver response to DP6, although liver fat content was significantly increased in adult rats;
- The study of dietary variations shows that compared to the standard diet (15 % casein, 5 % fat), the higher fat diet facilitates the increases of liver lipid content and the hyperglypemia in treated rats. In rats fed a low protein diet (3.5 % casein) the DP6 treatment stops the decrease in liver protein content and lipemia. Also, with this diet, the liver protein microsomal content is little increased. In vitamin A deficient rats the liver weight and protein content are unmodified when compared to rats fed a standard diet. However, in the vitamin A deficient rats liver lipid content is increased.

In both cases where the proteosynthesis is depressed (3.5 % protein and vitamin A deficiency) the liver response to DP6 ingestion is limited.

II. EFFECTS OF PHENOCLOR DP6 IN ESTURAINE FISH OF THE SPECIES (Chelon labrosus)

1°) Accumulation, storage and elimination

In fish fed a DP6 diet the PCB are rapidly stored in tissues. Liver and muscle are saturated within 8 days. However, PCB are accumulated in the carcass during an 8 days experimental period. When DP6 is removed from the experimental diet the disappearance of PCB from most tissues appears to be a biphasic process. Approximately 20 % of the compound is eliminated after 1 week, but only a 5 % loss is observed in the following 3 weeks. The bile contains relatively high concentrations of DP6 on the 30th experimental day, but the disappearance from this secretion is rapid and nearly complete. The chromatographic profiles of DP6 isolated from exposed mullets indicate that this PCB is not metabolized by the fish.

In DP6 fed fish the carcass DP6 concentration appears higher in older fish than in younger ones. This would indicate that PCB concentration in fish is dependent on the food intake. When fish are exposed to DP6 either from water or food the accumulation of DP6 in body tissues is higher from water than from food.

Metabolic effects : In the thirty days following the beginning of DP6 feeding, mullet show an increased liver weight and protein content, hyperglycemia, decreased glycogen content in liver muscle and heart. Total liver RNA remains stable during the entire experiment. When DP6 is removed from the experimental diet, most of the metabolic alterations disappear within 30 days, although the DP6 body tissue levels remain elevated. However, anemia and elevated liver glycogen continue to exist. The studies of the effects of DP6 ingestion on some enzyme activities indicate that PCB are inducers of fish hepatic drug metabolizing enzymes. In vitro experiments show that fish liver MFO activities are not significantly modified by Phenoclor DP6.

In vivo studies indicate that DP6 inhibits Mg^{++} and $(Na^{+} - K^{+})$ ATPase and succinodehydrogenase (SDHase) activities in kidneys, but the proteins of the microsomal and mitochondrial fractions of this organ are increased. Thus, the total kidney activities of these enzymes remain unaffected. In gills and brain the Mg^{++} ATPase and SDHase activities are increased.

In vitro studies exhibit an inhibitory effect of Phenoclor DP6 on gill $(Na^{+}- K^{+})$ ATPase. The effects of DP6 on in vitro osmotic water fluxes in gills of mullet demonstrate an increase of (m-s) permeability and a decrease of (s-m) permeability. These alterations could be harmful to mullet incurring rapidly a hypotonic medium.

CONCLUSION

I. TISSUE STORAGE METABOLISM AND EXCRETION

Experimental exposure of rats to dietary PCBs has revealed that the isomeric forms are found to various degrees in most body tissues with the highest values in fat tissue. Why isomeric forms are retained to various degrees is still not resolved. A possible explanation includes the differential metabolization and conjugation of the isomers. Fish accumulate DP6 from water 10^{3} times higher than from diet. Moreover no evidence of DP6 metabolism was observed in mullet. The turnover rate of DP6 in entire body tissues was estimated to T 1/2 = 52 days in rat and T 1/2 = 250 days in fish.

II. TOXIC EFFECTS

Our results suggest that the metabolic effects of Phenoclor DP6 seem to be higher than corresponding Aroclors. The most significant effects of DP6 to the liver of fish and rats included weight increase, fatty degeneration and necrosis. Increased liver weights are caused by the proliferation of smooth endoplasmic reticulum. Along with such structural changes, there are increased activities of some drug metabolizing enzymes such as N demethylase and hydroxylase correlated with cytochrome P_{450} increase. The term "enzyme induction" can be used in these processes which increase the rate of the microsomal synthesis. The dietary variations such as protein restriction and vitamin A deficiency limit the microsomal induction response.

The significant effects of DP6 on enzyme activities in fish such as ATPase and mixed function oxydase, suggest changes in fish response to variations of environmental parameters.

PUBLICATIONS AND ORAL COMMUNICATIONS ON CONTRAT RESEARCH

J.F. NARBONNE ; Effect of age and sex on liver response to Phenoclor DP6, a polychlorinated biphenyl in the Rat. Bull. Environ. Contam. Toxicol., 20, 662-667 (1978).

J.F. NARBONNE : Intoxication par les polychlorobiphenyles et facteurs nutritionnels. III. Influence d'un régime carencé en vitamine A sur l'accumulation des PCB chez le Rat. Toxicol. Eur. Res., 5, (1978) in press.

J.F. NARBONNE and M. BOURDICHON : Intoxication alimentaire par les PCB chez le Rat. V. Influence sur l'activité de quelques enzymes hépatiques Toxicol. Eur. Res., 6 (1978) in press.

J.F. NARBONNE : Turnover of liver proteins in Phenoclor DP6 treated rats. Toxicol. Appl. Pharmacol., 47, 103-110 (1979).

J.F. NARBONNE : Polychlorinated biphenyl accumulation in grey mullets (Chelon labrosus) : effect of age. Bull. Environ. Contam. Toxicol., 22 (1979) in press.

J.F. NARBONNE : Accumulation of polychlorinated biphenyl (Phenoclor DP6) by estuarine fish. Bull. Environ. Contam. Toxicol., 22 (1979) in press.

J.F. NARBONNE : Effect of polychlorinated biphenyls (Phenoclor DP6) on in vivo protein and RNA synthesis in rat liver. Bull. Environ. Contam. Toxicol. 23 (1979) in press.

J.F. NARONNNE and J.L. GALLIS : Effect of dietary polychlorinated biphenyl (Phenoclor DP6) exposure in Mullet (Chelon labrosus).
I. Distribution, storage and elimination;
II. Metabolic alterations and residual effects.Third meeting of the Marine and Esturaine Contact Group, Commission of the European Communities 8 and 9 March 1978, Marseille.

Contractor : I.N.R.A - Laboratoire de Recherches sur les Additifs Alimentaires.
Contract n° 188-77-1 ENV F

Project leader : G. BORIES

Title of project : Study of the water contaminants paraffinic and naphthenic hydrocarbons in fish : absorption, metabolism, accumulation, possible metabolic interactions and physiopathologic effects.

OBJECTIVE OF THE RESEARCH

Saturated hydrocarbons are widespread in the environment. They originate with plant biosynthesis and combined physico-chemical and biochemical transformations in sediments. The great rise of oil industries contributes to a large contamination by fossil hydrocarbons. Through the food chains, the human consumers come into contact with these substances ; however their fate, and even less their role in higher animals, are nearly unexplored.
Although they do not exhibit acute nor chronical toxicity, numerous physiopathological observations on Man, following the absorption of mineral oils, must be taken into account. The joint FAO/WHO Expert Committee on Food Additives (1974) underlined the need of investigations on the metabolic fate of such compounds and significance of their storage.

It must be pointed out that the aquatic food chain, either in fresh water or sea, undergoes a severe pollution by oil derivatives such as cutting oils, fuels, and mineral oils. The aim of this research was focused on Fish. This class of vertebrates is located at the end of the water food chain : from its metabolic capabilities will depend the nature and quantities of residues that will reach the human consumer. From an other point of view as it has been stated with mammals, hydrocarbons could exert biological and even physiopathological effects on these organisms. Lastly, on the basis of the extended results already obtained in our Laboratory in Rat and Chicken it was of major interest to enter on a comparative metabolic study.

The research project sponsored by the EEC Environmental Research Program provided the following points :

1) an in-depth investigation of the behaviour of paraffinic and naphthenic hydrocarbons in the Trout (Salmo gairdneri), i.e. study of absorption, transport, storage and mobilization,

2) the design of nutritional studies (growth, feed consumption, metabolism of lipids, absorption of carotenoïds) in order to reveal some eventual physiopathological effects.

MATERIALS AND METHODS

Saturated hydrocarbons belonging to the three classes (cycloparaffins, branched paraffins and normal paraffins) have been tested ; dodecylcyclohexane, pristane, and a mixture of odd and even n-paraffins with carbon chain lengths between C_{14} and C_{22} were chosen as models. These substances were introduced into the complete pelleted feed at a 1% level.

An experimental protocol was designed to study the effect of hydrocarbons on growth (fig.1). At the same time, slaughterings spread out all along the experimental period allowed to follow the storage and the eventual interaction of hydrocarbons with the metabolism of lipids.

A different experiment was designed to evaluate the digestibility of the diet, but also of proteins and lipids. The measurements were performed on

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groups of 12 trouts placed in tanks equipped for feces sampling.

Saturated hydrocarbons determination, and uncommon fatty acids isolation and identification have been carried out using methods performed in the laboratory.

Lipids metabolism parameters have been determined using the classical methods. The incidence of hydrocarbons on the absorption of the lipophilic carotenoïds used as feed additives was studied by measuring the muscle contents of these pigments.

RESULTS

1. Influence of saturated hydrocarbons on growth and feed consumption

Results are given in table I. Incorporation of 1 % hydrocarbons in the diet slows down significantly the growth of Trout. The effect is of limited extent with n-paraffins, but severe with the monocycloparaffin and dramatic with pristane. Food efficiency is decreased in the same way and the overall phenomenon is not related to a decrease of food intake.

When the administration of hydrocarbons is suspended, one can observe a recovery of growth rate, and even a compensatory growing which is the more intense as the slow down has been severe previously.

2. Effect of hydrocarbons on the digestibility of nutrients and carotenoïds

Digestibilities of the total diet, proteins and lipids, are not modified significantly by the hydrocarbons. Erratic results have been obtained concerning carotenoïds digestibility that involve the design of a new experiment.

3. Absorption of hydrocarbons

Absorptions of about 90 % have been determined with the three classes of hydrocarbons. Absorption of n-paraffins versus their carbon chain length shows a rapid decrease from 14 to 24 C atoms. (95 % and 30 % respectively).

4. Storage and mobilization of hydrocarbons

a) Storage : Results are given in table II. Aside the absolute quantities or percentage of hydrocarbon intake, a different calculation indicates the following concentration in the carcass : cycloparaffin 750 ppm, pristane 560 ppm and n-paraffins 960 ppm. The distribution into different tissues and organs indicates a major accumulation in fat (6200 to 12000 ppm), and much lower concentrations elsewhere,i.e. in the order : skin > spleen > muscle > kidney > liver.

Calculation of the quantities of stored hydrocarbons versus lipid content of tissues and organs showed that concentrations level off after a 3 months period, to respectively 12 mg (dodecylcyclohexane and n-paraffins) and 8,5 mg (pristane) per gram of lipids.

b) Mobilization : After hydrocarbon removal from the diet, it has been observed that 35 % of stored hydrocarbons disappeared after 2 months.

However fasting trouts did not mobilize stored dodecylcyclohexane and pristane after a 2 months period, at the very time when decrease of 30 % of n-paraffins was observed. It is noteworthy that neither weight lost nor lipid content decrease occurred at a considerable extent during the fasting period.

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5. Interaction with lipid metabolism

The evaluation of the lipid content of trout livers from the different experimental groups slaughtered at 160g live weight, shows a significant increase in the treated animals, emphazised with pristane (30 %). On the other hand the lipid content of muscles is diminished at a comparable extent.

Fatty acid composition of liver lipids does not exhibit any significant difference between the different groups. The same result is observed with the fatty deposits.

It must be noticed that, in the case of dodecylcyclohexane, cyclohexyldodecanoïc and cyclohexyldecanoïc acids have been identified in the liver and fat. In this tissue the concentration of these uncommon fatty acids reaches 2.3 %.

Compared with the control animals, the cholesterol concentration in fat was doubled in the n-paraffins and pristane groups, and 1.5 times higher in the dodecylcyclohexane group.

CONCLUSIONS AND REMARKS

The point of major concern raised by this study is the growth slow down observed in trouts fed a diet containing 1 % only of any of the three classes of saturated hydrocarbons. The branched paraffin pristane particularly, exerts a very severe effect. From the results of the investigation of different nutritional parameters it can be stated that neither a lack of appetency for the fortified diets, nor a decrease of feed consumption can explain the extent of such a phenomenon. The fact that the digestibility of the main nutrients is not modified, and that a compensatory growth occurs when the hydrocarbons are withdrawn from the diet, could support the hypothesis of a metabolic inhibitory effect.

The bioxidation of hydrocarbons into fatty acids being now well established in mammals, the first attempts have been directed on the investigation of lipid metabolism in the Trout. Although the fatty acid composition of liver or fat deposits is not significantly modified, the liver lipidosis and cholesterol increase in fat provide the first indications of such an interaction. The presence of cyclohexyl substituted fatty acids in lipids of trouts fed the diet containing dodecylcyclohexane could be of particular concern. The incorporation of such uncommon fatty acids in functional phospholipids should disturb some membrane properties.

The fate of hydrocarbons shows firstly an extensive absorption, that largely exceeds that measured in Rat or Chicken at a comparable level in the diet. No influence of the hydrocarbons on the absorption of the lipids of the diet and other lipophilic substances such as carotenoïds has been observed. A cumulative process occurs all along the experiment with a very preferential storage in fatty deposits. In fact, the hydrocarbon concentration in fat levels off after 3 months, and reaches values comparable to those measured in Rat ; the subsequent and absolute increase of stored quantities is closely related to fat deposition in the animal. The mobilization of stored hydrocarbons in the fasting trout is extremely limited, as the fatty reserves are not utilized so. It must be noticed that, on the contrary of mammals, fishes do not mobilize their fatty reserves at first, when fasting. However, after hydrocarbon withdrawal from the diet but *ad libitum* feeding, a depletion of the quantities previously stored occurs rapidly ; this gives evidence of an active lipid turn-over, even in fat reserves.

As a conclusive and prospective remark, one can say that saturated hydrocarbons exert a very physiological effect on trout. The first nutritional

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and biochemical investigations underline the hypothesis of a metabolic inhibitory process in which lipid metabolism seems to be involved. The significance of these first results require further investigations directed on the metabolic behaviour, and specially oxidative pathways, of the different classes of saturated hydrocarbons.

COMMUNICATIONS ON THE SUBJECT

1. Fate of hydrocarbon pollutants in fish;
   J. TULLIEZ. CEE Environment and raw materials research program. "Freshwater" contact group meeting. WINDERMERE (Angleterre) 1977.

2. Effects and storage of different types of saturated hydrocarbons in the growing trout.
   J. TULLIEZ. CEE Environment and raw materials research program. "Freshwater" contact group meeting. METZ (France) 1978.

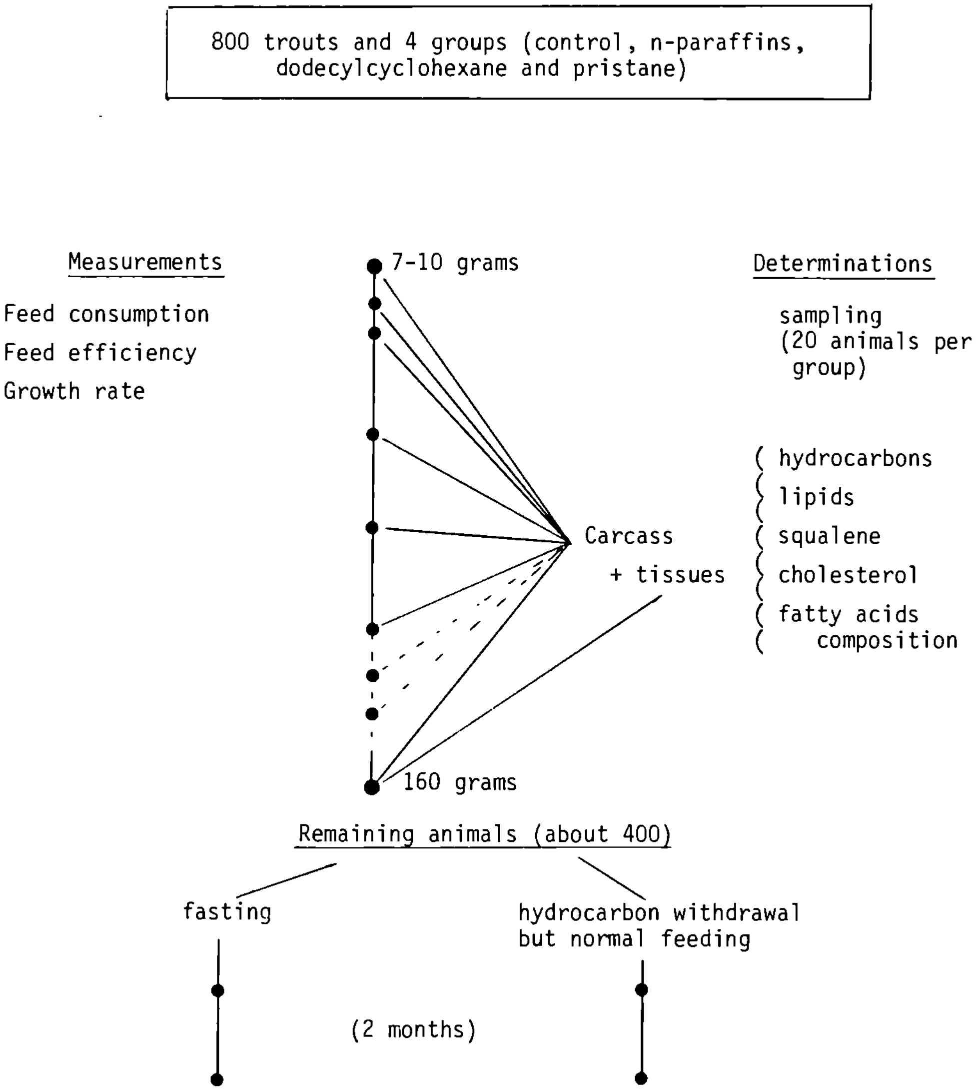

Figure 1 : Experimental protocol

Table 1 : Growth rate of trouts fed a diet containing 1 % hydrocarbon

Live Weight (g) Time required (weeks)	50	100	150
Control group	11	18	24
N-Paraffins	13	21.5	31
Dodecylcyclohexane	17	31	41
Pristane	18	32	48

Table 2 : Accumulation of hydrocarbons in the carcass (level at a live weight of 160g.)

	mg Hydrocarbon	% Hydrocarbon intake
N-Paraffins	143	5.07
Dodecylcyclohexane	110	3.29
Pristane	106	3.17

Contractor : Istituto di Scienze Chimiche, Università di Urbino

Contract n° : 214-77-1 ENV I

Project leader : Prof. F. Bruner

Title of project : Persistence and evolution of organic micropollutants in agricultural and industrial areas

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Summary of the Research Activity

The programme has been implemented according to the following objectives:

1) Identification and quantitation of chlorinated pesticides in the surface waters and in the soil of the province of Pesaro, that is a moderately industrialized area surrounded by an intensive agricultural activity with a background of mountains and forests.

2) Identification and quantitation of fluorochlorocarbons in the atmosphere of urban and rural areas.
Through such determinations the fate of halo-compounds in the environments can be inferred.
To achieve these goals, a critical evaluation of the existing analytical methodologies has been made. On the base of this, original samplings and analytical techniques have been implemented.

Pesticides and their derivatives in soil and surface waters.

Forthe determination of pesticides in waters and soil, particular attention has been devoted to sampling and sample treatment.

In particular a mixture of benzene and n-hexane has been proved as more effective for the extraction of chlorinated pesticides from soil samples.

In the case of waters, a procedure involving adsorption of pesticides on an appropriate adsorbent, graphitized carbon black has been implemented.

As a result, a substantial increase of the sample recovery has been observed with respect to the well established techniques such as that issued by EPA.

The results of GC analysis show that the distribution of pesticides in soil and waters, and the relative abundance of the single constituents as well, follow the composition of the pesticides mixtures used for the soil treatment and that, for example, DDT and its transormation products are present in the soil and water in spite of the fact that their use has been avoided since several years.

Determination of the halocarbons in the atmosphere.

The existing sampling and analytical techniques have been deeply modified to improve the reliability of halocarbon determination.

A sampling method based on the use of graphitized carbon black traps cooled at the dry ice temperature has been deviced and proved extremely effective for the concentration of all C_1-C_2 halocarbons.

This allows to inject into the GC column the halocarbon content of 6 to 30 litres of air through the heat-stripping technique.

Particular care has been devoted to determine that no decomposition of the halocarbon takes place during the heat treatment.

A new GC column that allows the separation of all C_1-C_2 halocarbons within 40 minutes has been prepared.

With the sampling and analytical tools described many air samples has been analyzed for their halocarbon content.

The main result of this research is that Fluorocarbon 21 ($CHCl_2F$) has been found in all the samples analyzed.

Such compound that, has no industrial use and is not produced in massive amounts has been identified without any doubt by

GC-mass fragmentography.

Its concentration has also been found substantially higher than it could be forecasted by the industrial production.

This implies its formation from other halocarbons in the troposphere via unknown mechanism.

Unfortunately the contract expired just when a strong need for further research in this field is felt.

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3) G. Crescentini and F. Bruner
"Identification of Trace Constituents of the Atmosphere: Evidence for the Presence of F21 by GC-Mass Fragmentography"
To be submitted to "Nature" (1979).

Contractor: Montedison S.p.A.
Contract n° 284-77-1 ENV I
Project leader: G. Pecenick
Title of project: Biodegradability of organic substances

Objective of the research

Concern for the impact of synthetic organic compounds on the natural environment has stressed the importance of reliable test methods and analytical techniques for the measurement of biodegradability - a major factor in determining their persistence and rate of accumulation. Of fundamental importance is the determination of "ultimate biodegradation" of these compounds and a number of methods based on unspecific analysis, e.g. BOD, COD, CO_2 - evolution, are already in use. A further technique which is both simple and applicable to all water-soluble compounds is TOC, i.e. the determination of soluble organic carbon; this method, moreover, has been shown to be valid for measuring the biodegradation of both pure compounds and their mixtures with other organic substrates (1,2). However, until now, no standard methods including TOC determinations have been adopted for general use; presently-used official OECD methods for detergents both anionic and nonionic, are capable of determining only what is defined as "primary biodegradation" (3,4). This research was carried out to establish the validity of the TOC technique and its application to the OECD Screening and Confirmatory Tests. To this end a comparison has been made between two proposed modifications of the dynamic test (5,1). In addition to TOC determination, in some instances, specific analytical methods were also used. An attempt was also made to investigate the biodegradation mechanism of ethoxylated alcohols by a pure bacterial strain isolated by enrichment culture on polyethylene glycols. Anionic and nonionic surfactants were used as test materials in this study.

Materials and Methods

Materials tested: Alcohol ethoxylate (AE-nEO), C_{12}-C_{15}, 70% linear chain, with mean n of 9,15,19 oxyethylene groups (EO) per molecule. Nonylphenol ethoxylate (NP-nEO) with mean n of 9,13,17 EO. Linear alkylbenzene sulphonate (LAS) C_{10}-C_{14}, with 29.9, 23.3, 16.9, 16.1, 12.1, 1.7% respectively of 2,3,4,5,6,7 phenyl isomers. These products and polyethylene glycols (PEG), 600 mean molecular weight, were obtained from Laboratorio Detergenti DIPI, Montedison, Milan. S^{35}-LAS, C_{12}, was obtained from the Radiochemical Centre, Amersham, UK.All other products eployed are reagent grade Carlo Erba, Milan.

Biodegradability tests: Modified Screening Test was used at test compound concentration of 10 mg/l TOC and 10 mg/l dry weight of sewage inoculum. Modified versions of the Confirmatory Test were carried out both with and without daily sludge-exchange, using two parallel laboratory activated-sludge units, blank and test unit, and test-compound concentration of 20 mg/l TOC. TOC determinations were carried out on a centrifuged sample (12.000 rpm x 10') with a Beckman TC Analyzer 915 A. UV spectrometry was carried out with a Philips SP 1800. Degradation values were obtained by multicomponent analysis considering NO_3-N and NO_2-N interferences (6). Sulphate determination was carried out both by radiometry using a Packard Tri-Carb 3320 spectrometer, in Lumagel scintillation mixture (Lumac, Supelchem, Milan) and by titration using an ion-selective electrode (Orion mod. 9482) in conjunction with a double-junction electrode (Orion mod. 900200). Selective extraction of ethoxylated alcohol degradation products was carried out according to Patterson (7) and Rudling (8).

Results

Biodegradability of nonionic surfactant: All alcohol ethoxylates tested are easily biodegradable, in the modified Screening test, regardless of their mean degree of ethoxylation; TOC removals higher than 90% were obtained, after 30 days, showing that polyoxyethylene chains have undergone microbial oxidation. On the contrary, only around 50% TOC removal, after 38 days, could be obtained when the ethoxylated nonylphenols were tested. Although inocula obtained from three different sources were used in the biodegradation experiments, slight differences in rate and extent of biodegradation were observed only for NP 17 EO. When a PEG 600 - acclimatised pure - strain, tentatively identified as a Flavobacterium sp., was used for studying biodegradation mechanism of a C_{16} AE-10EO, the hydrophilic moiety was the first to disappear. Neither polyglycols, nor carboxylated oxyethylene chains could be detected (after 5 days incubation) by either TLC or IR analyses of selectively extracted fractions. The acidic fraction was found to consist mainly of fatty acids derived from the hydrophobic chains. Carboxylated polyglycols, however, were detected in the degradation products extracted from the effluent of a laboratory ac-

tivated-sludge unit treating AE-9EO.
The applicability of TOC to the dynamic test has been evaluated by following the biodegradation of the three test alcohols. Although great care was devoted to maintain similar operating conditions in the two parallel units, only in the case of the 9 EO adduct were degradation values comparable, being 92±2 and 96±6% respectively with and without daily sludge-exchange. When the 15 EO and 19 EO adducts were tested, degradation values were found to be 70±2 and 66±1% when the sludge-exchange version was run, and 107±4 and 34±5% without sludge-exchange. These diverging results were obtained even when physiological conditions of the sludges, as checked by nitrification and oxygen uptake rate, were found to be comparable in the two runs. In one trial, however, addition of 10 ppm TOC of the 9 EO adduct to the test unit, was found to upset both physiological parameters only when the version without sludge-exchange was performed. Moreover, effluent TOC steady state values are preferably achieved with the sludge-exchange version.

Biodegradability of anionic surfactant: The most commercialised type of anionic surfactant, LAS, was used in the study. Biodegradability experiments were carried out using sewage inoculum both freshly collected and acclimatised for two weeks. In both cases, MBAS and TOC removals were respectively 100 and 70%, after 15 days incubation. Only in the case of UV absorptions are the final values appreciably different, being 80% with acclimatised inoculum and 60% with the fresh inoculum. Disappearance of soluble organic carbon and of UV absorption, specific for benzene ring, show a similar trend. Similar behaviour and similar MBAS and TOC removals were observed when S^{35}-LAS was used; 80% reduction of UV absorption was obtained in this case, after 22 days. Evidence for ring oxidation was also given by the immediate release of inorganic sulphate: 86% of the expected sulphate was recovered. No blank TOC was used in this case, for calculating TOC removal, otherwise inconsistent values were obtained. TOC removals reported can, therefore, reasonably assumed to be underestimated, if contribution of cell lysis to soluble carbon is considered.

Conclusions

TOC technique can be easily applied to the official Screening Test; modifications only involve test compound and inoculum concentrations.

However, when applied to the Confirmatory Test, this technique requires strict control of sludge condition to obtain reliable results. Moreover, the proposed modifications are rather laborious and show poor reproducibility. TOC removal values obtained with this method may differ widely from those obtained in static conditions using the same compound. Other tests should be devised for treatment-plant simulation if unspecific analyses are to be applied. Inoculum acclimatisation seems to be an important factor in determining the type of metabolites formed in the oxidation of alcohol ethoxylates. Metabolism of these products may be strictly related to the microbial population involved in the biodegradation process. In the case of LAS, biodegradation does not seem to involve definite steps, microbial attack taking place on different constituents of the molecule simultaneously. Oxidation of LAS extends far beyond the aliphatic chain and affects the benzene ring as well.

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TOPICS 12 & 14 : ORGANIC MICROPOLLUTANTS & NEW CHEMICALS

Health effects

Contractor: Gesellschaft für Strahlen- und Umweltforschung, Abteilung für Genetik, Neuherberg, Federal Republic of Germany

Contract No.: 136 - 77 - 1 ENV D

Project Leader: Dr. U.H. Ehling

Title of Project: Chemically-Induced Mutations in Mice

The main scope of this project is a) the mutagenicity testing in vivo with mammals, b) the determination of the sensitivity of the different methods for selected compounds, c) the quantification of genetic risk to man.

Dominant Lethal Mutations

To determine the sensitivity of the dominant lethal assay, $(101 \times C3H)F_1$ hybrid male mice were injected intraperitoneally with 1 ml of the test compound. The animals weighted between 27-30 g and did not vary from the nominal value by more than 5%. Immediately after treatment, each male was mated sequentially to one $(101 \times C3H)F_1$ virgin female at 4-day intervals up to 48 days. The age of the mice when first mated was 89-98 days. Uterine contents of the females were examined 14-17 days after conception. With this mating schedule we can detect, with a sample size of 45 animals per mating interval and per dose, a mutagenic effect of 10%. For different compounds the lowest effective dose, in a sample size of 50 animals, was as follows: methyl methanesulfonate (MMS) - 10 mg/kg, mitomycin C - 0.3 mg/kg, Natulan - 100 mg/kg.

Atrazine, suspended in olive oil and administered by oral intubation with a stomach tube, induces dominant lethal mutations in spermatozoa and spermatids in the dose range 1500-2000 mg/kg.

Heritable Translocations

The induction of heritable translocations was evaluated for MMS and mitomycin C in those stages of germ cell development most sensitive to dominant lethal induction. For MMS the most sensitive germ cell stages are late spermatids and early spermatozoa. For mitomycin C the spermatocytes are most sensitive to cell killing and induction of chromosomal damage.

The heritable translocation test was performed in two ways. The commonly used protocol selects translocation suspects among the F_1 progeny by fertility testing. Only those animals with reduced fertility are analysed cytogenetically. The new protocol for the heritable translocation test (Adler, 1978) was used in the second experiment with mitomycin C. All F_1 progeny were analysed cytogenetically and no fertility selection was performed.

With MMS the frequency of translocation carriers derived from treated late spermatids and early spermatozoa was 20 in 245 progeny (8%) for 40 mg/kg and 7 in 546 progeny (1.3%) for 20 mg/kg. In the controls there was no translocation carrier among 1065 animals tested. The translocation frequency of both groups is significantly different from the control value ($P < 0.001$).

With mitomycin C the frequency of translocation carriers derived from treated spermatocytes was one in 490 progeny in the first experiment and, again, one in 487 progeny in the second experiment. No translocation carrier was found among 1400 controls. The total frequency of 2 translocation carriers in 977 animals tested is not significantly different from the control ($P = 0.17$).

Bone Marrow Chromosome Analysis and Micronucleus Test

Chromosome analysis and the micronucleus test in mouse bone marrow were performed with MMS, mitomycin C and Natulan in order to establish the lowest dose recognized in both test systems. The mutagenic effect of atrazine was tested by chromosome analysis only.

Both tests were performed after a single interperitoneal injection of the test compound, except for atrazine which was administered as a suspension in olive oil by oral intubation. Simultaneous controls for each experiment were injected with the solvent. Bone marrow was sampled 24 hours after treatment. For chromosome analysis the animals were injected with colchizine 1 hour prior to preparation of the bone marrow. In both tests 4 treated animals were used per dose and 125 mitoses or 2000 polychromatic erythrocytes were analysed per animal.

MMS, mitomycin C and Natulan had a chromosome breaking effect in mouse bone marrow. In the chromosome test the lowest effective doses were 5 mg/kg for MMS, 0.625 mg/kg for mitomycin C and 50 mg/kg for Natulan. While with MMS the lowest recognizable dose was reached at 5 mg/kg, the dose response curves for mitomycin C and Natulan indicate that doses below the ones used in our experiments can still be recognized with the sample size applied in the present protocol. Atrazine gave a positive response in the chromosome analysis at the only dose tested. 2000 mg/kg of atrazine induced 4% chromosome breaks in bone marrow as compared to 0.7% in the controls.

In the micronucleus test the lowest effective doses were 10 mg/kg for MMS, 25 mg/kg for Natulan and 0.625 mg/kg for mitomycin C. While for MMS the chromosome analysis is more sensitive than the micronucleus test, this conclusion is not yet possible for mitomycin C and Natulan. Both systems will be used again with lower doses of the chemicals.

Specific Locus Mutations

Natulan-induced specific locus mutations fulfill the criteria for estimation of the doubling dose, i.e. the dose necessary to induce as many mutations as occur spontaneously. The doubling dose of Natulan in A_s spermatogonia is 114 mg/kg. The therapeutic dose for Natulan is about 215 mg/kg. If man and mouse are equally sensitive, this dose would induce 1.9 times a many mutations as arise spontaneously.

The calculation of individual risk is of great importance for the treatment of patients with drugs. In addition to the individual risk, it is necessary to quantify the risk to the population by a mutagen. From the incidence of patients with Hodgkin's disease (1 : 42.000), we can calculate a population dose of 5.12 microgram/kg of Natulan. Assuming equal sensitivity between mouse and man and between both sexes, we can calculate, for an estimated number of 30.000 genes for man, the induction of new mutations due to Natulan treatment. Given a mutation rate in the mouse of 0.7×10^{-10} per microgram/kg Natulan, based on the data of the 600 mg/kg group and 30.000 mutable loci (3×10^4), we compute, for one million births (10^6) after a parental exposure of 5.12 microgram/kg procarbazine, a number of new mutants equal to $0.7 \times 10^{-10} \times 3 \times 10^4 \times 10^6 \times 5.12 \times 2 = 21.5$. The final factor of 2 comes from the fact that both parents are exposed (Ehling and Neuhäuser, 1979). Experiments for improving extrapolation from mouse to man are in progress.

Summary: Mutagenicity in Mice

Compound	Test systems							
	Dominant Lethals	Heritable Translocations	Micro-nuclei	Chromosome aberrations			Somatic Mutations in vivo	Specific Locus Mutations
				Bone marrow	Spermatocytes	Spermatogonia		
MMS	+	+	+	+	n.t.	n.t.	+	+
Natulan$^{(R)}$	+	n.t.	+	+	n.t.	+	+	+
Mitomycin C	+	(-)	+	+	+	+	+	+
Atrazine	+	n.t.	n.t.	+	n.t.	n.t.	(-)	n.t.

n.t. = not tested

Completed studies funded under contract no. 136 - 77 - 1 ENV D

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Biochemisches Institut für Umweltcarcinogene - Prof.Dr.Grimme
Contract n° : 138-76-10 ENV D
Project Leader : Prof.Dr.G.Grimmer
Title of project : Investigations on the Carcinogenic Action of used lubricating Oil of Passenger Cars

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Objective of the research : (1) Assessment of the amount of carcinogenic polycyclic aromatic hydrocarbons (= PAH) that leak out to environment by improper discharge of the used lubricationg oil.

(2) Assessment of the carcinogenic risk for man in contact with used lubricating oil by experiments with animals (skin carcinogenic activity). These experiments on animals would give answer to the question whether carcinogenic substances other the PAH are present in the used lubricating oil. In this case, a PAH-free fraction would show carcinogenic activity too.

Materials and methods :

Samples of used (20 samples) and unused (18 samples) of lubricating oil has been investigated on their content of PAH by a gas-chromatographic method (G.GRIMMER & H.BÖHNKE : Enrichment and gas chromatographic profile-analysis of PAH in lubricating oils, Chromatographia 9, 30-40, 1976).

For experimental studies of the carcinogenicity by skin test on mice, a sample of used oil (10 000 km) has been separated in PAH-containing and PAH-free portions. To compare the skin-carcinogenicity of a sample of used oil with fractions thereof and furthermore with the reconstituted oil (reconstitution of these fractions) by the chronic application on the skin of mice (2 times weekly for 2 years, 3 dosis each) the following fractions are produced for the investigation in 3 dosis

(a)	Sample of the used oil	(100.0 %)
(b)	PAH-free portion of the same oil	(91.6 %)
(c)	PAH-containing fraction (2 and 3 rings)	(7.3 %)
(d)	PAH-containing fraction (4 to 7 rings)	(1.1 %)
(e)	Reconstituted oil	(99.1 %)

For the bioassay, the oil and fractions thereof are tested in 3 dosis. Used oil : 65 mg, 195 mg and 585 mg/anno, containing 15 µg, 45 µg and 135 µg BaP. Fractions thereof : in corresponding amounts (91.6 %, 7.3 % or 1.1 % of the total oil). 65 CFLP mice for each dosis.

Results (chemical part)

Table 1 (encl.) shows the content of 14 PAH in used lubricating oils from 10 different passenger cars. Distance with the same oil : 1000 - 6000 km;

Content of benzo(a)pyrene in the samples after 1000 - 6000 km was between 5.2 mg and 35.1 mg BaP/kg. There is no correlation between the distance and the PAH-content of the oil.

The results of the PAH-analysis of 10 samples of mixed used oil (mixed from different cars), collected in different service-stations are shown in tab. 2. The PAH-content of the mixed samples are in the same range as in tab. 1.

Tab. 3 and 4 shows the PAH-content of fresh lubricating oil samples. In this case the content of benzo(a)pyrene ranges beween 0.008 mg - 0.266 mg BaP/kg.

Results (biological part)

Experiments on animals with used lubricating oil and fractions thereof are not finished. Therefore it is not possible to give a comprehensive final report.

Preliminary results after 55 weeks : By application of the total used oil 38 % of the mice developed tumors on the highest dosis (585 mg/anno), 9 % tumors by 195 mg and non tumor after application of 65 mg oil.

PAH-contaning fraction (4-7 rings, 1.1 % of the total oil) produce 12 % of the mice with tumors (about 6 mg/anno), respectively in 6 % of the mice by the medium dosis.

The PAH-free fration (91.6 wt-%) produce no tumors at all.

tab. 1

Content of Polycyclic Aromatic Hydrocarbons in Lubricating Oils Samples from Passenger Cars

mg/kg

Sample	1	2	3	4	5	6	7	8	9	10
Car model	Simca	VW1302	Kadett	VW1300	VW 1303	VW1600	Merced	Kadett	VW1302	Mer220
mileage (km)	3300	33300	12000	55200	30500	110300	140000	64000	73000	100000
distance with the oil (km)	1000	1300	1600	4000	2300	5000	4000	6000	4900	6000
Lubricating Oil producer	AE	AE	MS	Pe	AE	AS	AE	AE	AE	AE
Fluoranthene	16.6	3.4	16.4	49.9	53.5	63.7	47.3	109.0	29.9	99.0
Pyrene	32.7	5.7	35.0	141.0	135.0	133.0	89.3	326.0	62.1	184.0
Benzo(a)anthracene	14.8	10.2	17.8	46.8	45.3	67.4	27.4	53.7	19.0	77.5
Chrysene	11.0	8.7	17.3	34.3	39.6	54.0	28.7	68.7	18.1	74.0
2-Methylchrysene	8.2	1.7	5.7	9.2	12.8	17.2	15.8	19.8	8.1	9.0
Benzofluoranthenes	5.7	14.4	14.3	27.9	27.3	28.2	39.3	44.3	16.6	43.4
Benzo(e)pyrene	6.4	15.1	17.9	34.9	35.6	30.8	40.0	48.9	27.3	43.5
Benzo(a)pyrene	5.2	9.7	10.9	19.6	22.6	23.2	24.2	24.2	14.2	35.1
Perylene	1.9	3.1	2.9	4.9	6.4	5.7	6.2	7.2	4.6	10.0
Methylene-benzo(e)pyrene	0.9	2.4	2.0	3.9	3.2	4.8	4.2	4.9	2.3	6.7
Indeno(1,2,3-cd)pyrene	2.1	4.9	4.8	8.9	8.5	10.9	10.8	8.7	5.5	12.5
Benzo(ghi)perylene	4.4	27.4	34.3	33.0	67.7	61.4	85.2	56.4	34.8	69.8
Anthanthrene	1.6	3.7	3.5	8.3	8.8	9.4	3.7	4.7	3.0	10.8
Coronene	2.8	9.7	10.7	29.4	24.3	24.7	17.5	12.1	10.0	23.5

AE = Aral Elastic, MS = Mobil Super, Pe = Pentolup, AS = Aral Super Special

tab. 2

Content of Polycyclic Aromatic Hydrocarbons in Mixed Oil Samples from Different Service Stations

mg/kg

	01	02	03	04	05	06	07	08	09	10
Fluoranthene	115	32	74	99	39	11	171	119	49	6
Pyrene	275	75	155	198	76	18	263	347	121	11
Chrysene+Benzo(a)anthracene	116	90	59	87	63	8	52	84	33	9
2-Methylchrysene	10	16	7	23	21	5	7	24	15	5
Benzofluoranthene (b+k+j)	60	29	34	49	15	4	52	71	27	4
Benzo(e)pyrene	69	30	40	51	17	4	34	72	29	3
Benzo(a)pyrene	34	17	22	30	9	3	42	35	16	3
Perylene	13	8	9	12	3	1	13	10	5	1
Methy lene-benzo(e)pyrene	5	4	6	6	2	1	11	27	3	1
Indeno(1,2,3-cd)pyrene	16	9	12	16	6	1	28	12	8	2
Benzo(ghi)perylene	103	46	55	67	27	6	51	104	49	5
Anthanthrene	11	7	8	12	4	1	25	10	6	1
Coronene	25	10	14	21	5	1	20	36	18	1

Mixed oils from : 01 = Fa. Knobloch, Harburg
02 = ARAL Kreizazek, HH 93
03 = FINA Mittelstein, HH1
04 = SCG Stellingen, HH 54
05 = ESSO Remseger, HH 26
06 = ESSO, Billwerder Steindamm
07 = Reeperbahngarage, HH 4
08 = ESSO Staak, HH 93
09 = BP Bensemann, HH 90
10 = ARAL Vogelhüttendeich, HH

tab. 3

Polycyclic Aromatic Hydrocarbons in unused Samples of Lubricating Oil (fresh oils)

mg/kg

	01	02	03	04	05	06	07	08	09
Fluoranthene	0.074	0.031	0.071	0.550	0.008	0.068	0.042	0.057	0.051
Pyrene	0.182	0.201	0.168	0.897	0.039	0.062	0.175	0.162	0.100
Chrysene+Benzo(a)anthracene	0.528	0.740	0.400	0.582	0.318	0.207	0.753	0.591	0.295
2-Methylchrysene	0.477	0.955	0.432	1.026	0.361	0.188	0.329	0.478	0.235
Benzofluoranthenes (b+j+k)	0.064	0.078	0.026	0.702	0.054	0.027	0.052	0.063	0.081
Benzo(e)pyrene	0.076	0.181	0.057	0.402	0.243	0.054	0.099	0.085	0.120
Benzo(a)pyrene	0.027	0.048	0.023	0.266	0.025	0.008	0.021	0.027	0.049
Perylene	0.063	0.026	0.061	0.224	0.009	0.007	0.038	0.078	0.064
Methylene-benzo(e)pyrene	0	0	0	0	0	0	0	0	0
Indeno(1,2,3-cd)pyrene	0	0	0	0	0.020	0	0	0	0
Benzo(ghi)perylene	0.093	0.050	0.081	0.073	0.057	0.019	0.016	0.021	0.018
Anthanthrene	0.005	0.002	0.007	0.006	0.005	0.008	0.005	0.009	0.008
Coronene	0.010	0.002	0.013	0.008	0.016	0.002	0.001	0.002	0.001

01 = ARAL Super Elastic 20W50
02 = ARAL Super 10W30
03 = ARAL Spezial 20W30
04 = A AL Super Elastic 15W50
05 = ARAL 2T-Motor Oel
06 = BP Zweitakter Spezial
07 = BP Visco 2000 15W50
08 = Engergol BP 20W20
09 = Visco Static BP 20W50

tab. 4

Polycyclic Aromatic Hydrocarbons in Unused Samples of Lubricating Oils (fresh oils)

mg/kg

	10	11	12	13	14	15	16	17	18
Fluoranthene	0.620	0.063	2.750	0.033	0.316	0.024	0.205	0.227	0.120
Pyrene	1.350	0.437	6.530	1.060	0.466	0.219	0.578	0.442	0.348
Chrysene+Benzo(a)anthracene	2.860	1.180	13.400	0.565	0.338	0.720	1.060	2.090	1.270
2-Methylchrysene	1.420	2.400	7.010	0.703	0.138	0.756	0.424	0.994	0.679
Benzofluoranthenes (b+j+k)	0.085	0.110	0.234	0.110	0.013	0.181	0.148	0.320	0.140
Benzo(e)pyrene	0.137	0.198	0.312	0.214	0.035	0.030	0.033	0.583	0.317
Benzo(a)pyrene	0.051	0.033	0.061	0.064	0.012	0.020	0.077	0.181	0.089
Perylene	0.064	0.022	0.032	0.043	0.020	0.010	0.011	0.127	0.062
Methylene-benzo(e)pyrene	0	0	0.009	0	0	0	0	0	0
Indeno(1,2,3-cd)pyrene	0	0	0.014	0	0	0	0	0	0
Benzo(ghi)perylene	0.051	0.013	0.025	0.061	0.017	0.058	0.019	0.139	0.088
Anthanthrene	0.019	0.006	0.010	0.027	0.004	0.030	0.004	0.009	0.008
Coronene	0.009	0.002	0.001	0.005	0.004	0.002	0.003	0.016	0.007

10 = Shell Rotello TX HD Öl (Diesel) SAE 30
11 = Shell Super SAE 15W50
12 = Shell Myrina SAE 15W40
13 = Shell X 100 SAE 20W20
14 = Shell Super 2 T
15 = MOBIL Super 15W50
16 = ESSO Uniflo 15W50
17 = ESSO Plus SAE 30
18 = ESSO Extra SAE 20W50

Contractor: University of Freiburg, GFR, Forstbot.Institute
Contract n° 144-77-1 ENV D
Project leader: Professor Dr.Dr.H.Marquardt
Title of project: Further improvement of a genetic prescreening test pattern for carcinogenic effects of environmental chemicals.

Introduction and methods

During the first contract a pattern of genetic and cytogenetic mutagenicity tests has been developed with the aim to obtain extensive mutagenicity and first pharmacokinetic data of a test compound, and additional information for the correlation of mutagenic and carcinogenic properties of a given substance. In a diploid strain of Saccharomyces cerevisiae (D4-RDII) the induction of mitotic gene conversion (intragenic recombination) as the genetic endpoint on the molecular level was examined using the following four assays: (I.) direkt assay (liquid test), (II.) in vitro liver microsome test, (III.) host-mediated peritoneal assay, and (IV.) urinary assay. On the cytological level three different cytogenetic effects in bone marrow cells in vivo were studied: Induction of micronuclei in mice, chromosome aberrations and the induction of sister-chromatid-exchanges (SCE) with a newly developed test both in Chinese hamsters.

With this battery of genetic tests the following questions can be answered. (I.) Is the test substance in a mammal acting directly or only after metabolization. (II.) How long do the metabolites stay in an active state, i.e. are able to induce genetic or cytogenetic effects. (III.) The amount of metabolites and the velocity by which they are excreted via urine. (IV.) Are those metabolites bound to glucuronic or sulfonic acid.

Results and discussion

By using methylcholanthrene-induced microsomes and applying the test battery to the polycyclic aromatic hydrocarbons 7,12-dimethylbenzanthracene (DMBA), 3,4-benzo(a)pyrene (BaP) and phenanthrene (Ph) genetic activity in the gene conversion system was detected only in the case of DMBA and BaP (Tab. 1) (5). The noncarcinogenic phenanthrene was inactive in all yeast test systems. The negative results in the host-mediated assay allow the conclusion that the concentration of the active metabolites which reach the lymph tract is too low to show a genetic effect. The results of the cytogenetic tests indicate that the concentration of the long-lived active metabolites of DMBA which reach the bone marrow is sufficient to yield positive results in all three tests (Tab. 1) (3,6). In the SCE-test however, merely BaP showed a distinct effect, whereas Ph yielded a weak effect only with high doses. This result is not reflected by other tests because of the unique sensitivity of the in vivo SCE-test.

Furthermore the test pattern was applied to the three nitrofuran derivatives nitrofurantoin (1-5-nitro-2-furfuryliden)-1-aminohydantoin), Carofur (1-(5-nitro-2-furyl)-2-(6-amino-3-pyridazyl)-ethylene-hydrochloride) and FANFT (2-formylamino-4-(5-nitro-2-furyl)thiazol). The first two compounds are drugs, the latter a strong carcinogen. All the three substances are acting directly (Tab. 1). The two compounds tested in the in vitro microsome test, nitrofurantoin and carofur, showed reduced activity when compared with the direct test. This allows the conclusion that they act as a mutagen in the original form and that metabolism deactivates them partially. The urinary assay shows a fast excretion in the first 3-4 hours: Only nitrofurantoin yields a weak but positive reaction in the host-mediated assay because of the quick excretion of the other compounds. The cytogenetic tests produce a similar weak reaction, too. Since the pharmacokinetics of nitrofurantoin and FANFT are well investigated in rats and man the conclusions of our genetic results could be reexamined: We found total concordance (7). Using Salmonella as test organisms the correlation between pharmacokinetic and genetic data depends on the strain used.

Mutagenicity assays as prescreening tests for carcinogenicity

The combination of these genetic and pharmacokinetic data obtained with our test battery allows an additional conclusion on a possible carcinogenic risk of nitrofurantoin: Even though the quick excretion of unmetabolized nitrofurantoin into the urine, the very low and quickly decreasing concentration in the blood is sufficient for a weak effect in the host-mediated assay and in the cytogenetic tests. Hence, a quick excretion into the urine does not mean that the substance is genetically inactive (cf. cyclophosphamide). A weak carcinogenic effect of nitrofurantoin in mammals can therefore not be excluded by the genetic results, despite negative results in carcinogenicity experiments with rats. It seems desirable to extend carcinogenicity tests to other species than rats and to continue epidemiologic studies on patients treated chronically with nitrofurantoin (Furadantin). In the case of Carofur our communication to the producer (Boehringer, Mannheim, GFR) of the genetic results initiated more detailed experiments on carcinogenicity and it turned out to be active (communication of the producer). The carcinogenic activity of FANFT was without question.

In the case of the polycyclic hydrocarbons the carcinogenic properties of DMBA and BaP are reflected by clear mutagenicity data in the liver microsome test. The noncarcinogenic Ph is negative in all the genetic tests. In the cytogenetic tests we find a clear correlation for DMBA only. Merely in the SCE test BaP yielded a distinct effect. The positive effect of Ph in the SCE test occured only with high doses and it is not correlated to the negative effect in other tests. So the reliability of genetic tests on the molecular level is much higher than the reliability of cytogenetic test in respect to the correlation between mutagenicity and carcinogenicity.

Substances of current interest

Some other compounds were not applied to the entire test pattern of genetic and cytogenetic tests (Tab. 1). Procarbazine (Natulan) was tested in the liquid and in the liver microsome test with non-induced and phenobarbitone-induced microsomes of NMRI-mice. All tests whowed clear negative results (4). Of the two pesticides Benomyl and Atrazine only Benomyl was active. The effects were dose dependent, both in the direct and in the liver microsome test. The only cytogenetic test (SCE) applied so far to Benomyl, showed a slight but not significant increase. Mitomycin C was tested only in the direct liquid test by means of the gene conversion test strain. It induced gene conversion at high frequencies: Mitomycin C could be considered as a highly active compound.

Further improvement of the SCE test in vivo

The crucial prerequisite for the differential staining of chromatids to show sister-chromatid-exchanges (SCE) is the incorporation of 5-bromodeoxyuridine (BUdR) in the DNA instead of thymidine. To ensure a continuous incorporation of BUdR a steady state of a minimum concentration was reached by repeated injections of BUdR during one S-phase (1, 2). Instead of these injections Allen et al. (Cytogenet. Cell Genet.18,1977) suggested the use of a 50 mg tablet of pure BUdR, which was to be implanted subcutaneously into the animals. By using cyclophosphamide these two variations of the in vivo SCE-test were compared. The tablet technique revealed a slight incrase in untreated controls compared to the injection method from 3.2 ± 0.07 to 3.58 ± 0.1 SCE per cell. Both variations showed a clear dose response relationship of SCE induced by cyclophosphamide. The employment of the BUdR-tablets in the SCE test offers the following advantages: (I) The percentage of differentially stained metaphases is incrased from a maximum of 30% in the old method to a maximum of about 70% in the new version, (II) the differential staining shows a better contrast and (III) the implantation of the tablet is tolerated better by the animals than repeated injections.

Comparative testing of bleomycin with special regard to the induction of SCE.

Several investigations have shown a close correlation between the induction of SCE and the induction of chromosome aberrations. In the present time the genetical relevance of a SCE is not yet clear and it is therefore of great theoretical interest to find substances that do not show this correlation. This would lead to more detailed information as to whether sister-chromatid-exchanges are genetical relevant processes.

Bleomycin, an antitumor antibiotic, seems to be such an exception from the rule (Gebhardt and Kappauf, Mutation Res. 58,1978). In the direct test it induces mitotic gene conversion of medium strength (Tab. 1). In the micronucleus test bleomycin was found to induce micronuclei in the bone marrow already at a comparatively low doses of 50 mg/kg bodyweight, while the extend of induced SCE's was low. These preliminary data show that bleomycin is a genetically acting substance, inducing both molecular and chromosomal alterations. Its ability to induce SCE is lower than that of other substances which induce chromosome aberrations or micronuclei.

Tab. 1
Induction of mitotic gene conversion in Saccharomyces cerevisiae, micronuclei, chromosome aberrations and sister-chromatid-exchanges (SCE) in the bone marrow of Chinese hamsters with several compounds.

compound	direct test	microsome test	urine assay	host-med. assay	micronucleus test	chromos. aberr. test	SCE test
Cyclophos.	-	++	+++	++		+	++
7,12-DMBA	-	+	-	-	+	+	+
3,4-BaP	-	+		-	-	(+)	+
Phenanthr.	-	-		-	-	-	+
Nitrofurant.	+	-	+++	(+)		(+)	+
Carofur	+++	++	+	-		++	+
FANFT	++		+	-			
Procarbaz.	-	-					
Benomyl	+	+				- (+)	
Atrazine	-	-					
Mitom. C	+++						
Bleomycin	++				+	+	

(+) increased over control, not significant, + weak, ++ medium, +++ strong effect

List of publications

1. Bayer,U. and Bauknecht,T., Experientia 33,25 (1977)
2. Bauknecht,Th., Vogel,W.,Bayer,U. and Wild,D., Human Genet. 35, 299-307 (1977)
3. Bayer,U. in: Carcinogenesis, a comprehensive survey, Vol. 3: Polynuclear aromatic hydrocarbons: Second international symposium on analysis, chemistry and biology, p. 423-428, Raven Press,New York, 1978
4. Bayer,U., Mutation Res. 56, 305-309 (1978)
5. Bayer,U., Dissertation, Freiburg i.Br. (1978)
6. Kaths,S.,Diplomarbeit, Freiburg i.Br. (1978)
7. Siebert,D.,Bayer,U. and Marquardt,H., The application of mitotic gene conversion in Saccharomyces cerevisiae in a pattern of four in vitro and in vivo assays for mutagenicity testing, Mutation Res submitted for publication

Contractor: Ministerium für Wissenschaft und Forschung des
Landes Nordrhein Westfalen, vertreten durch den
Rektor der Universität Düsseldorf

Contract n^{o} 175-77-1 ENV D

Project leader: Prof.Dr.G.Röhrborn

Title of project: Analysis of non-disjunction processes

I. Investigator: A.Basler, G.Röhrborn

Compound:
Benomyl (pur.) purchased from Du Pont de Nemours

Genetic system:
Analysis of metaphase-II (mII) chromosomes in mice

Method:
Female mice of the strain NMRI were treated orally with Benomyl (2x500 mg/kg b.w.; 24 h apart; per stomach tube). The preparation of metaphase II chromosomes were performed 16 h after the last application by the method of RÖHRBORN and HANSMANN (1971).

Results:
No induced effects (structural chromosome aberrations or aneuploidies) could be ascertained.

Analysis of metaphase II chromosomes:

Series	n animals	n mII	hyper-ploid mII	hypo-ploid mII	structural aberrations mII
control	83	127	0	3	2
Benomyl	62	75	0	5	1

Conclusions:
Our results agree with those of SEILER (Mut.Res.40 (1976), 339-348) who performed the micronucleus test and those of SHERMAN et al. (Toxicology a.Applied Pharmacology 32 (1975), 305-315) who performed a teratogenicity study. Mutagenic effects detected in Aspergillus, are not transferable to mammals.

II. Investigator: A.Basler, G.Röhrborn, W.Seite

Compound:
ethanol; purchased from Merck

Test system:
a) Teratogenic effects
b) Analysis of metaphase II (mII) chromosomes of mice

Methods:
a) Female mice were treated for a period of 50 days with 15% ethanol (in drinking water). Thereafter they were mated with untreated males. The females were treated with ethanol also during pregnancy. Teratogenic effects (litter size, weight of the embryos, postnatal mortality) were analysed.

b) Female mice were treated for a period of 50 days with 15% ethanol (in drinking water). In the mean the females received 11 mg ethanol/g body-eight/day. At the end of the treatment metaphase II chromosomes of unfertilized oocytes were analysed. The preparation of chromosomes was performed by the method of RÖHRBORN and HANSMANN (1971).

Results: a) Teratogenic effects

Series	number of living animals per litter at birth	number of living animals per litter after 3 month	weight (g) of living animals at birth	weight (g) of living animals after 2 month
control	12.4	10.2	1.64	30.3
ethanol	10.9	2.3	1.36	23.4

Results: b) Chromosome aberrations in metaphase oocytes

Series	n animals	n mII	hyperploid mII (n=21) n	hyperploid mII (n=21) %	mII with structural aberrations n	mII with structural aberrations %
control	100	202	0	0	3	1.5
ethanol	100	260	2	0.8	6	2.3

Conclusions:
There is a clear teratogenic (see a) effect after the chronic application of alcohol. Alcohol does not increase the yield of structural aberrations. However, two of 260 metaphases II were hyperploid (n=21). This was never observed in the conrol.

III. Investigator: A.Basler, M.Brucklacher, F.Nobis, G.Röhrborn

Compound:
Trypaflavin^{R}= 3,6-diamino-10-methylacridinumchlorid

Test system: dominant lethal assay (DL-test)
analysis of metaphase-II chromosomes in mice

Method:
a) Pregnant C3H mice were orally treated with 50 mg Trypaflavin/kg body-weight on day 7, 11, 14 or 15 post conceptionem. The embryos were thus exposed to the compound or its metabolites in utero. At the age of 10 weeks the dominant lethal assay was performed with F_1 females.

b) Female C3H mice were treated with Trypaflavin (2mg/kg for 50 days; dissolved in drinking water). These mice were caged with untreated males. The DL-test was performed.

c) Female NMRI mice were chronically treated with Trypaflavin (2mg/kg for 50 days by stomach tube). Metaphase II chromosomes of unfertilized oocytes were analysed.

Results:

a) Dominant lethal mutations were induced only in those litter treated in utero on day 7 post conceptionem.

b) Chronic treatment increased the percentage of preimplantation egg loss and the yield of dead implants.

c) In metaphases II of unfertilized oocytes the yield of all observed aberration types (aneuploidies, gaps, satellite associations, breaks and fragments, deletions and interchanges) slightly was increased.

Conclusions:

1) Trypaflavin is a weak mutagen.

2) Oogonia (treatment on 7 days p.c.) are more sensitive to the induction of chromosome aberrations than oocytes (treatment on day 11, 14 or 15 of prenatal stage).

3) The effects - detected with the dominant lethal assay- were due to induced chromosome aberrations in mezaphase II oocytes.

IV. Investigator: A.Basler, G. Röhrborn

Compound: Methyl methanesulfonate (MMS)

Genetic system: Micronucleus test with Syrian hamster
bone marrow

Treatment:

Female Syrian hamster, 8-12 weeks old, were intraperitoneally injected with 40 mg/kg of MMS. Bone marrow was sampled 16 h after treatment for analysis of micronuclei in polychromatic erythrocytes to measure the proportion of normochromatic to polychromatic erythrocytes.

Results:

Series	proportion normochromatic: polychromatic erythrocytes	polychromatic erythrocytes with micronuclei per 1000 cells
control	1000 : 720	2.1
MMS	1000 : 199	33.1

p 0.01

Conclusions:

MMS induces a bone marrow depression evidenced by a decrease of the ratio of normochromatic to polychromatic erythrocytes. A single treatment of Syrian hamster with MMS induced - besides these cytotoxic effects - structural chromosome aberrations. The number of polychromatic erythrocytes with micronuclei was significantly increased.

V. Investigator: A.Basler, A.Hübinger, G.Röhrborn

Compound: Mitomycin C; purchased from Kyowa

Test system: diaplacentar mutagenesis
analysis of metaphase II chromosomes

Methods:
a) Pregnant mice were treated with MC (1.25, 2.5 or 5 mg/kg, i.p.) on day 7, 11 or 15 post conceptionem (p.c.). The embryos were thus treated in utero with the test compound. 8 weeks after birth of diaplacentar treated F_1-females their metaphase II chromosomes of unfertilized oocytes were analysed.

b) Adult female mice, 8 weeks old, were pretreated with hormones (PMS and HCG) to synchronice the estrus. MC was injected intraperitoneally at the preovulatory phase, e.g. together with the HCG application. The doses were 5, 10 or 20 mg MC/kg. The analysis of metaphase II chromosomes was performed 16 h after the MC injection.

Results:
a) Cytotoxic effects were induced only in those mice treated in utero on day 11 of fetal life. From 275 F_1-females no oocytes were found which might be caused by severe cell killing. This effect is limited to this stage og oogenesis; application on day 7 or 15 does not decrease the number of analysable oocytes. Experiments with lower doses shall clarify whether this cytotoxic effect is due to chromosome aberrations.
The yield of metaphases with chromosome aberrations seems to be increased slightly in females beeing treated with MC at day 7 or 15.

b) MC, injected at the preovulatory stage, induced chromosome aberrations. A dose-effect-dependency could be demonstrated. The yield of metaphases with aberrations ranged from 0% (control), 3.3% (5 mg MC/kg), 5.0% (10 mg MC/kg) to 6.3% (20 mg MC /kg).

Discussion and conclusions:
In embryonic stages of mice, the oogenesis ranges from primitive germ cells (oogonia) to oocytes in the dyctiotene stage. Primordial germ cells can be detected in 8 days old mouse embryos. The period of DNA synthesis is dividung oogonia occurs from dya 10 to day 13 p.c. On day 13-15 of fetal life the germ cells perform the last regular DNA-synthesis during oogenesis and enter the leptotene stage of meiosis. Subsequently, the application of a compound like MC, well known as an agent that effects the DNA synthesis, should be effective mainly, if applicated at the days 10-13. This could be shown in our experiments. These results correlate with those in spermatocytes (ADLER, Mut.Res. 35; 1976, 247-256) and in the DL-test (KRATCHOVILOVA, J, Mut. Res. 21; 1973, 192). Also in these investigations 5 mg MC/kg led to a lack of spermatocytes and unfertilized females respectively.

The sensitivity of oocytes beeing in the preovulatory stage and oocytes beeing in the leptotene/zygotene stage do not differ from each other. 5 mg MC/kg, given at the 15th day of fetal stage (oocytes in the leptotene/zygotene stage) induced 2.9% of aberrations. 5 mg MC/kg, given in the ovulatory stage to adult mice, induced 3.3% aberrations. Both stages, however, were not as sensitive as mitotic dividing cells undergoing DNA replication, like oogonia.

VI Investigator:A.Basler, G.Röhrborn, G.Roszinsky-Köcher

Compound:Benzo(a)pyrene = BaP; purchased from Eastman

Test system: a)Analysis of metaphase II chromosomes in mice; b) Chromosome aberrations in Chinese hamster's bone marrow cells; c) Sister chromatid exchange in vivo

Methods: We used female mice of the strain NMRI. 450 mg BaP/kg b.w.,suspended in olive oil, was given per stomach tube. Metaphase II chromosomes were prepared using the method of RÖHRBORN and HANSMANN (1971), 40 h after the application. Chinese hamsters were treated twice with 450 mg BaP/kg b.w. BaP was given either per os or i.p. 24 h after the last application, bone marrow chromosomes were prepared using the method of SCHMID and STAIGER (1969). To analyse in vivo induced SCE we used the method of VOGEL and BAUKNECHT (1977).

Results: a) Chromosome aberrations in metaphase II chromosomes

Series	n animals	n mII	% mII with aberrations excl.gaps	incl.gaps
control	45	105	0.95	0.95
BaP; per os	45	75	9.33	5.33

b) Analysis of chromosome aberrations in bone marrow cells of Chinese hamsters

Series	n animals	n metaphases	metaphases with aberrations incl.gaps n	%	excl.gaps n	%
control	7	1.400	37	2.6	5	0.4
BaP; per os	4	800	20	2.5	4	0.5
BaP; i.p.	7	1.400	80	5.7	12	0.9

c) Induction of SCE in vivo

Series	n hamsters	n metaphases	SCE/cell
control	18	900	4.16
BaP;per os	8	400	4.78
BaP; i.p.	8	400	10.57

Conclusions: BaP, given orally, induced aberrations only in metaphase II chromosomes of mice, but not in bone marrow cells of Chinese hamsters. With repect to bone marrow, the SCE test seems to be more sensitive than the usual chromosome analysis.

Contractor:	Med. Institut für Lufthygiene und Silikoseforschung an der Universität Düsseldorf
Contract no.:	289-76-10 ENVD, Project 1
Project leader:	Prof.Dr.med. H.-W. Schlipköter Priv.-Doz.Dr.med. F. Pott Dr.rer.nat. R. Tomingas
Title of project:	Sampling, analysis of polycyclic aromatic hydrocarbons (PAH) and testing of emissions and immissions for carcinogenicity.

Objective of research

In these investigations, we are concerned with two questions:

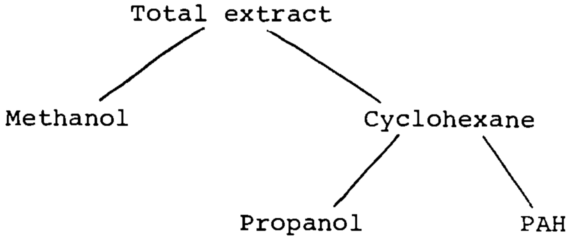
1. The relative proportion of PAH in suspended particulate matter (SPM).

2. The carcinogenicity of the total extract in SPM, of the PAH fraction, of the non-PAH fraction of the total extract and of the propanol fraction of the extract.

Materials and methods

Collection of SPM on a folded glass fibre filter (Dräger), surface area: 15 m^2, air flow rate: 1700 m^2/h, collection of particles > 0.3 µm in size with an efficiency of 99.998 %. Locations: 4 urban areas (Düsseldorf, Duisburg-Neuenkamp, Duisburg-Hamborn, Münster); 1 rural area (Krahm). Collection periods: winters 1975/76, 1976/77 and 1977/78, for a period of between 11 and 15 weeks at each location.

Analysis: Extraction of the filters with methanol, liquid-liquid partition of the extract between water and cyclohexane, fractionating of the cyclohexane phase on an aluminia column according to the following scheme:



Final determination of 8 PAH by direct measurement of the fluorescence intensity on the thin-layer plate. Abbreviations: Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(e)pyrene (BeP), Benzo(a)pyrene (BaP), Perylene (PER), DIbenz(a,h)anthracene (DBahA), Benzo(ghi)-perylene (BghiP), Coronene (COR).

Animal experiments: Extracts and fractions thereof were dissolved or suspended in tricaprylin. A single subcutaneous injection of 0.5 ml was administered to female NMRI mice; Histoacryl was used to seal the point of perforation. Dose levels refer to the BaP content or to corresponding fractions which contain no BaP. Tumours and tumoric tissue found at the application site were analysed histologically.

Results

Figs. 1 and 2 show the results from the PAH analysis and the calculation of the relative proportions of the 7 PAH to BaP (= PAH profiles).

In Fig. 3 and Table 1 are shown the dose-response-relationships of the extracts and fractions thereof.

Conclusions

The PAH profiles analysed from 5 sampling stations in the western Federal Republic of Germany during winter 1976/77 and winter 1977/78 showed on the whole great similarity. A high correlation factor was even obtained with the results from winter 1975/76 (1,2). It would appear that the widely varying PAH profiles of the emissions found in the atmosphere become relatively uniform when found in the suspended particles. Apart from that, the long collection period of ca. 3 months causes the fluctuations in the PAH profile to level out. Nevertheless, the results are inadequate for general application.

The total extracts from the urban areas collected during the winter 1975/76 induced a significantly higher tumour rate than the PAH fractions isolated thereof (Fig. 3). This finding supports the view that, in addition to PAH, there exist other tumour-inducing substances. On the other hand, the carcinogenic PAH from the rural area appear to be inhibited by accompanying substances; similar effects have already been found in previous investigations using automobile exhaust condensates.

The total extracts from Duisburg-H. and Münster collected during the winter of 1976/77 were not more active than the PAH fractions. But the propanol fraction from both cities showed a carcinogenic effect which was less active than that of the PAH. This finding is to be investigated.

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1. Tomingas, R., F. Pott and G. Voltmer (1978): Profiles of Polycyclic of Aromatic Hydrocarbons in Suspended Particles of Different Cities in the Western Federal Republic of Germany. Zbl. Bakt. Hyg., I. Abt. Orig. B 166, 322-331.

2. Tomingas, R.: Profiles of Polycyclic Aromatic Hydrocarbons in Suspended Particles. International Congress on Analytical Techniques in Environmental Chemistry, 27-30 Nov. 1978, Barcelona, Spain; in press.

Table 1: Examination of the carcinogenicity of extracts and fractions of suspended particles from Duisburg-H. and Münster, sampling period winter 1976/77, using the subcutaneous test on mice (60 mice per group; provisional results 20 months after injection.)

Declaration of extract or fraction	Dose+) (µg)	mice with s.c. tum.	
		(no.)	(%)
Tricaprylin (carrier)	(0.5 ml)	1	1.7
Extract of unused filter	0.04	2	3.3
" " " "	0.16	2	3.3
" " " "	0.64	2	3.3
Total extract, Duisb.	0.04	6	10.0
" " , "	0.16	4	6.7
" " , "	0.63	6	10.0
" " , "	2.5	7	11.7
Cyclohexanephase, Duisb.	0.04	2	3.3
" , "	0.16	4	6.7
" , "	0.63	9	15.0
" , "	2.5	12	20.0
PAH-Fraction, Duisb.	0.04	0	0
" , "	0.16	3	5.0
" , "	0.63	6	10.0
" , "	2.5	18	30.0
Propanol-Fraction, Duisb.	0.16	1	3.3
" , "	0.63	5	8.3
" , "	2.5	12	20.0
Total extract, Münster	0.04	6	10.0
" " , "	0.16	9	15.0
" " , "	0.63	8	13.3
" " , "	2.5	0	0
Cyclohexanephase, Münster	0.04	4	6.7
" , "	0.16	7	11.9
" , "	0.63	11	18.3
" , "	2.5	11	18.3
PAH-Fraction, Münster	0.04	4	6.7
" , "	0.16	10	16.7
" , "	0.63	9	15.0
" , "	2.5	17	28.3
Propanol-Fraction, Münster	0.16	5	8.3
" , "	0.63	4	6.7
" , "	2.5	11	18.3

+) BaP per animal or declaration of the BaP amount which would be in the fraction if PAH were not eliminated.

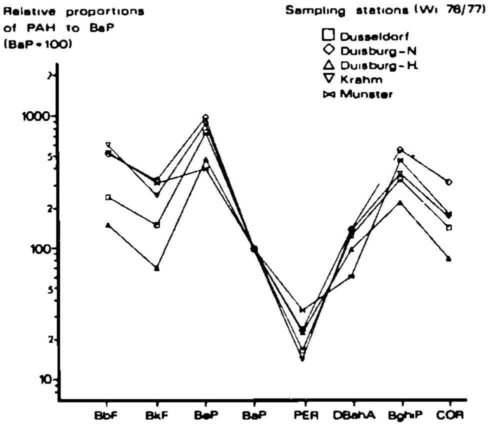

Figure 1: PAH in suspended particles of 5 sampling stations; relative proportions of 7 PAH to BaP (BaP = 100). Sampling period winter 1976/77. (Abbr. see methods.)

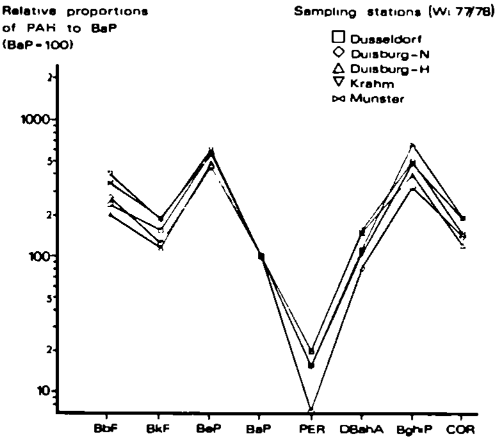

Figure 2: PAH in suspended particles of 5 sampling stations; relative proportions of 7 PAH to BaP. Sampling period winter 1977/78. (Abbr. see methods.)

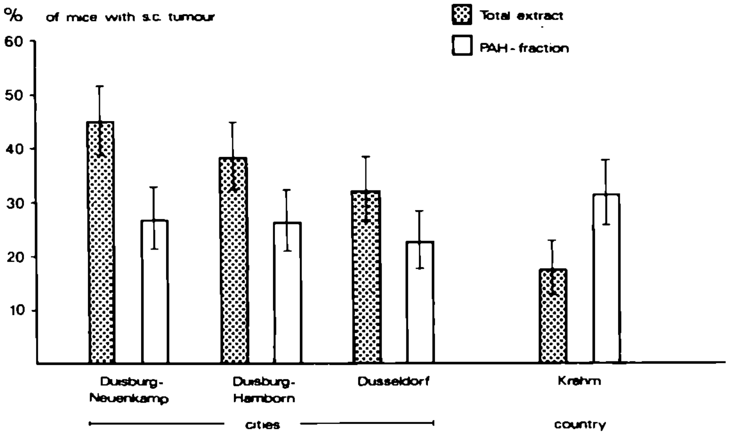

Figure 3: Tumour incidence (percentage with 95 % confidence area) in mice after subcutaneous injection of extracts of suspended particulate matter (total extract and PAH-fraction of 4 sampling stations; winter 1975/76). Each column represents all tumours of 4 groups per 60 mice treated with different doses of extracts containing benzo(a)pyrene amounts of 0.16 µg, 0.63 µg, 2.5 µg and 10 µg.

CONTRACTOR : Lab. de Médecine Expérimentale de l'Institut de Recherche Scientifique sur la Cancer - CNRS - VILLEJUIF France.

CONTRACT N°: 176-77 - 1 ENV F

PROJECT LEADER : Ivan CHOUROULINKOV

PROJECT TITLE : Evaluation of the cell mutability during the transformation induced with environmental chemicals.

Research Objectives

The objectives of the research on transformation and mutability of cells in culture were a) to study the similarity in transformation behavior of the cells in vivo and in vitro, b) to study the relationship between transformation criteria, tumorigenicity or malignancy, and the mutability of the cells, and c) to argue in favor of the use of the cell model in fundamental and applied research chemical carcinogenesis.

Materials and Methods

Cells : Fibroblasts from rat, Syrian hamster, rabbit, and Chinese hamster.

Carcinogens : 7,12-dimethylbenzanthracene (DMBA), benzo(a) pyrene (BaP).

Promoter : TPA (12-O-tetradecanoyl-phorbol-13-acetate) and croton oil.

Mutagens : Methylmethanesulfonate (MMS), cyclophosphamide, procarbazine (Natulan), mitomycin C (MMC), diethylnitrosamine (DEN), N-methyl-N-nitro-N-nitrosoguanidine (MNNG).

Cell transformation : Transformation of cells treated with carcinogens alone or in association with promoters is verified by the following major criteria : formation of abnormal colonies, formation of colonies in semi solid medium, and formation of tumors after inoculation into isologous or immunodepressed animals. The effects are evaluated in terms of the time in culture or the number of subcultures.

Mutagenic effects are evaluated by a) the incidence of gene mutations by use of 8-azaguanine, 6-thioguanine and ouabaine as mutant selectors and b) the rate of sister chromatid exchanges (SCE).

Results

a) Similarity in behavior of the cells in vivo and in vitro, in terms of transformation.

It is well-established that species differ in sensitivity to the sarcomagenic effect of carcinogenic hydrocarbons. The rat is extremely sensitive, the Syrian hamster less sensitive, and the rabbit nearly resistant. Experiments with cultured fibroblasts of these three species showed that fibroblasts of the :

rat are transformed spontaneously (50-60 passages) or after a single treatment with DMBA or BaP(45-46 passages).

Syrian hamster are not transformed spontaneously or after a single treatment with DMBA or BaP (do not establish).

rabbit are spontaneously transformed only after 172 subcultures. This transformation is not accelerated by a treatment with DMBA alone or even in association with treatments with a promoter.

In addition, it has been established that chemical carcinogenesis on mouse skin occurs in two phases : an initiation phase during which a weak dose of carcinogen must operate, and a promotion phase during which a promoter must intervene to enable the initiated state to be expressed as a tumor. The experiments with rat and Syrian hamster fibroblasts showed that this sequential aspect of carcinogenesis is reproducible with cultured cells. Transformation, as evaluated by various criteria, is most precocious when initiation with DMBA or BaP is followed by a treatment with TPA or croton oil.

b) - Relationship between transformation criteria and malignancy

Tumorigenesis in cultured cells is a criterion observed after a long period which, depending on the species, can be from 3 months to 3 years. Transformation criteria such as cloning efficiency, formation of abnormal colonies, or formation of colonies in semi solid medium are observed in a shorter time. It is therefore important to know to what extent these more quickly observed criteria are associated with tumorigenicity. Results of the experiments with fibroblasts from the rat, Syrian hamster, and Chinese hamster showed a correlation among these criteria, but they are not necessarily associated. On a practical level, transformation criteria can be used to indicate carcinogenic potential, but the most significant criterion is still tumorigenicity or the formation of tumors after injection into animals. On a fundamental level, the precise significance of transformation criteria still needs to be clarified. Preliminary results indicate mutation-type phenomena.

c) - Relationship between transformation and cell mutability

The problem was to determine whether during the course of transformation, which is a progressive phenomenon, cells showed a susceptibility to chemical mutagens, also progressive.

Results from experiments with kidney fibroblasts from the Chinese hamster showed that the incidence of gene mutation (ouabaine and 8-azaguanine resistance) induced by MNNG varied considerably from one subculture to another and from one line (treated or not) to another. Evaluation of spontaneous SCE on the same lines showed significant variation both among passages and among lines. However, these variations tended to stabilize at a comparable level for all the lines, i. e., after a period of 15-18 subcultures, the time corresponding to the stabilization of the lines as expressed by an increased cloning efficiency and formation of abnormal colonies, in some cases with malignant transformation.

Assays with normal rat and Syrian hamster fibroblasts also produced variable results concerning gene mutations induced by mutagens such as MNNG or MMS.

These results indicate that normal cells in culture do not lend themselves to studies on gene mutations. This finding explains the widespread use of established cells, abnormal and transformed, for evaluation of the frequency of gene mutations induced by chemical agents. It thus seems that normal cells are better armed against mutagenic effects (gene mutation type) from chemical agents from the environment.

Such is not the case for the SCE. The increased rate of SCE at the time cells are cultured, the variations in this rate until the establishment (stabilization), indeed the transformation, of the cell "line" indicate that the cells are undergoing a period of adaptation to the culture medium and that the SCE are a natural means for cells to reprogram their genetic code in order to face the adversity of the environment. Furthermore, the SCE parameter seems to be extremely sensitive to the effects of mutagens and carcinogens. The spontaneously increased rate of SCE in cell cultures does not seem to lessen the sensitivity of the test which allows the use of normal or transformed cells, as shown by results with the substances listed below.

Lowest effective concentration inducing significant increase of SCE

Substances	mouse b.m. cells mg/kg	human cells	normal C.H. cells	V79 ch.H. cell line
MMS	25	10mg/l	lmg/l	-
Natulan	50	-	5mg/l	-
MMC	-	lµg/l	lµg/l	5µg/l
BaP	-	-	10µg/l	10µg/l

MMS - methylmethanesulfonate ; MMC - mitomycin C ; BaP benzo(a) pyrene.

Conclusions

The use of cultured cells in fundamental and applied research on chemical carcinogenesis is justified.

In the framework of fundamental research, the mechanisms of initiation and promotion can be better defined and studied from a biochemical point of view.

In the framework of applied research, the cell model lends itself to the study of carcinogenic potential of environmental substances, by use of transformation criteria and mutagenesis tests.

It seems, however, that gene mutation tests performed on cells that are already abnormal do not reflect the true response of a normal cell. But on a preventive level the results will probably be more favorable.

SCE seem to be a normal means of cells survival. The rate of SCE is suggested as an indicator of carcinogenic potential. Carcinogenesis, as a multifactorial and progressive phenomenon, would seem to have a mechanism of rearrangement or reprogramming of genes which the SCE seem to indicate.

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Progress reports 1977 et 1978.

Contractor: IARC, Unit of Chemical Carcinogenesis
Contract no.: 190-77-1 ENV F
Project leaders: Dr H. Bartsch and Dr R. Montesano
Title of project: Prescreening of Environmental and Industrial Chemicals in a series of Short-Term Tests for the Detection of Potential Carcinogens

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Objectives of the research

The overall goal of this research project is to contribute to a long-term programme of primary cancer prevention, aimed at identifying and minimizing human exposure to environmental and industrial carcinogens and mutagens by systematic screening in a series of short-term tests.

Since vinyl chloride was recognized as a human carcinogen, there has been increased concern about a number of halogen-substituted ethylenes, butadienes and butenes, because of (*a*) the extensive worldwide production of some of these compounds, (*b*) the common structural element, (an) olefinic double bond(s), which could undergo oxidative metabolism by mammalian enzymes to yield oxiranes, as potentially reactive mutagenic or carcinogenic intermediates and (*c*) the notable lack of data with regard to carcinogenicity for some of these compounds. We have therefore investigated the mutagenic and alkylating properties of several such halo-olefins (Fig. 1) and the nature of their reactive metabolites. The data have been published in detail (Bartsch *et al.*, 1979; Kuroki *et al.*, 1979).

Material and Methods

Mutagenicity tests in *S. typhimurium* were carried out as plate incorporation assays (Method A) as described by Ames *et al.* (1975) or by the adapted procedure to test volatile compounds (Bartsch *et al.*, 1979) (Method B). Quantitation of the test compound dissolved in the aqueous phase was achieved by gas-liquid chromatography. Mutagenicity in V79 Chinese hamster cells was determined in two genetic loci, comprising resistances to 8-azaguanine, and ouabain. Experimental procedures were adopted with a modification from the assay system for nitrosamines reported elsewhere (Kuroki *et al.*, 1979; Drevon *et al.*, 1977).

Determination of alkylating activity of halo-olefins and related epoxides with 4(4-nitrobenzyl)pyridine (NBP) (Method C). Various concentrations of the test compounds were incubated with NBP dissolved in either acetone or ethylene glycol under O_2 or N_2 at 37°; after 30 min, the absorption spectra were recorded against an appropriate blank and absorbance at λ_{max} was plotted.

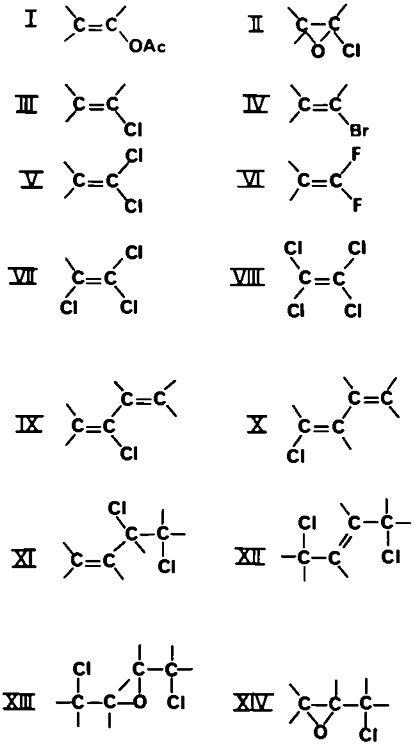

Figure 1: Chemical structures of halo-ethylenes, chlorobutadienes, dichloro butenes and related epoxides studied.
Vinyl acetate (I), chloroethylene oxide (II), VC (III), vinyl bromide (IV), vinylidene chloride (V), vinylidene fluoride (VI), trichloroethylene (VII), tetrachloroethylene (VIII), CBD (IX), 1-chlorobutadiene (X), 3,4-dichlorobutene-1 (XI), 1,4-dichlorobutene-2 (XII), 1,4-dichloro-2,3-epoxybutane (XIII), epichlorohydrin (XIV).

Results

1.1 Mutagenicity of halo-olefins and related epoxides in *S. typhimurium*

The compounds in Fig. 1 were tested for their mutagenicity in *S. typhimurium* strains using Method A or Method B. Mutagenicity, expressed as the number of revertants per µmole of test compound per hour of exposure, was estimated in two strains of *S. typhimurium* TA1530 and TA100 in the presence of a postmitochondrial mouse liver supernatant, following exposure to vapours of one of the olefins. Their activity was in the following descending order: 3,4-dichlorobutene-1>1-chlorobutadiene (technical grade) >2-chlorobutadiene>vinyl bromide>vinylidene chloride>vinyl chloride;

marginal mutagenicity was detected in the presence of 1,1,2-trichloroethylene and 1,1-difluoroethylene, and none with tetrachloroethylene and vinyl acetate.

1.2 Mutagenicity assays in *S. typhimurium* in the presence of human liver fractions

Investigations on the comparative metabolism in animal and human tissues may assist in making extrapolations from data obtained in experimental animals to processes of tumour induction in humans. In this study, we measured the enzymic capacity of liver specimens from different human subjects (taken for diagnostic reasons) to convert a number of halogenated hydrocarbons into mutagenic intermediates. Liver fractions from humans converted vinyl bromide, vinyl chloride, vinylidene chloride and 2-chlorobutadiene into mutagens. In experiments with vinyl chloride, a ten-fold variation was noted among the specimens studied; their mean activity was 85% of that observed with liver from untreated rats. Thus, the majority of the human liver samples tested possessed microsomal enzyme activity for converting these halo-olefins into electrophiles capable of binding to bacterial DNA.

1.3 Comparison of alkylating, mutagenic and carcinogenic activities of halo-olefins

Alkylating activity towards 4(4-nitrobenzyl)pyridine (Method C), mutagenicity in *S. typhimurium* TA100 in the presence or in the absence of a NADPH-generating system (Method A, B) and the available data on the carcinogenicity of these halo-olefins and related epoxides in animals are compiled in Table 1. Since many halo-olefins require microsomal activation and because of possible interference by bacterial metabolism, no good correlation was found between their mutagenic and alkylating activities. However, good correlations were observed for vinyl chloride, vinyl bromide and 2-chlorobutadiene when fortified mouse liver microsomes were added to the assays. These data support the hypothesis that an oxidation of the double bond in certain halo-olefins, which is dependent on microsomal monooxygenases, is a common pathway in the formation of biologically reactive intermediates. Although an excess of lung and skin cancer in humans has been related to exposure to 2-chlorobutadiene, no carcinogenic effect has been reported in rats and mice (IARC, 1978). Since this compound is mutagenic, further experimental and epidemiological evidence should be obtained in order to assess its biological hazard. Owing to the limited nature of the animal data, no evaluation of the carcinogenicity of vinyl bromide has been made (IARC, 1978); however, its mouse and human liver microsome-mediated mutagenic effects indicate its potential carcinogenicity. Vinylidene fluoride, 1-chlorobutadiene, 3,4-dichlorobutene-1 and 1,4-dichloro-2,3-epoxy-butane were also mutagenic, to varying extents and should be tested further in short-term assays and in animals, if human exposure is found to occur.

1.4 Mutagenicity of vinyl chloride, vinylidene chloride and chloroprene in V79 Chinese hamster cells

The mutagenicity of vinyl chloride, vinylidene chloride and 2-chloro-1-butadiene was also tested in V79 Chinese hamster cells in the presence of a 15 000 x *g* liver supernatant from phenobarbitone-pretreated rats and mice. Mutations in two genetic loci, in terms of 8-azaguanine- and ouabain-resistance, were induced in a dose-related fashion by exposure to vapour of vinyl chloride in the presence of liver supernatant from phenobarbitone-pretreated rats. Vapour of vinylidene chloride and chloroprene induced a dose-related toxicity in the presence of liver supernatant from phenobarbitone-pretreated rats but these two compounds were not found to be mutagenic in V79 Chinese hamster cells. The results confirm previous observations on the mutagenicity of vinyl chloride in bacteria, yeasts and insects.

Conclusions

In addition to this important class of chemicals, studies are under way on the parameters (e.g. microsomal concentrations, different systems of metabolic activation systems, etc.) that influence the mutagenic response in the various test systems. A correct knowledge of these parameters appears essential for the proper evaluation of the results in short-term assays.

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Compound	Alkylating activity (reaction with NBP)	Mutagenicity in *S. typhimurium* TA100	Evidence for carcinogenicity in animals
Vinyl acetate	n.d.	0	no
Vinyl chloride	+	+	yes
Vinyl bromide	+	+	?
Vinylidene chloride	0	+	yes
Vinylidene fluoride	0	±	
Trichloroethylene	0	±	yes
2-Chlorobutadiene	+	+	?
1-Chlorobutadiene	+	+	
3,4-Dichlorobutene-1	+	+	
Tetrachloroethylene	n.d.	0	yes
1,4-Dichlorobutene-2	+	+	yes
1,4-Dichloro-2,3-epoxybutane	+	+	
Epichlorohydrin	+	+	yes

Table 1: Comparative alkylating, mutagenic and carcinogenic activities of halo-olefins and related epoxides (n.d. not determined); sufficient evidence for the presence or absence of carcinogenicity in animals ('yes' or 'no') is supplied by published references. Limited evidence for carcinogenicity is indicated as a question mark.

Contractor : Istituto Superiore di Sanità, Roma

Contract n° : 177-77-1 ENV I

Project leaders : Profs. A. Carere, G. Morpurgo and I. Camoni

Title of project : Mutagenicity of pesticides as pure compounds and after plant metabolism

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Objective of the research

The most important aims of this project were:

- to set up a battery of " in vitro " short-term tests able to reveal any kind of genetic damage induced by environmental chemical mutagens and/or carcinogens;
- to perform mutagenicity studies on pesticides as pure compounds and after plant metabolism;
- to perform studies to relate the genetic activity of chemicals to their structural formula
- to continue the comparative testing of the chemicals jointly selected by the participants of the E.E.C. programme.

Materials and methods

The following mutation, recombination and repair tests have been so far set up in our laboratory:

Procaryotic systems: induction of back-mutations (AMES test)[1] and forward-mutations (8-AG resistance)[2] in *S.typhimurium*; induction of forward-mutations in *S.coelicolor*.[3-5]

Eucaryotic systems: induction of two kinds of forward-mutations , mitotic segregation (crossing-over and non disjunction) and lethal recessives in *A.nidulans*;[6-10] stimulation of unscheduled DNA synthesis (UDS) in "in vitro" EUE cell cultures.[11,12]

Technical details can be found in the papers cited.

Other persons involved in the project: Dr. D. BELLINCAMPI, Dr. R. BENIGNI, Dr. M. BIGNAMI, Mr. O. CERVELLI, Dr. P. COMBA, Mr. G. CONTI, Mr. L. CONTI, Dr. R. CREBELLI, Dr. E. DOGLIOTTI, Mr. C. GALLI, Dr. G. GUALANDI, Mr. A. NOVELLETTO

Results

Induction of 8-Azaguanine resistance in S.typhimurium

We have developed a forward-mutation assay, based on induction of 8-AG resistance in S.typhimurium by simply modifying the protocols proposed by Shopek et al.[13] and by S.Thompson[14]. We assayed the sensitivity of our test system with both frame-shift and base-substitution mutagens; we could observe a rather similar efficiency in detecting base-substitutions mutagens when compared with the AMES test and a general minor sensitivity in detecting frame-shift mutagens. Mitomycin C however, an agent inducing a more generalized DNA damage turned out to be more active in the forward-mutation assay. The most interesting results were obtained with the herbicides diquat and paraquat; both chemicals turned out to be negative in the Ames test and positive in the forward-mutation assay. The results obtained are in publication.[15]

An "in vitro" method to test plant metabolism

Further studies were necessary in order to complete this investigation began in the previous program. At the end of this study the following conclusions can be drawn: the method, based on the use of "in vitro" Nicotiana alata cell cultures, is rapid and simple; moreover the results obtained with five pesticides (Atrazine, Dichlorvos, tetrachlorvinphos, Kelevan and Maleic hydrazide) suggest that the "in vitro" method simulates well the metabolism of the whole plant in the field. This procedure was also successfully applied to the genetic systems of A.nidulans; one pesticide, Atrazine, turned out to induce gene mutations and mitotic crossing over only after N.alata metabolism.

The results obtained are in publication.[16]

A quick method to test lethal recessives in A.nidulans

A simple method to test lethal recessives in A.nidulans was set up. The method scores the recessive lethals on the first, the third and the fifth chromosome which represent about 40% of the total map of A.nidulans. Two examples of induced lethals, with U.V. light and MMS were studied. The frequency of lethals may reach 36% of the total population with U.V. irradiation.

The results of this study have been published.[17]

Mitotic non disjunction in A.nidulans

Considerable efforts have been done in order to improve the analysis of non disjunction in A.nidulans. To this aim we have set up a new method which permits the treatment in liquid suspension of quiescent or germinating conidia with the chemical under test. The induction of non disjunction is revealed by plating on complete medium and by scoring for the abnormal colonies . The advantages of the new technique over the old one (Plate test) is a higher sensitivity and the possibility to obtain a perfect quantization of the induced genetic damage; moreover it permits correlation of the induced non disjunction with the physiological conditions of the cell and with the induction of all other possible genetic parameters, i.e. point mutation, crossing-over and gene conversion. The results obtained with MMS, 4-NQO, nitrogen mustard, Benomyl, ethyl alcohol and p-fluorophenylalanine suggest that by utilizing simple systems of analysis it is possible, in A.nidulans, to establish if a given chemical induces non disjunction by interfering with the DNA or with the spindle. Actually alkylating agents like MMS, which acts on DNA, induce non disjunction either on quiescent or germinating conidia and at the same time induce point mutation and crossing-over; on the contrary, agents able to induce non disjunction not acting on the DNA, like for instance Benomyl which binds to tubulin, or the aminoacid analog p-fluorophenylalanine, are inactive on quiescent conidia and do not induce point mutation and crossing-over.

The results obtained in this field are in press.[18]

Mutational studies with diquat and paraquat

Further mutational studies were necessary in order to elucidate controversial results previously obtained in the Ames test with the two widely used herbicides diquat and paraquat. Both chemicals were assayed in the following tests: AMES test, 8-AG resistance and repair test in S.typhimurium; Streptomycin resistance in S.coelicolor; gene mutations and lethal recessives in A.nidulans; stimulation of UDS in "in vitro" human epythelial-like (EUE) cell cultures with autoradiographic techniques. From the results obtained we can derive the following conclusions: both herbicides are negative in the AMES test but positive in forward-mutations tests either in S.typhimurium or in A.nidulans; moreover both chemicals induce aspecific genetic damages as the induction of lethal recessives in A.nidulans and the UDS in "in vitro" EUE cell cultures. This supports the basic in dication that diquat and paraquat share a mutagenic potential . The results obtained are in publication.[19]

Mutational studies with halogenated alyphatic hydrocarbons

Seven halogenated alyphatic hydrocarbons - di- and tri-chloroethanol, mono-, di- and tri-chloroacetaldehide (the last in the hydrate and anhydrous forms) and allyl chloride - were tested for their ability to induce gene mutations of the base-substitution type in S.typhimurium (TA1535 and TA100 strains) and A.nidulans. The aims of this research were: to compare their genetic activity in procaryotic and eucaryotic microorganisms; to assess the importance of alfa-halogenation on their biological activity; to correlate their genetic potency to their structural features. The results obtained can be summarized as follows: halo-acetaldehides: In S.typhimurium 2-chloroacetaldehide and 2,2-dichloroacetaldehide are weakly mutagenic on TA100 strain with and without S-9 mix., mostly in spot test experiments; 2,2,2-trichloroacetaldehide was, on the contrary, clearly positive (more then ten-fold increase) in plate tests with and without S-9 mix. In A.nidulans 2-chloroacetaldehide seems to exert its mutagenic activity mostly at toxic levels; 2,2-dichloroacetaldehide is, on the contrary, clearly mutagenic at high survival rates in spot and plate tests; 2,2,2-trichloroacetaldehide was weakly positive only in the hydrate form. Haloethanols: di- and tri-chloroethanols were negative in the AMES test with and without S-9 mix.; both chemicals showed a weak genetic activity in A.nidulans (2.5-fold increase). Allyl chloride: in both strains of S.typhimurium used weak but clearcut and repetitive positive effect was observed in spot test experiments with and without S-9 mix.
The high volatility and citotoxicity of this chemical made hard to obtain clear results in A.nidulans.
These results are in form of a manuscript to be sendet for publication.

Conclusions

The comparative results up to now obtained allow the following considerations: 1) it is important to use both forward- and back-mutations systems for what concerns the induction of gene mutations for screening purposes; 2) particularly interesting is the analysis of genetic events like mitotic crossing-over and non disjunction in A.nidulans although the extrapolation to higher organisms isin the case of non disjunction still to be elucitated.

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Contractor : Istituto di Ricerche Farmacologiche "Mario
Negri" - Via Eritrea 62 - 20157 Milan, Italy

Contract n.: 191-77-1 ENV I

Project Leader : Prof. Silvio Garattini

Title of project : The capacity of liver and other tissues from different animal species to form and metabolize epoxides

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Objective of the research

Metabolic activation of the aromatic and oleifinic double bond through the formation of unstable arene oxides, diol epoxides and alkene oxides has been suggested as the reason why polycyclic hydrocarbons, styrene, vinyl chloride and other enviromental contaminants have mutagenic and/or carcinogenic activity (1-3).
It has been demonstrated that the formation of these strongly electrophilic intermediates is catalyzed by microsomal mixed function oxidase enzymes and that their toxic effect is due to their covalent binding to nucleic acid and other cellular macromolecules (4-5). The possibility of these reactive intermediates forming and being inactivated in the liver and extrahepatic organs, which, in some cases, are the target of these compounds' carcinogenic action, prompted us to investigate the distribution of the activities of mixed function oxidase enzymes capable of activating styrene to styrene oxide and the possibility of epoxide hydrase transforming this latter to phenylethylene glycol.
Styrene was chosen as model compound because together with styrene oxide it is widely used in large amounts in industry and because in its metabolic pathway the passage to styrene oxide is accepted as being the crucial step, since this compound is thought to be the toxic intermediate.

Materials and Methods

Adult male and female animals of the following species were used in all experiments : CD-COBS rats (150-180 g) and CD_1 mice (20-25 g) were obtained from Charles River (Calco, Como, Italy); New Zealand rabbits (2-5 kg) and Dunkin Hartley guinea pigs (300-350 g) were obtained from Allevamento Padre Antonio

(Mariano Comense, Como, Italy).

Hepatic microsomal cytochrome P-450 and/or P-448 was induced with phenobarbital (80 mg/kg, i.p., in 0,9% NaCl once daily for 3 days) or 3-methylcholanthrene (40 mg/kg, i.p., in corn oil daily for 3 days).
Microsomes from different tissues were prepared according to Kato and Takayanaghi (6).
Styrene monooxygenase and epoxide hydrase activities were measured by a gas-chromatographic method using styrene or styrene oxide as substrate (7).

Results and Discussion

Styrene monooxygenase and epoxide hydrase activities were studied in the hepatic and extrahepatic microsomal fractions of male and female rats, mice, guinea pigs and rabbits. The highest specific activity of both enzymes were present in the liver where levels of epoxide hydrase were higher than monooxygenase. The two enzyme systems are present in all the tissues of both sexes of the species considered. A particularly high ratio of monooxygenase to hydratase activities was found in mouse and rabbit lungs.
Furthermore the <u>in vivo</u> metabolism of styrene was studied in the rat after i.p. administration of the cold and ^{14}C-labelled compound. In addition to phenylethylene glycol, mandelic acid, benzoic acid and hippuric acid, phenolic metabolites, namely, 4-vinylphenol, p-hydroxy-mandelic acid, p-hydroxybenzoic acid and p-hydroxyhippuric acid, were identified in the urine of the treated animals. Results of covalent binding studies of ^{14}C-phenylethylene glycol to rat liver microsomal protein suggest that these phenolic compounds may be formed as a result of chemical rearrangements of unstable arene oxides.
These findings, together with the studies of Watable et al.(8) on the comparative mutagenicity towards <u>Salmonella typhimurium</u> TA 100 of styrene, styrene-7,8-oxide and styrene-3,4 oxide, suggest that this new metabolic pathway leading to the formation of phenolic products during styrene biotransformation, even if occurring only to a limited extent, may play an important role as far as the toxicity of this compound is concerned.
Finally styrene-7,8-oxide was incubated with glutathione in the presence of rat liver cytosolic fraction. The product of this enzymatic reaction was purified and submitted to mass spectrometry and PMR analysis. The results indicate that conjugation of glutathione with styrene-7,8-oxide leads to the formation

of two isomers, namely S-(1-phenyl-2-hydroxyethyl)glutathione and S-(2-phenyl-2-hydroxyethyl)glutathione in a ratio of approximately 60 to 40. These data are in good agreement with those obtained in vivo by Seutter-Berlage et al. (9) and may indicate that for other compounds too in vitro incubation with cytosolic S-glutathione trasnferase may be predictive of a more complex metabolism of epoxides.

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Isolation and structure determination of enzymatically formed styrene oxide glutathione conjugates.
Chem. Biol. Interactions, in press

Contractor :	Istituto Superiore di Sanità, Roma
Contract n° :	262-77-1 ENV I
Project leader :	Prof. V.G. Longo
Title of project :	Electroencephalographic investigation of the effects of organophosphorous pesticides and their metabolites on the central nervous system

Electroencephalographic (EEG) and behavioural investigations have been carried out inour laboratory to elucidate threshold doses which induce EEG effects in animals, and also to evidentiate whether there is a mechanism of action of long-acting anticholinesterase agents different from cholinesterase inhibition.

Previous experiences have shown that methyl-parathion induce at low doses (0,5-2% of LD_{50}) an increase in power of intermediate (7,5-16H_z) frequency bands, followed by a depression of the same bands lasting up to 24 hours. Successive treatments with the same doses induced significant cumulative effects (Zapponi et al, 1979). Higher doses (up to 10-15% of LD_{50}) induced depression in several frequency bands of the spectrum and in total power. However, no clear acute dose/effect relationship could be shown, and a slight depressive effect induced by solvent (sesame oil) on some EEG bands which could interact with depressive effects seen soon after high doses of methyl-parathion, was shown. In 1978 EEG effects induced by 4 doses of physostigmine, ranging from 0,1 to 0,8 mg/kg have been studied.

A significant, dose-dependent decrease of power in three frequency bands (ranging from 7 to 20 H_z) and of total power of the spectrum was shown (0,8 mg/kg i.p. corresponds about to 80% of LD_{50} in DBA male mice).

MATERIALS AND METHODS

DBA/2 male mice weighing 22-24 grams were chronically implanted with cortical electrodes ; after 7 days the animals were recorded for 2 hours, then were challenged with drug (physostigmine, ranging from 0,025 to 0,8 mg/kg) or saline, four animals per dose, and recorded for 2 more hours.

RESULTS AND DISCUSSIONS

Low doses of the drug (0,025 and 0,05 mg/kg) did not induce any effects on gross behaviour or EEG tracings in mice. EEG power spectral analysis performed on computer showed a depression in power of 7,5-12 H_z frequency band, although this effect was not statistically significant.

Significant, dose-related depression on EEG power bands was evident only after doses of physostigmine of 0,1 mg/kg (corresponding to 10-15% of LD_{50}) to 0,8 mg/kg (corresponding to 80-100% of LD_{50}). However, lower doses of methyl-parathion (corresponding to 1-5% of LD_{50}) did induce increase of power in some EEG bands (7.5 to 16 Hz) soon after administration, while corresponding doses of physostigmine (measured as % of LD_{50}) did not.

To our knowledge this effect has not yet been described. It cannot be due to solvent alone (sesame oil), which never induced increase in power of EEG bands, but only slight decrease ; and it may probably not be due to some pharmacological interference exerted between sesame oil and methyl-parathion effects on EEG, because of totally different effects are induced at different doses. Further data are needed, however, on mechanism of action of effects exerted by physostigmine or organophosphorus compounds on electrical activity of the brain.

Experiences are in progress according to the following schedules :

1) EEG analysis on acutely implanted rabbits, challenged with paraoxon, in doses of 0.01 to 0.1 mg/kg i.v. (12 rabbits recorded to date)

2) EEG analysis on chronically implanted mice, challenged with paraoxon (not yet started)

3) EEG analysis on chronically implanted rabbits, challenged with physostigmine (not yet started).

This work has been made with the contribution of the following people :
P. Ceccarelli, E. Deodati, G. Gallozzi, A. Loizzo, E. Ortolani, S. Palazzesi, A. Pezzola, G.A. Zapponi.

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Contractor : Laboratorio di Genetica, Università di Pisa

Contract n° : 267-77-1 ENV I

Project leader : Prof. N. Loprieno

Title of project : Investigations on the relationship between metabolic conversion and mutagenic activity of environmental chemicals with potential cancerogenic activity

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Objective of the research

It has been demonstrated that genetic risk may be the consequence of che exposure of human population to industrial pollutants, such as vinyl chloride, chloroprene, etc. (J.K., WAGONER, 1978): these compounds are known to produce all kind of mutagenic events in laboratory organisms and animals, such as point mutations, chromosome mutations, mitotic recombinations, etc. It is therefore relevant in mutation research to develop different experimental procedures to detect all types of genetic end points which may be produced by a chemical agent: these procedures should include all possible types of exposure protocol for investigate the potential mutagenic activity of chemico-physically different agents and all possible variables of biological factors which might influence the metabolic fate of the compounds.

By developing different short-term tests for the mutagenic evaluation of chemical pollutants, it is moreover higly important to identify quantitative criteria for a comparison of the results obtained with different test-organisms.

Materials and methods.

We have attempted in our laboratory to develop investigations on the potential mutagenic activity of known mutagens (methylmethanesulfonate, ethylmethanesulfonate, dimethylnitrosamine, mitomycin c, hycanthone) and also of a group of environmental contaminants in industrial use, such as styrene, styrene oxide, trichloroethylene and its putative metabolite, epoxy 1,1,2-trichloroethane, difluoromonochloromethane, epichlorhydrine, or in agricultural use, such as the herbicide atrazine, or in medical use, such as the antischistosomal drug praziquantel.

For this study we have developed six different mutagenicity assay systems which include *Salmonella typhimurium* (reverse gene-mutation), *Schizosaccharomyces pombe* (forward gene-mutation), *Saccharomyces cerevisiae* (mitotic gene conversion), chinese hamster cells grown in vitro (forward gene-mutation), human cells grown in vitro (Unscheduled DNA synthesis) and mouse bone marrow cells, in vivo cytogenetic analysis (chromosome mutation). The mutagenicity test systems which make use of organisms (bacteria, yeast and mammalian cells) grown *in vitro*, include also different methodologies of treatment with the chemicals, in presence of metabolic activation systems of animals and plant origin. Moreover, some of the microbial mutagenicity tests, such as those based on *Salmonella* and on yeast, may be used *in vivo*, in combination with animals, in the so called host mediated assay, which allows the exposure of the microbial cells in the animal body, in close contact with different animal organs such as the liver, the kidney, the lung, etc. This analytical procedure is particularly indicated for the study of the organ-specificity of some carcinogenic compounds which might depend their activity on the specific metabolism of a particular animal tissue.

Results and conclusions

Styrene and styrene oxide. The styrene-3,4-oxide, the main metabolic product of styrene, has resulted positive in all tested mutagenic systems: it produces base-pair substitutions, as well insertion/deletion type mutants, mitotic recombination, UDS and chromosome mutation. Styrene, on the contrary, has proven to be completely inactive, with the exception of yeast cells tested in vivo, in the host mediated assay, where a weak mutagenic activity was evaluated. Present considerations from the critical analysis of the literature conclude that many methodological procedures are inadequate for studying compounds like styrene, as the metabolic activation system do not allow a persistent action of monooxygenase liver function and therefore the formation of an active concentration of the metabolic form, the epoxide. On the basis of such results, styrene might be considered as a human hazard, since it is possible for it to produce in vivo some type of mutational event.

Difluoromonochloromethane and praziquantel have been shown by an extensive analysis to be completely devoid of a mutagenic potential.

Trichloroethylene has been considered a potential human carcinogen on the basis of long-term animal experiments. The composition of the sample used for the carcinogenic study did include however other potential carcinogens, such as epichlorhydrine and 1,2-epoxybutane. By using yeast and chinese hamster mutational assays trichloroethylene has resulted inactive; acute treatments of mice have not produced, moreover chromosome aberrations. Moreover experiments performed in vivo, by means of the host mediated assay procedure, have demonstrated that trichloroethylene samples containing epichlorohydrine are mutagenic, whereas those devoid of epichlorohydrine are inactive. Epichlorohydrine alone is mutagenic in vitro as well in vivo and might justify the mutagenic activity of trichloroethylene impure samples. The present experiments are far for demonstrate the safety of trichloroethylene, but they suggest the development of more adeguate long-term study with this chemical of so wide use.

Atrazine has been shown to be activated to some extent to a mutagenic metabolite in vitro by a plant metabolic activation system, but not by an animal metabolic activation system. Experiments in vivo do show however that this compound could be converted by the animal metabolism into a metabolite with some mutagenic activity.

Other investigations have included the development of an in vivo intrasanguineous host mediated assay with the yeast S.pombe which has been found particularly useful in the evaluation of the mutagenic activity of the dimethylnitrosamine: this system is being applied for the study of the in vivo formation of the nitroso-compounds; for a better evaluation of the induction of unscheduled DNA synthesis in mammalian cells, data base have been developed in a new test which makes use of embricnic rat or mice cells which are able to detect both direct and indirect chemical mutagens, as they are able to perform a satisfactory metabolic reaction.

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Contractor: Istituto Superiore di Sanità, Rome, Italy
Contract n°: 268-77-1 ENV I
Project Leader: Professor Gian Luigi Gatti
Title of project: Analysis of the mechanisms of the neurotoxicity of organophosphorus pesticides in relation to the inhibition of the isoenzymatic profiles of acetylcholinesterase

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In view to obtain an organic picture of the results this report presents separately two main lines of the research:
Part 1. Standardization of the procedure to obtain the electrophoretic profile of the molecular forms of AChE in whole brain of the rat. Alterations of isoenzymatic profiles following an acute intoxication with DFP and the differential recovery of various molecular forms within 25-days.
Part 2. Standardization of the procedure to obtain the electrophoretic profile of the molecular forms of AChE of the rat and human blood cells.

PART 1.

Objective of the research: The main objective of the present research was the study of the alterations of the normal pattern of acetylcholinesterase (AChE) isoenzymes induced by pesticides for contributing to the comprehension of the mechanisms of their neurotoxicity. As concerns brain AChE, previous studies have been limited to a restricted range of experimental conditions. Vijayan and Brownson (1), using polyacrylamide gel electrophoresis, showed that 2 hr after a high dose of parathion (2.5 mg/kg i.p.) three rat brain isoenzymes were affected similarly while after a lower dose (1.25 mg/kg) the isoenzyme accounting for the largest portion of AChE activity was inhibited more than the others. Srinivasan and coworkers (2) found that synaptosomal and microsomal AChE isoenzymes from rat brain were differentially affected by intracerebral DFP treatment, and that their recovery took place at different rates. Recently, Overstreet and coworkers (3) confirmed that 4 hr after DFP (1 mg/kg i.m.) five isoenzyme forms were not inhibited to the same extent by organophosphorus exposure.

Materials and methods. Sprague-Dawley male albino rats (160-200 g) were treated with DFP at a dose of 1.1 mg/kg (in arachis oil) subcutaneously. Separate groups of six rats were sacrificed at 3 hr, 18 hr, 4, 12 and 25 days after DFP treatment. Appropriate groups treated with the vehicle were run in parallel.

Whole brain, including cerebellum, was removed, frozen and thawed three times. One hemisphere was homogenized using tris-HCl buffer (pH 8.5, 0.038 M; tissue:buffer ratio 1:6) to obtain the soluble fraction of AChE (corresponding to about 20% of total AChE in crude homogenate) and the other using the same buffer containing 0.1% Triton X-100 in order to obtain a partially solubilized fraction (corresponding to about 40% of total AChE in crude homogenate). The homogenates were centrifuged at 100.000 g for 1 hr in a Beckman L 2 65B ultracentrifuge. The total AChE activity of the supernatants was determined according to the method of Ellman and coworkers (4).

The separation of the isoenzymes was carried out by disc polyacrylamide gel electrophoresis. Cylindrical double layer gels were prepared in 12 cm glass tubes (diameter 0.5 cm). A complete gel consisted of a 9 cm layer of 7.5%separating gel and a 1 cm layer of 3% spacer gel. A continous tris-glycine buffer (pH 8.1, 0,05 M) was used for electrophoresis. Samples of supernatant (15 μl corresponding to about 6 nmoles of acetylthiocholine hydrolyzed per min) were electrophoresed with a running time of 150 min at 1 mA per gel.

In order to carry out the enzymatic reaction and staining, the gels were incubated for 1 hr at 30° C with shaking in a solution of 0.2 M maleic acid, 0.002 M $CuSO_4$, 0.01 M glycine and 5.2 mM acetylthiocholine iodide (pH 6.5) and placed in 30% Na_2SO_4 overnight. Finally, after washing, gels were stained in a saturated solution of dithioxamide containing 1.64 g/l of sodium acetate. The gels, immersed in water,were then scanned at 600 nm in a Gilford 2400 spectrophotometer and peak areas integrated using a Hewlett-Packard (9804) digitizer. In some experiments, in order to inhibit pseudocholinesterase, present in little amounts in preparations from whole brain,0.01 mM iso-OMPA was added to the incubation mixture. The quantitative isoenzymatic profile obtained did not differ from that obtained in the absence of iso-OMPA.

The statistical elaboration of the data was performed by variance analysis.

Results. In order to achieve the standardization of the procedure the effect of various factors (freezing and thawing of brain; ratio tissue:buffer for the homogenization; gel concentration; amount of the sample electrophoresed; running time and current intensity of the electrophoresis; concentration of acetylthiocholine and the incubation period of the enzymatic reaction) was studied separately(5).As a result of this preliminary study the procedure described in Materials and Methods was found the most satisfactory which ensured highly reproducible profiles of AChE from untreated rats both in soluble and solubilized fraction (Fig.1). Three main molecular forms were detected accounting for about 63%(slow-migrating), 19%(medium-migrating) and 18% (fast-migrating) of total AChE activity. This profile was very similar in the

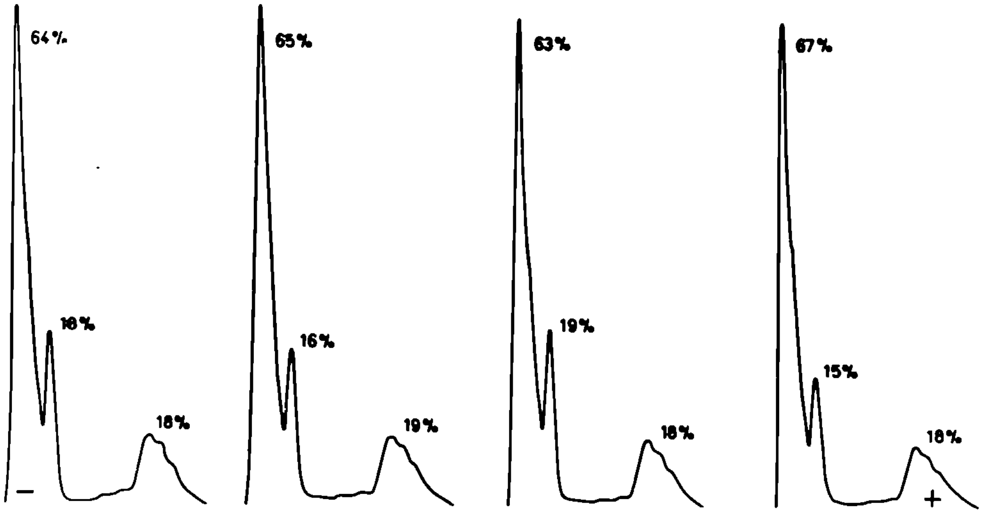
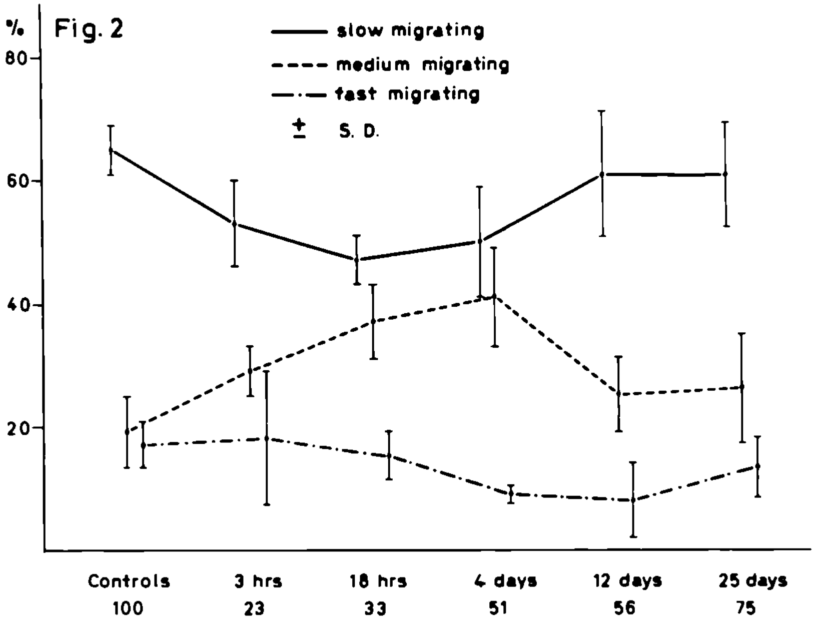


Fig.1. Densitometric recordings of AChE isoenzymes (soluble fraction). Samples from four rats were electrophoresed on different days.

two fractions and did not differ substantially from that found by Vijayan and Brownson (6) for the normal rat brain using flat bed electrophoresis.

The results on the alterations of the isoenzymatic profiles of soluble AChE induced by an acute treatment with DFP are presented in Fig. 2. It appears that in the early phase of the intoxication i.e., after 3 hr when there is a strong inhibition - about 80% - of total AChE, a slight modification of the profile may be observed. This modification tends to become more pronounced over a period of four days after treatment which goes hand in hand with the initial portion of the recovery curve for total AChE activity. This means that after 18 hr, when total AchE shows a 1.5 fold increase over the level measured at 3 hr (from 23 to 33% of control level), the slow-migrating molecular forms are inhibited proportionately more than forms migrating at intermediate speed while the percentage accounted for by the fast migrating forms appears to be unaltered. Therefore the recovery at 18 hr is due mainly to a re-increase of medium speed migrating isoenzymes. This alteration of the profile is the most pronounced after four days when total AChE shows an increase to 50% of the control level and the contribution of medium-migrating molecular forms reaches over 40% (respect to 19%in controls) at expenses of slow-migrating forms, and, to a lesser extent of the fast-migrating. As a matter of fact, when absolute levels, rather than percentage contribution to total activity of various forms are considered, it turns out that medium-migrating form, after having shown a reduction of 65% at 3 hr, and of 36% at 18 hr returns to the control level within four



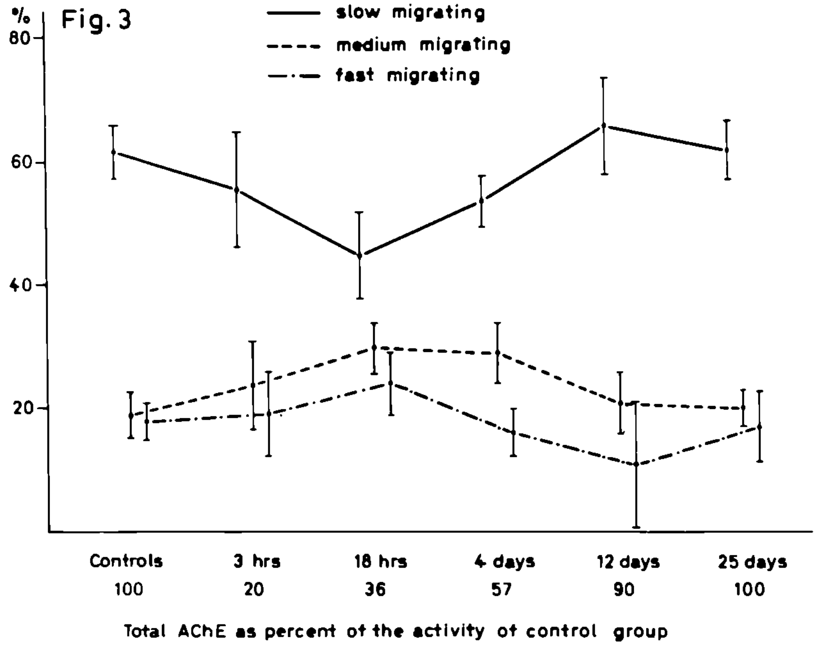


Fig. 2 and 3. Percentage contribution to total AChE activity actually present of individual molecular forms in the soluble (Fig. 2) and solubilized fraction (Fig.3) of controls and after a single treatment with DFP (1.1 mg/kg in arachis oil s.c.). N=6 per group. Averages ± standard deviation.

days. In the late stage of the intoxication, the shift in isoenzymatic pattern is gradually reversed. However, after 25 days the percentages of forms slow- and medium-migrating are still somewhat different from those observed in untreated animals(7).

The results on the alterations of the isoenzymatic profiles in the partially solubilized fraction obtained from the brain of the same rats are presented in Fig.3. It appears that the changes in profiles over time are similar to those found in the soluble fraction although less pronounced(7).

Conclusions and comments. The results indicating a differential recovery of AChE isoenzymes, which extend the observation period until a normalization of both total AChE and of quantitative profiles of its molecular forms completes the data obtained by other investigators dealing with organophosphorus exposure (1, 2,3). This means that different pools of enzymatic molecules having differential metabolic activity should exist. It has not to be excluded, however, that medium-migrating molecular forms at lower molecular weight are intermediates in the building up of high molecular weight (and slow migrating) isoenzymes. The important finding that selective recovery appears more pronounced in the soluble fraction than in the solubilized (i.e., soluble +a portion of membrane bound) AChE supports the hypothesis that the enzymatic molecule once synthesized remains in a soluble form and, as a second step, binds up to membranes, if its synthesis has to be considered the main mechanism in the return of activity in an intoxication by irreversible antiChE compounds. This finding is in agreement with some recent lines of evidence indicating that the cytoplasmatic(i.e.) soluble) AChE prevails during the period of its intense synthesis such as the early post-natal development (8) or in muscle cells culture after an intoxication by DFP, and that, generally speaking, this portion of enzymatic activity is metabolically more active.

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Contributors: G.BIGNAMI, G.M.BISSO, G.CARRO-CIAMPI,A.MENEGUZ H.MICHALEK and technicians L. AMORICO and M. BASTIANELLI.

PART 2. Objective of the research: Human erythrocyte AChE has been widely studied as far as activity levels, association with the erythrocytic membranes (e.m.) and composition in molecular forms (1-5). Scarce information is available regarding the characteristics of erythrocyte AChE from other mammalian species;in particular, little characterization work has been done on the AChE of rat erythrocytes (1,5). This paucity of data represents a serious limitation for the use of rats in experimental models for the study of blood cholinesterases as a complement of research on the mechanisms involved in chronic intoxication with anticholinesterase agents. It was therefore decided to perform a comparative study on the characteristics of AChE from rat and human erythrocytic membranes.

Materials and methods: AChE was solubilized for extraction from human and rat e.m. by means of one of the following procedures: a) treatment with 0,5% Triton X-100; b) repeated freezing and thawing in liquid nitrogen; c) a combination of treatments a) and b).

AChE activity was determined with the method of Ellman and coworkers (6); protein with the method of Lowry and coworkers (7).

AChE, solubilized according to treatment c),was subjected to electrophoresis in cylindrical gels of 6% polyacrylamide in tris/glicine buffer pH 8,1; The enzymatic activity was revealed with a procedure based on the histochemical method of Koelle (8), and the bands so formed were read densitometrically at 566 nm. The molecular weights were determined by gel electrophoresis at different polyacrylamide concentrations, according to the method of Hedrick and Smith (9).

Results: Rat e.m. AChE activity levels were approximately 4 times lower than human; treatment with Triton X-100 activated the enzyme from human e.m. more than that from rat e.m. (around 115 and 60% respectively). The experiments with different extraction procedures showed that rat e.m. AChE requires more drastic conditions in order to become solubilized. Treatment c) was finally chosen because it allowed around 95% solubilization of the enzyme from both sources.

After polyacrylamide gel electrophoresis of the solubilized enzyme, in both man and rat the enzymatic reaction revealed two bands, not coinciding in mobility, which are inhibited by 10^{-5}M eserine. Of the two bands the slower moving one (band II) showed more intense enzymatic activity and appeared constantly, while the faster moving one (band I) as less intense and did not constantly appear.

Gel electrophoresis at different polyacrylamide gel concentrations allowed preliminary conclusions to be reached about the molecular weight of the enzyme corresponding to band II,i.e.

315,000 for rat and 240,000 for man e.m. AChE.

Conclusions and additional comments: In rat erythrocytes AChE is more firmly associated with the cell membrane than in human erythrocytes, and its active site seems to be more exposed on the membrane surface. The two components of human e.m. AChE, separated in the present work by means of polyacrylamide gel electrophoresis, may be compared with the two bands separated from the same material by Shafai and Cortner (10), by means of electrophoresis on cellulose acetate. On the other hand Rieger and coworkers (5) observed only one peak of activity after centrifugation in a density gradient of AChE extracted from rat e.m.These results are not in contrast with our data.

The observed variability in appearance (both in man and rat) of band I could be due to genetic factors controlling its expression, as suggested by Das and Lo (11).

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Contributors: N. FRONTALI, G.F. SCARSELLA, L. SETTIMI, M.E. TRAINA and technicians P. CONTI and D. TRUCCHI.

Contractor : Dept. Rad. Genetics and Chem. Mutagenesis, Univ. Leiden

Contract No.: 139 - 77 - 1 ENV N

Project Leader : Prof.Dr. F.H. Sobels; Dr. E. Vogel

Scientists : W.G.H. Blijleven (MSc.); M.J.H. Kortselius (MSc.);
J.A. Zijlstra (MSc.)

Title of project : Studies on the induction of genetic damage by chemical mutagens in Drosophila (Project 1)

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1. OBJECTIVE

Of critical importance in the assessment of any genetical screening test is its ability to identify mutagenic activity of compounds that require enzymic activation. The objectives of this research program are to (i) establish the extent to which procarcinogens can be detected as mutagens in Drosophila; (ii) explore the features of activating enzymes in this system; (iii) isolate and characterize mutagen-sensitive mutants and examine their utility for a better identification in Drosophila of carcinogenic aromatic amines and polycyclic hydrocarbons; (iv) establish the resolving power (and thus the relative reliability) of different genetic end points used as a measure of mutagenicity; and (v) investigate the relation between chemical reaction pattern and mutagenic properties of mutagens.

2. METHODS AND RESULTS

2.1. MUTAGENICITY OF PROCARCINOGENS IN DROSOPHILA.

It has become clear that Drosophila has the enzymic potential for converting procarcinogens into genetically active metabolites. During the contract period, a series of procarcinogens were assayed for the induction of point mutations (recessive lethals) in tester strain Berlin K. The following carcinogens scored positive in the test. 1-Chlorobutadiene-1,2, 1-chlorobutadiene-1,3; vinyl chloride; vinyl bromide; methylphenylnitrosamine; N-nitrosopyrrolidine; N-nitrosopiperidine; N-nitroso-N'-methylpiperazine; N-nitrosomorpholine, procarbazine; 4-dimethylamino-trans-stilbene; and estragole.

On the other hand, several aromatic amines and polycyclic hydrocarbons were decidedly negative or produced only marginal effects when assayed in the recessive lethal test. Thus several attempts have started aimed at improving the detection capacity of Drosophila for this important class of environmental carcinogens.

One possibility to tackle this problem is an analysis of the enzymes involved in the activation of polycyclic compounds, and the usage of enzyme inducers. In experiments which were conducted in close cooperation with the Pharmacology Department of the University of Leiden (Prof.Dr.D.D.Breimer), several types of cytochrome P-450 could be identified in whole body homogenates of numerous Drosophila strains. Microsomal fractions also showed aryl hydrocarbon hydroxylase activity and hydratase action, while the postmicrosomal supernatant contained glutathione-S-transferase activity. Treatment with phenobarbital resulted in both qualitative and quantitative changes in the content of cytochrome P-450. The changes in enzymic pattern were less pronounced following exposure of larval stages or adult flies to arochlor or 3-methylcholanthrene; but the effects were clearly distinct from those obtained with phenobarbital. Experiments are currently in progress to examine the effects on mutagenicity by polycyclic compounds of arochlor and 3-methylcholanthrene.

An alternative approach to improve the sensitivity of Drosophila for polycyclic compounds is the development and standardization of more sensitive tester strains. The mutagen-sensitive mutants mei-9^{L1}, isolated in our laboratory, and mei-9^{a} mei-41^{D5} have an improved detection capacity relative to wild-type tester strains, when MMS, EMS, 2,4,6-trichloro-(1-phenyl-3,3-dimethyltriazene) and several polycyclic compounds were compared in these strains. This was indicated by the positive response in strains mei-9^{L1} or mei-9^{a} mei-41^{D5} of 2-acetylaminofluorene (2-AAF), benzo(a)-pyrene and 7,12-dimethylbenzanthracene.

2.2. THE RELATION BETWEEN CHEMICAL REACTION PATTERN AND MUTAGENIC PROPERTIES OF ALKYLATING AGENTS IN DROSOPHILA

Previous work from our laboratory with Drosophila has demonstrated the existence of mutagens which are efficient in the induction of gene mutations, but fail to induce chromosome breakage events, or cause chromosome aberrations only at toxic levels of exposure. An extreme example is the procarcinogen DEN (diethylnitrosamine). Since the alkylating species derived by metabolic conversion from DEN is supposed to be identical with that formed by chemical activation from ENU (ethylnitrosourea), the question was whether ENU would show a similarly low chromosome-breaking efficiency in Drosophila, and if so, whether this then could be related to its low binding affinity to centers of high nucleophilic strength in DNA.

Thus, our present work is an attempt aimed at comparing in Drosophila the mutagenic activity of a series of structurally related monofunctional alkylating agents (AA) with known Swain-Scott s factors. The compounds chosen for comparison were the alkyl methanesulfonates MMS, EMS, and iPMS, the nitrosamides MNU and ENU, the nitrosamines DMN and DEN, and one dialkylsulfate, DMS. The six directly acting AA constitute agents with widely differing s value on the Swain-Scott scale. The parameters applied for measuring their biological reactivity were (i) mortality of treated adult males, (ii) production of X-chromosomal recessive lethal mutations, (iii) induction of reciprocal translocations between the chromosomes 2 and 3, and (iv) induction of loss of ring-X- or Y-chromosomes, and Y-chromosome rearrangements.

The data are interpreted as indicating for these AA a causal connection between the pattern of primary alkylation of DNA and the quality of genetic damage observed:

(1) The agent with the lowest s value, ENU, and its pendant DEN, failed to produce translocations, at mutation frequencies which reached up to 44% in the case of ENU. Highest chromosome-breaking activity was attributed to AA with high s, MMS and DMS (Table 1). For MMS, the proportions of translocations (T) : mutations (M) approximately reached a 1 : 1 ratio in stored spermatozoa, at a recessive lethal frequency of 14%. Ability to break chromosomes, as indicated by the T : M ratios, decreased in the sequence MMS $\geq$ DMS, MNU > DMN > EMS > iPMS > ENU = DEN.

(2) Nearly the reversed sequence in relative mutagenic effectiveness was obtained when arranging the (directly acting) AA on basis of their DC /LC_{50} ratios (DC - the exposure condition doubling the frequency of recessive lethals after injection): ENU > EMS > iPMS, MNU > MMS = DMS (Table 1).

(3) In the ring-X loss test, the ability to cause chromosome losses decreased in the sequence MMS = DMS > MNU = DMN > EMS > iPMS > ENU = DEN. This ranking is consistent with the sequence in effectiveness in producing translocations.

(4) The low frequencies of Y-chromosome rearrangements made any meaningful comparison between the AA impossible. The diagnostic value of such tests is poor, since even at toxic concentrations large numbers of offspring must be scored to obtain statistically significant effects.

3. CONCLUSIONS

To summarize the outcome of the enzyme analysis, it is clear from the data that there exist several types of cytochrome P-450 in Drosophila. The results of testing carcinogens in mutagen-sensitive strains show that these mutants are a useful tool for mutagen testing of aromatic amines and polycyclic hydrocarbons.

The overall conclusion from the experiments on the relation between chemical reactivity and mutagenic effectiveness of AA is that two variables have major significance in determining the quality as well as the frequency of genetic damage induced by monofunctional AA in Drosophila. These two parameters are dose (intensity of alkylation) and reaction pattern (site of alkylation). For each AA a "dose", as measured by the yield of point mutations (recessive lethals), can be defined below which detection of chromosomal aberrations is hardly possible.

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Table 1. Relationship between reaction pattern of monofunctional AA and chromosome-breaking efficiency

Compound	s[a]	Doubling Concentr.[b] recessive lethals (mM)	LC_{50} (mM)	DC : LC_{50} rec.lethals	Critical "dose level" for induction of[c]		
					Transloc.	Ring-X loss	Y-rearrangements
MMS	0.86	0.10	7.8	~ 1 : 100	~ 5	~ 5	> 25
DMS	0.86	0.10	7.7	~ 1 : 100	5 - 10	5 - 10	> 25[d]
EMS	0.67	0.50	197	~ 1 : 400	25 - 50	~25	> 100
MNU	0.42	0.18	26	~ 1 : 150	10 - 30	10 - 20	> 35
iPMS	0.31	0.50	72	~ 1 : 150	50 - 100	~ 50	> 100
ENU	0.26	0.06	28	~ 1 : 450	> 100 - 200[d]	~100	> 100

[a] Swain-Scott s factor;

[b] extrapolated from CM_4 values (concentration producing 4% point mutations after injection)

[c] "dose" expressed as n-fold increase in the frequency of point mutations (recessive lethals) above spontaneous background

[d] close to LC_{50}; DC, Doubling Concentration; LC_{50}, Lethal Concentration -50 (50% survival).

Contractor : Dept. Rad. Genetics and Chem. Mutagenesis, Univ. Leiden

Contract No. : 139 - 77 - 1 ENV N

Project Leader : Prof.Dr. F.H. Sobels

Scientists : Dr. J.W.I.M. Simons, Dr. A.A. van Zeeland, Dr. A.G.A.C. Knaap

Title of project : **Studies on the induction of point mutations by chemical mutagens in mammalian cells in vitro (Project 2)**

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1. OBJECTIVE

The research with mammalian cells in vitro, V79 Chinese hamster cells and L5178Y mouse lymphoma cells, has been directed to (1) the extension of the number of genetic markers to be used in the assay systems, (2) the possible synergistic effect of non-mutagenic agents on the frequency of induced mutations, and (3) the assessment of the genetic activity of some known and some potential mutagens for comparative purposes.

2. RESULTS

2.1. During the contract period an assay system using L5178Y mouse lymphoma cells has been taken into use in which the induction of mutations could be determined simultaneously at two loci, namely the locus for thymidine kinase (TK) and the locus for hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT). Reconstruction experiments and expression time experiments have been carried out to establish the conditions under which reproducible and reliable determinations of mutant frequencies can be ascertained.

Several mutagenic treatments with a different mode of action were assayed in this system. Induction of mutations both at the TK-locus and the HGPRT-locus was observed after treatment with X-rays, UV, ethylmethanesulphonate, diepoxybutane, diethylnitrosamine and natulan (the last two with activation), the locus for TK being approximately two times more mutable. In contrast, treatment with ethidiumbromide , a frameshift mutagen, does enhance the mutant frequency for TK deficiency whereas induction of HGPRT-deficient mutants could not be shown. Moreover the induced mutant frequency at the TK-locus does decrease with time. It is indicated that the mutations induced by ethidiumbromide are at a selective disadvantage in the population.

The results stress the need for examining the genetic activity of a compound at several loci and, preferably, at several time intervals after treatment.

2.2. The genetic damage which is observed after treatment with mutagenic agents is the ultimate result from the initial, premutational damage and different repair processes. In order to get more insight in the repair processes in mammalian cells, we examined the effect of caffeine and ethionine on the survival and the induced mutant frequency for HGPRT-deficiency in V79 Chinese hamster cells.

Caffeine was chosen because it may act synergistically with mutagens by interference with post replication repair processes. It was shown that: (1) caffeine itself does not induce point mutations, (2) post treatment with caffeine potentiates the cell killing of EMS and UV, and (3) post treatment with caffeine does not alter the frequency of HGPRT-deficient mutants induced by EMS and UV. These results suggest that in mammalian cells caffeine does not interfere with repair processes which lead to point mutations.

Ethionine was chosen because it is a potent liver carcinogen, which could not be shown to be mutagenic in a variety of test systems and it may act synergistically with mutagens, due to the disturbance of the methylation-instructed mismatched repair. So far, the experiments showed that: (1) ethionine itself does not induce point mutations in mammalian cells, (2) ethionine enhances the cell killing effect of EMS and 6-hydroxyaminopurine (HAP), and (3) ethionine treatment decreases the frequency of HGPRT-deficient mutants induced by EMS and HAP.

2.3. Once the mutagenic activity of a compound has been shown in a particular test system, the next question to be asked will be how mutagenic is this compound. We therefore compared the mutagenicity of the compounds tested under the EEC-contract, on the basis of the mutation induction per mM per hour of treatment in two cell lines and at two loci. For the calculation of the mutagenic potency the shape of the dose-response curves, i.e. linear, plateau or humped, has been taken in account.

Table 1. Mutagenic potency of some chemicals (units)

Cell line	L5178Y	L5178Y	V79
Locus	TK	HGPRT	HGPRT
Chemical:			
EMS	2.5×10^{-5}	1.1×10^{-5}	0.9×10^{-5}
MMS	not tested	1.1×10^{-5}	2.2×10^{-5}
Mitomycin C	not tested	not tested	3400×10^{-5}
DEN *	0.7×10^{-5}	0.4×10^{-5}	0.6×10^{-5}
Natulan *	2.6×10^{-5}	1.2×10^{-5}	2.3×10^{-5}
Atrazine *	not tested	not tested	not mutagenic

* after metabolic activation.

The mutagenic potency of a compound is expressed as induced mutant frequency per survivor per mM per hour of treatment.

3. CONCLUSIONS

The assay system for TK-deficiency in L5178Y mouse lymphoma cells has been evaluated and has proven to be a useful mutation selection system in addition to the selection system for HGPRT-deficiency. The simultaneous determination of mutation induction at these two loci in the same cell line enhances the reliability of the test system and facilitates comparative mutagenesis.

The experiments on the possible synergistic effect of caffeine show that caffeine does not alter the mutagenic effect of UV- and EMS-treatment, whereas it does enhance their cellkilling effect. It is concluded that caffeine does not interfere with processes which lead to point mutations.

In the protocol for testing the mutagenic potential of chemicals, a system for metabolic activation has been included for the detection of indirect mutagens. The mutagenic activity of chemicals has been compared on the basis of their mutagenic potency, i.e. the induced mutant frequency per survivor per mM per hour of treatment.

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Contractor : Dept. Rad. Genetics and Chem. Mutagenesis, Univ. Leiden
Contract No. : 139-77-1 ENV N
Project Leader : Prof.Dr. F.H. Sobels
Scientists : A.D. Tates and A.T. Natarajan

Title of project : Studies on the induction of chromosome aberrations in mammalian germ cells and somatic cells by chemical mutagens (Project 3)

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1. OBJECTIVES

The large contribution of chromosome aberrations (structural and numerical) to human fetal wastage, the high percentage of newborns (0.5 - 1%) with mental/ physical defects resulting from various types of chromosomal changes together with the absence of rapid screening systems for the detection of induced point mutations covering a substantial part of the genome, make cytogenetic studies with mammalian cells indispensable. In the context of the EEC programme the major objectives of the cytogenetics studies with mammalian cells are to (1) accumulate information on the induction of structural and numerical chromosome aberrations as well as sister chromatid exchanges (SCE's) in somatic cells and germ cells of mammals by chemical mutagens/carcinogens using a variety of assay systems; (ii) develop and standardize in vivo and in vitro cytogenetic techniques for detecting directly and indirectly acting mutagens/carcinogens; (iii) investigate the relationship between reaction kine tics and clastogenic action of monofunctional alkylating agents in mammalian cells, to establish correlation between a) the concentration of mutagens such as EMS and DEN (mM or mg/kg) with the number of ethylations per nucleotide in the DNA of the target cells, and b) the number of ethylations per nucleotide with different cytogenetic parameters. The latter studies are being carried out in collaboration with colleagues using other test systems and genetic endpoints as well; (iv) study the relationship between reaction kinetics and mutagenic action of monofunctional alkylating agents in mammalian cells.

2. RESULTS

2.1. IN VIVO STUDIES

2.1.1. Studies with methyl-phenylnitrosamine and MMS in mice

Studies were conducted with methyl-phenylnitrosamine (MPNA) and MMS. When two i.p. injections of MPNA were given (2 x 30 mg/kg - 2 x 120 mg/kg; 1 day interval) the lowest effective concentration (LECT) tested for cytogenetic damage in bone marrow cells was 2 x 60 mg/kg for both chromosome aberrations and micronuclei. It is of interest to note that parallel in vitro studies in our laboratory showed that MPNA could not induce chromatid aberrations and SCE's in CHO cells in the presence or absence of rat liver microsomes. Thus, the MPNA results point to the importance of using in vivo cytogenetic methods for agents that register negative in in vitro tests even when supplied with a metabolic activation system. In continuation of earlier in vivo work with MMS (single i.p. doses 10-160 mg/kg) where the LECT for the micronucleus test was 40 mg/kg and where no significant increase of chromatid aberrations in bone marrow cells could be detected at any of the concentrations tested, the effect of MMS was tested with the new in vivo SCE test (subcutaneous BrdUrd tablet of 55 mg). The superiority of the SCE test with bone marrow cells over the micronucleus test with the same cells was clearly demonstrated by the low LECT (1 mg/kg) for the former test.

2.1.2. Chemical dosimetry study with EMS in mice

In the context of a large chemical dosimetry study involving several genetic end points following treatment with EMS in diverse systems (Salmonella, E. coli, yeast, Neurospora, mammalian cells in vitro) two in vivo experiments have been carried out with mice (single exposures of 50, 150 and 250 mg/kg). Frequencies of SCE's in bone marrow were significantly increased following 50 mg/kg, whereas the frequency of micronuclei in polychromatic erythrocytes was significantly increased after an exposure to 150 mg/kg. Induced chromosome aberrations in the bone marrow were detected but the frequencies were not significantly different from the controls. Very few aberrations were detected in spermatogonia.

Parallel experiments with tritiated EMS were carried out to measure the dose of EMS (ethylations per nucleotide in DNA) in various tissues of male mice. The results indicate a uniform distribution of the mutagen in different tissues of the body (testis, liver, bone marrow). Alkylation appears to be higher in the bone marrow, which can be attributed to the special nature of the cells, i.e. loose cells with very high proliferative activity. It is remarkable that the levels of alkylation detected in the tissues of mice are similar to those observed with mammalian cells in culture at the same concentration in terms of mg/kg of solution. More data are needed before firm conclusions can be drawn with respect to the relationship between ethylations/nucleotide and any genetic endpoint.

2.1.3. Studies with rat liver cells in vivo

A technique was developed for detecting micronuclei (as indicator for chromosome breakage phenomena) in freshly isolated liver cells. It consists of the following steps: (i) in vivo treatment with a mutagen before or after hepatectomy; (ii) partial hepatectomy; (iii) liver perfusion followed by isolation of liver cells at different time intervals after hepatectomy; and (iv) preparation of slides, followed by fixation and staining (Feulgen + Naphtol yellow S) for the detection of purple micronuclei in a yellowish cytoplasm. The sensitivity of this technique was tested, using diethylnitrosamine (DEN), a potent carcinogen which is negative in classical cytogenetic tests for testis and bone marrow cells. With the liver micronucleus test DEN was clearly positive under the following experimental conditions: (i) One i.p. injection (50 or 150 mg/kg) one week before hepatectomy and perfusion 24 h or 72 h after hepatectomy; (ii) five i.p. injections (6.3 mg/kg) with one week intervals. Hepatectomy was performed one week after the fifth injection and the cells were isolated 48 h later; and (iii) DEN (40 mg/kg) was administered 24 h after hepatectomy and liver cells were isolated 48 h or 120 h later.

The positive response with DEN was later on confirmed with DMN as well. These results clearly indicate that the liver cell micronucleus test is particulary useful for demonstrating clastogenic effects of compounds which undergo metabolic activation in the liver and which form mutagenic metabolites that for one reason or another do not reach the common target cells aminable for cytogenetic assays.

2.1.4. Nondisjunction studies with the field vole Microtus oeconomus

Microtus is eminently suited for cytological studies on nondisjunction of sex chromosomes in male germ cells, because of its unique distribution pattern of heterochromatin. With the C-banding technique that stains specifically heterochromatin, one can demonstrate that the Y-chromosome of Microtus is completely heterochromatic. The X-chromosome, which exhibits a block of centromeric heterochromatin that is larger than that of the autosomes, can also be recognized. This special distribution pattern of the heterochromatin in Microtus cells makes it possible to identify the X- and Y chromosomes in postmeiotic cells, particularly in the early spermatid stage. In addition to studies with X-rays, eleven chemicals have been tested for the induction of sex chromosome nondisjunction and the production of diploid spermatids. With respect to the induction of sex chromosome nondisjunction positive results - which need further confirmation - have been obtained for MMS, parafluorophenylalanine, vincristine, procarbazine, carbendazim and bleomycin. Nocodazole, benomyl, colcemid, 6-marcaptopurine and halothane were all negative at the concentrations tested. For the induction of diploid spermatids, positive response was only obtained for MMS and parafluorophenylalanine. In view of these data, it seems that the Microtus system is a useful tool for analyzing factors contributing to the occurrence of nondisjunction in male germ cells.

3. CONCLUSIONS

3.1. IN VITRO STUDIES WITH CHINESE HAMSTER CELLS

3.1.1. Studies with ethylphenylnitrosamine (MPNA), atrazine and procarbazine

In contrast to the in vivo study (see 2.1.1.) where MPNA was found to increase the frequencies of chromosome aberrations and micronuclei in bone marrow cells, this compound (1.3 - 10 mM) did not induce chromosome aberrations and SCE's, both with and without metabolic activation. Atrazine was tested with and without metabolic activation. At the concentrations tested (12.5 - 100 mM) atrazine did not induce cytogenetic damage. The large series of in vitro experiments with procarbazine has been completed and the outcome of these experiments is rather complex and the following conclusions have been drawn: (i) the metabolic activation system (rat liver microsomes, S-9 mix) is capable of converting procarbazine into a metabolite(s) that can induce low frequencies of chromosome aberrations as well as SCE's; (ii) the amount of metabolite produced saturates at relatively low concentrations of procarbazine and (iii) at high concentrations of procarbazine the S-9 mix captures or detoxifies part of this compound, because in the absence of S-9 mix high concentrations of procarbazine induced many more aberrations than in its presence. In most in vitro experiments chromosome aberrations were scored in the first cell cycle after treatment with procarbazine and SCE's in the second cycle. Recently we developed a method permitting the scoring of both cytogenetic parameters in the same population of treated cells. The procedure is as follows: (i) culture cells for one cell cycle in medium with BrdUrd; (ii) treat cells for 1 or 2 h in the dark with the mutagen; (iii) culture cells for one cycle in medium with excess thymidine in the absence of BrdUrd, and (iv) fix and stain the cells. When procarbazine (without S-9 mix) was tested with this method it was found thatthe techniques for scoring chromosome aberrations and SCE's are equally sensitive. This finding, which was also confirmed for other chemicals, shows that the intrinsic sensitivity of the SCE test for mmamalian cells in vitro is not greater than that of the classical chromosome aberration test.

3.1.2. Relationship between reaction kinetics and mutagenic action of monofunctional alkylating agents

The relationship between chemical reaction patterns of several monofunctional alkylating agents described in terms of primary alkylation patterns in DNA and proteins as well as Swain Scott's factor and their biological effects in mammalian cells (CHO) in vitro were studied. Three end points were considered, namely the concentrations required (i) to give 50% reduction in colony formation, (ii) to double the frequency of spontaneously occurring chromosome aberrations, and (iii) to double the frequency of spontenaous sister chromatid exchanges (SCE's). The chemicals with their reaction type and S-values were as follows: ethylnitrosourea (SN_1; 0.26), methylnitrosourea (SN_1; 0.42), ethylmethanesulfonate (SN_1/SN_2; 0.67), methylmethanesulfonate (SN_2; 0.83) and dimethylsulfonate (SN_2; 0.83).

The following conclusions can be drawn:

(i) For cell killing (as determined by colony formation) there is no direct correlation between the effective concentration and the mode of action of the chemicals tested (i.e. SN_1 or SN_2);

(ii) However, if one compares methylation vs. ethylation, it is clear that the former is more effective than ethylation, irrespective of the mode of action;

(iii)Similar relationships hold true for production of chromosome aberrations and SCE's; and

(iv) The concentration to double the frequencies of chromosome aberrations and SCE's are similar in each case, which indicates that the two cytogenetic endpoints arise from the same primary lesion in the DNA.

4. PUBLICATIONS (funded by contract 139-77-1 ENV N).

Aaron, C.S., A.A. van Zeeland, G.R. Mohn, A.T. Natarajan, A.G.A.C. Knaap, A.D. Tates and B.W. Glickman, Molecular dosimetry of the chemical mutagen ethylmethanesulfonate: comparative studies of mutation induction in Escherichia coli, V-79 Chinese hamster cells, L5178Y mouse lymphoma cells and some in vitro and in vivo cytological results. Mutation Res. 1978, in press.

Vogel, E. and A.T. Natarajan, The relation between reaction kinetics and mutagenic action of monofunctional alkylating agents in higher eukaryotic systems. I. Recessive lethal mutations and translocations in Drosophila, Mutation Research 1978, in press.

Vogel, E. and A.T. Natarajan, The relation between reaction kinetics and mutagenic action of monofunctional alkylating agents in higher eukaryotic systems. II. Total and partial sex-chromosome loss in Drosophila, Mutation Research, 1978, in press.

Aaron, Charles S., A.A. van Zeeland, G.R. Mohn and A.T. Natarajan, Molecular dosimetry of the chemical mutagen ethyl methanesulfonate in Escherichia coli and in V-79 Chinese hamster cells, Mutation Research 50 (1978) 419-426.

Tates, A.D., Microtus oeconomus (Rodentia), a useful mammal for studying the induction of sex-chromosome nondisjunction and diploid gametes in male germ cells, Environmental Health Perspectives, 1979, in press.

Contractor : Medical Biological Laboratory TNO
P.O. Box 45
2280 AA Rijswijk, The Netherlands
Contract n° : 192-77-1 ENV N
Projectleader : P.H.M. Lohman
Co-workers : G. Veldhuisen, J.v.Hemmen, I.E. Mattern
Title of the project: IMPROVEMENT OF THE INCORPORATION OF LIVER METABOLISM IN TEST-SYSTEMS WITH MAMMALIAN CELLS

Objective of the research

Many compounds which by themselves are not mutagenic are converted by liver oxygenases into substances with genotoxic properties. Therefore liver extracts are often added to in vitro testsystems to provide for the metabolic conversion of the substance to be tested. However, some cell systems react unfavourably to the presence of liver homogenates, the activity of these extracts rapidly decreases with time and frequently the active metabolites are very short lived and are hardly able to reach and enter the target cells.
Within the scope of the contract two approaches have been followed to overcome one or more of the above mentioned limitations of metabolic conversion systems.

I. The use of cells with endogenous metabolic capacities.

The genetic system (end point) in this study has been the induction of sister chromatid exchanges (SCE) in rat liver cells. Hepatocytes cultured in vitro would have been the system of choice to overcome all above mentioned problems. However, hepatocytes in vitro do not carry out the necessary two rounds of replication. Therefore, as an alternative, rat hepatoma cell lines were used. These cells have preserved at least part of the most important enzyme systems responsible for the metabolic conversion of xenobiotics in normal rat liver cells.

II. The use of porous membranes to separate cells from liver homogenates.

In this second approach fresh liver homogenate is brought into a system with mammalian cells by pumping it through capillaries with porous walls on the outside of which the cells are located. In principle, these hollow fibers (Amicon "VITAFIBER tissue culture systems"- see ref. 1.) allow the transport of compounds (metabolites or xenobiotics) of low molecular weight through the walls of the capillaries, owing to the relatively large pores, whereas larger molecules (enzymes) remain on the inside (exclusion limit approx. 10.000 dalton). Mammalian cells can grow in high densities on the outer surface of these membranes. Because of the intensive contact of growing mammalian cells with the outside of the membranes it was expected that the test compound and possibly also short-lived metabolites can reach the cells when this compound together with fresh liver homogenate is pumped through the interior of the fibers. Moreover, it was assumed that this system in certain respects would mimic the in vivo exposition (a constant suppley of freshly formed metabolites) in a much better way than the static batch procedure commonly used in the incubation of cells with the rapidly deteriorating liver homogenates.

Results and conclusions

I. The enhancement of sister chromatid exchanges (SCE) in Reuber Hepatoma H35 cells treated with genotoxic agents.

SCE induction in H35 rat hepatoma cells (cell line H-4-II-E) was compared to that in control rat cells (R1, rhabdomyosarcoma cells originating from non-hepatic tumor tissue). The results are indicated in Table 1 and can be summarized as follows:

a. Methyl methanesulphonate (MMS; directly active) induces SCE's in both cell-lines with equal efficiency.
b. Di-ethylnitrosamine (DEN; requires bioactivation) induces SCE in H35 but not in R1.
c. 3-methylcholanthrene (3MC; requires bioactivation) induces SCE in H35 (but less efficient than DEN) and not in R1.
d. 4-nitroquinoline-N-oxide (4NQO; activated by target cell) at low concentration induces SCE in R1 and not in H35. For a comparable effect a 50-100 times higher concentration of 4NQO is needed in H35 than in R1. Also on the basis of survival H35 is much more resistant than R1.

The conclusions from these data is that H35 cells can be used as an indicator for the genotoxic activity of chemicals which need metabolic activations. However, the 4NQO results suggest that H35 cells are also capable in detoxifying directly active chemicals. So the exclusive use of H35 might yield false negative results.

In order to compare H35 and R1 cells with respect to their capacity to activate chemicals, the arylhydrocarbon hydroxylase activity (AHH) in both cell-lines was measured. The background AHH activity (uninduced) in H35 was only slightly higher than in R1; but after cultivation of the cells in 1 µM 3MC, the AHH activity in H35 increased 20-40 fold while no increase in activity was found in R1. This result indicates that the used H35 cell-line has indeed maintained at least part of the most important enzymes responsible for the metabolic conversion of xenobiotics in rat liver cells.

II. The use of porous membranes to separate cells from liver homogenates.

For this study first a number of introductory investigations had to be performed. Firstly the growth of the cells on the surface of the fibers had to be mastered. In particular the estimation of the progress of growth without being able to perform a cell count or analogous procedure caused a problem. It appeared possible, however, to judge growth from the changes in the glucose concentration in the medium that passed through the fibers. This is demonstrated in Fig. 1. Chinese Hamster Ovary (CHO) cells were grown on Amicon VITAFIBER no. 3P10. Per 3P10 unit 10^6 cells were inoculated and at time points indicated in Fig. 1, samples were taken from the medium which had passed through the fibers. Duplicate glucose determinatios were made with a glucose analyzer (Yellow Springs Instrument Co., model 23A). At the same time point cells were detached from the fiber by trypsinization and counted to follow the growth of the cells. The results shown in Fig. 1 demonstrate that it is possible to follow the growth of CHO cells on the fibers by measuring the glucose consumption in the medium.

The second introductory investigation was needed to find out whether liver homogenates could be pumped through the very narrow fibers (as indicated in Fig. 1) and whether the chemicals (or their metabolites) could pass through the pores in the walls of the various types of fibers in use (for protein retention of fibers see Fig. 1).

The chemicals used in this study were ethidium bromide (EB), cyclophosphamide, mitomycine C and MMS. It was found that in spite of the large pore size not all low molecular weight compounds can freely pass through the membranes; in particular polycyclic aromats, like EB and mitomycine C, are greatly impeded, probably due to adsorption to the walls of the hollow fibers. Presumably this will be also the case with the metabolites of this type of compounds (see below). Subsequently the properties of the system were tested with bacteria in the outer compartment (Salmonella typhimurium

TA100) to study whether these could be mutated. For a comparison the induction of revertants was also determined with bacteria which were incubated in direct contact with the compound (with or without liver homogenate). It was found that MMS (without liver homogenate) did pass through the walls of the hollow fibers and induced mutations in the bacteria, although somewhat less efficient than in the control experiment. Cyclophosphamide (see table 2), however, did fail to increase the number of revertants in the outer compartment, in contrast to the results obtained on direct contact. The most likely explanation is that also the active metabolites of cyclophosphamide were adsorbed to the material of the hollow fibers and did not reach the bacteria. Despite the lack of succes with some of the polycyclic compounds we still considered it worthwhile to continue our experiments with mammalian cells. When mammalian cells are grown on the fibers they attach to and (partly) even grow inside the pores of the membrane as shown by electron microscopy. Because of this intensive contact with membranes it appeared possible that mutagens or metabolites have a better chance to reach these cells than in case of bacterial cells, which were suspended in the fluid outside the membranes. This will be certainly relevant for short lived metabolites, but also when adsorbtion occcurs a favourable effect might be expected. This investigations are in progress at this moment in the ongoing programme.

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TABLE 1 SCE INDUCTION IN H35 AND R1 CELLS

	Concentration (mM)	H35(1)	R1(1)
methyl methanesulphonate	0.1	1.13 ± 0.048	1.67 ± 0.067
(MMS)	0.03	0.53 ± 0.040	0.85 ± 0.037
	0	0.22 ± 0.018	0.22 ± 0.015
Di-ethylnitrosamine	10	-	0.24 ± 0.022
(DEN)	3	0.63 ± 0.069	0.28 ± 0.024
	1	0.70 ± 0.083	0.22 ± 0.018
	0.3	0.49 ± 0.021	0.27 ± 0.021
	0	0.28 ± 0.021	0.27 ± 0.023
3-Methylcholanthrene	0.01	0.53 ± 0.028	0.29 ± 0.020
(3MC)	0.003	0.38 ± 0.031	0.21 ± 0.025
	0.001	0.27 ± 0.024	0.23 ± 0.016
	0.0003	0.28 ± 0.028	0.24 ± 0.021
	0.0001	0.22 ± 0.016	0.21 ± 0.024
	0	0.16 ± 0.011	0.23 ± 0.021
4-Nitroquinoline-N-oxide	0.0017	0.74 ± 0.036	-
(4NQO)	0.0005	0.48 ± 0.047	-
	0.00017	0.38 ± 0.032	-
	0.00005	0.32 ± 0.067	-
	0.000017	0.26 ± 0.032	0.81 ± 0.045
	0	0.15 ± 0.027	0.27 ± 0.021

1) SCE per chromosome ± s.e.m. (N=20-25)

TABLE 2 "AMES" TEST WITH CYCLOPHOSPHAMIDE (Salmonella typhimurim TA100) in the presence or absence of "S9 mix".x)

Concentration (mM)	Mutants per plate[xx)] Direct method - S9	Direct method + S9	Exposed via Amicon VITAFIBERS[xxx)] - S9	Exposed via Amicon VITAFIBERS[xxx)] + S9
0	25	54	21	34;66
0.018	35	43		
0.017	16	37		
0.050	24	48		
1.63	24	103		
3.4			37	61
4.7	22	181		
1.55	28	65		63
17.0			22	28;63;35

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x) S. typhimurium cells were exposed to cyclophosphamide either directly or via the hollow fibers system, with or without "S9 mix" (centrifugated rat liver homogenate).

xx) numbers averaged over 3 plates/points

xxx) Bacteria were suspended in medium in the outer compartments of 3P10 units. Test compound ± "S9 mix" in buffer was circulated through the inside of the fibers for 1 hr. at 37 °C in the indicated concentrations. With "S9 mix", after this period approx. 10% of the cyclophosphamide could still be detected in the circulated buffer.

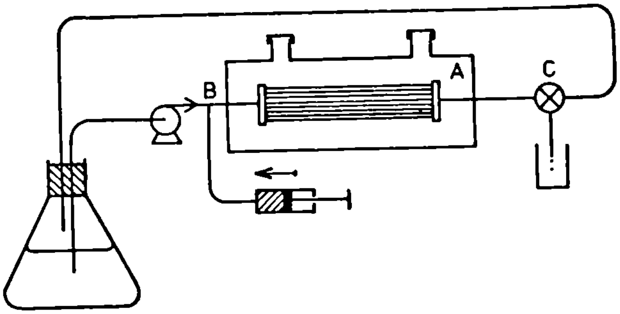

Fig. 1a. System used for growth and exposition of cells with VITAFIBER-units.

Cells are inoculated in the outer compartment of the hollow fiber unit (A). The fibers are continuously perfused with growth medium. Additional substances (e.g. liver extracts) can be admixed to the medium just before the entrance of the unit (B). Monitoring of the effluent of the capillaries may occur via the outlet directly after the unit (C). When exposition to freshly produced metabolites is intended the effluent is not fed back to the stock solution.

Four types of VITAFIBER-units can be used, differing in pore size and material, viz.

3P10	(polysulphone; exclusion limit for protein:	10.000 MW)
3X50	(acrylic copolymer;	50.000)
3X100	(acrylic copolymer;	100.000)
3S100	(polysulphone;	100.000)

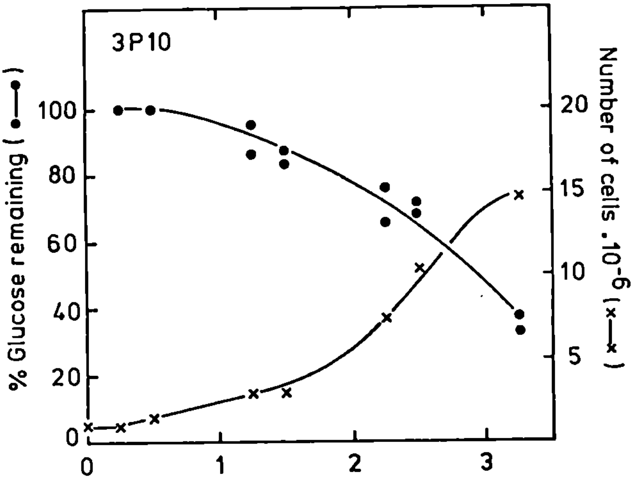

Fig. 1b. Determination of cell-growth in hollow fiber units by monitoring glucose consumption.

Chinese Hamster Ovary cells were grown in 3P10 VITAFIBER units for various periods. Each unit was inoculated with 10^6 cells in the outer compartment (6 ml). Medium with glucose (200 ml) was circulated through the capillaries (180 ml/hr). At the end of the indicated periods the number of cells was determined (X——X) as well as the glucose concentration in the medium (O——O).

Contractor : Etat Belge (Ministère de la Santé Publique), Institut d'Hygiène et d'Epidémiologie

Contract N° : 261-77-6 ENV B
Project Dealer : Dr. R.F. MOUTON
Title of Project : DNA MISREPAIR AND CARCINOGENESIS : Latent Cytotoxicity of Repair Inhibitors of the Human Environment.

OBJECTIVE OF THE RESEARCH

In the last years, the assessment of Cancer risk has begun with a systematic assay of the mutagenic potency of chemicals by reverse of foward mutation tests either in vivo or in vitro.
Beside the problems dealt with the metabolic activation of indirect mutagens, we pointed out (1) the importance of the interaction of non-mutagenic/carcinogenic chemicals with the repair processes of DNA lesions. It was further demonstrated that Caffein, a potent inhibitor of DNA repair of UV, MMS, MMC induced lesions, can act synergistically with DNA-damaging agents both on the Mutation frequency and on the Cell Transformation index (2,3).

Our specific aim is the development of screening tests for the detection of synergistic genetic toxicities of chemicals. We therefore investigate possible synergies between DNA-damaging agents and inhibitors of DNA repair. Following our previous work, this goal requires a clear-cut definition of DNA-damaging properties of the chemicals tested. Accordingly, we have taken up the extremely simple test just developed in 1977 by R.B. PAINTER (4-6). That test seems convenient to detect successfully DNA-damaging agents without false positive results.

We present here some of the results obtained with such procedure applied to human and monkey cells in culture, and to the fast growing eukaryote Tetrahymena pyriformis. We used the CEE recommended chemicals as references.

Following our previous work with caffein, we also report the study of Quinacrine and Chloroquine toxicity on the kinetics of the UV-lesion repai in T. pyriformis using our original immunoautoradiographic procedure.

Abbreviations : DNEM : Dulbecco's modification of Eagle's medium
NCS : New-born calf serum; PBS: Dulbecco's phosphate buffer saline pH 7.2; $^{14}C/^{3}H$-TdR: $^{14}C/^{3}H$-Methyl-thymidine; TCA: trichloroacetic acid; UV: Ultraviolet light at 254 nm; MMS: Methyl-methane-sulfonate; MMC: Mitomycine C; DEN: diethyl nitrosamine; NAT: procarbazine, Natulan; ATRA: atrazine; MNNG: methyl-nitro-nitroso-guanidine; 4-NQO: 4-nitroquinoline-N-oxyde; BP:benzo(a)pyrene; CAF: Caffein; HU: Hydroxyurea.

Aknowledgments: W.M. thanks Prof.MERCIER for the gift of S9 fractions and J.ARANY for discussions.
M.OTTOGALI, J.NISOLLE and F.TERSAGO are aknowledged for valuable technical assistance.

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MATERIAL AND METHODS

Chemicals : were purchased from : Sigma : MMS, Chloroquine, Quinacrine-Aldrich : MMC, HU, BP, MNNG - ICN-Pharmaceuticals : 4-NQO - Merck: CAF - Amersham: ^{3}H-TdR (46 Ci/m mol), ^{14}C-TdR (52 mCi/mmol) - Atrazin was a gift of CEC - Procarbazin was a gift of Dr. HENNAU (Roche S.A.) Chemicals to be tested were dissolved in DMSO, excepted for NAT, CAF, HU which were dissolved in PBS.

Biological material : Human epidermoïd carcinoma KB cell line ATCC CCL 17
Monkey Kidney cells -VERO ATCC CCL 81
Tetrahymena pyriformis GL from Carlsberg Institute.

Cell culture : Cells were routinely grown at 37°C in "Corning" plastic flasks between 6.10^3 and 10^5 cells/cm^2 in DMEM (Gibco 196 G) supplemented with 10 % NCS (Gibco) and 50 µg/ml Gentamycin. Medium was buffered at pH 7.2 with 10 % CO_2 in the water-saturated atmosphere of a "Forma" water-jacketed incubator. Cultures were screened for PPLO contaminations by autoradiography (7).

Protozoan culture : The protozoa were routinely grown and experimented in the Zeuthen's medium (8) as previously described (9).

PAINTER's test :

i. The DNA-damaging properties of the chemicals tested were assayed exactly according to R.B.PAINTER (4) for cell treatment and labelling conditions and according to W.MOENS (10) for DNA extraction and liquid scintillation conditions.

ii. PAINTER's procedure was followed with slight modifications in the case of protozoa : ^{14}C-labelling was replaced by cell number counting using a "Coulter-Counter ZF", jauge 200 microns. The cells were continuously labelled at 0 time after chemical treatment. The results are expressed as the ratio of the radioactivities of the control to the treated samples x 100 at time of harvesting. Pulse labelling gave similar qualitative results.

Immunoautoradiography of Thymidine dimers removal in T. pyriformis GL
The effect of CAF, Quinacrine, Chloroquine on the UV-photolesions repair process have been measured in T. pyriformis according to T. LAKHANISKY (11).

RESULTS

I. Detection of DNA-damaging-agents using PAINTER's test.

An early reponse to DNA damage in mammalian cells is the inhibition of DNA synthesis. PAINTER has shown (4) that DNA-damaging and non-DNA-damaging agents can be distinguished by specific changes in the rates of DNA synthesis after treatment : with a DNA-damaging agent, the rate of DNA synthesis continues to decrease, but with a non-DNA-damaging agent the rate of DNA synthesis increases after treatment. The test measures thymidine incorporation into the DNA of the cells at various times after treatment with a suspected mutagen or carcinogen.

Using the human KB cell line, a similar type of cell line such as the Hela one used by PAINTER, we have confirmed the results obtained by this author with MMS, MMC, 4-NQO, MNNG. The test is not restricted to the use of human cells since monkey kidney cells are also convenient.

Table I illustrates the kinetics of rate inhibition of DNA synthesis following cells treatment with MMS, MMC, 4-NQO, MNNG. The inhibition was in all cases dose-dependent. The activity of doses as low as 5.10^{-4} M (MMS), 10^{-9} M (MMC), 5.10^{-7} M (MNNG), 5.10^{-8} M (4-NQO) were significantly detected.
DEN, NAT, ATRA, BP were not active in absence of metabolic activation system. (data not shown). With KUROKI's system of activation (12) we have succeeded in activating DEN cells with concentrations between 5.10^{-3} M and 10^{-2} M. Preliminary results have shown that NAT, ATRA and BP require special conditions of metabolic activations.

The response of DNA replication to DNA-damaging agents is supposed to be the consequence of the organisation of mammalian DNA into replicons (13,4). Human cells have been used by PAINTER as a representative biological system. However, T. pyriformis' DNA synthesis is also based on "replicon" structures (14); moreover as this protozoan presents cost and handling advantages over mammalian cell culture, we have applied PAINTER's procedure to it.
Table II. illustrates the kinetics of DNA synthesis inhibition after treatment of T. pyriformis with UV light, MMS, 4-NQO. The kinetics were similar to those measured in KB or VERO cells. The first fast phase was however completed sooner than in the case of the mammalian cells (30 min. with continuous labelling Table II. instead of 1-1.5 H with human cells Table I.). We have also found a reversible inhibitory effect of HU on DNA synthesis similar to that observed by PAINTER with Hela cells (Table III.). Caffein at antirepair concentrations (2.10^{-4} M) did not inhibit DNA replication. Growth inhibiting concentrations of Caffein (10^{-3} to 10^{-2} M) inhibit DNA synthesis but in a reversible way after treatment (Table III.).

II. Detection of inhibition of UV photolesions repair

In the previous CEC publication, we have described the inhibition by Caffein 2.10^{-4} M of the release of thymidine dimers after UV treatment of growing of T. pyriformis using an immunoautoradiographic method. We show here that Quinacrine (10^{-5} M) and Chloroquine (2.10^{-5} M) do not affect the thymidine dimers release (fig 1) (for technical details see 11).

DISCUSSION

A general method for screening chemicals from a genetic toxicological viewpoint has been proposed by PAINTER (4). We have confirmed it on human, monkey and protozoa cells. In all these cell types we have observed the specific response of DNA replication to DNA-damaging or non-DNA-damaging agents. We have drawn a parallel between human cells and T. pyriformis applying PAINTER's test to both systems : T. pyriformis DNA synthesis seems to be based on "replicon" structures too (14); moreover the protozoa present cost and handling advantages over mammalian cells.
Problems of metabolic activation are under study. We have already induced rat liver microsomial enzymes by various procedures in order to isolate active microsomial fractions for NAT, ATRA, BP metabolisation. On the other hand, T. pyriformis seems to have its own system of detoxification (resistance to MMC, MNNG, Aflatoxine B1). Work is under progress to describe these metabolisms.

The inhibitory effect of Caffein on thymidine dimers release has been shown previously. Quinacrine and Chloroquine are known as anti-repair drugs (15, 16). But using the same immunocytological method, these chemicals do not influence excision but rather a further step of DNA photolesions repair. This points out the need for a global measurement of DNA repair in order to detect all DNA repair inhibitors.

CONCLUSION

The toxicity of MMS, MMC, 4-NQO, MNNG, DEN, NAT, ATRA, BP, HU, CAF has been tested using PAINTER's test with various eukaryotic cell types. Among the chemicals, MMS, MMC, 4-NQO, MNNG are directly active; DEN, NAT, ATRA, BP require metabolic activation. HU and CAF are non-DNA-damaging agents.

The toxicity of Quinacrine and Chloroquine has been measured for their effect on UV photolesions release. As no measurable toxicity has been observed, we suggest that these chemicals affect DNA repair at another level than excision. Metabolic activation studies and development of other methods assessing DNA repair are under progress.

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PUBLICATIONS AND ORAL COMMUNICATIONS

1) Th. LAKHANISKY, B. HENDRICKX, R.F. MOUTON, J.J. CORNELIS (1979)
   - A serological study of removal of UV-induced photoproducts in the DNA of Tetrahymena pyriformis (GL) influence of Caffein, Quinacrine and Chloroquine.
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   - Detection of UV-induced lesions in T. pyriformis's DNA by radio-labelled antibodies.
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TABLE I : Effect of direct mutagens on the rate of DNA replications of human (KB) or monkey (VERO) cells.

Time after treatment	MMS 1mM	1.8mM	MMC 10^{-8}M	MNNG 10^{-9}M	10^{-5}M	5.10^{-5}M	4-NQO 10^{-9}M	10^{-6}M	510^{-6}
0	42	13	82	105	85	59	100	91	57
30 min	34	11	48	NT	79	18	NT	100	30
90 min.	31	9	26	107	75	37	100	79	31
150 min.	41	16	23	NT	61	27	NT	73	29
210 min.	43	24	25	NT	NT	NT	NT	NT	NT

NT : non tested.

Cells were at 10^4 cells/cm^2 in 2.5 ml medium containing 0.02 µCi/ml C-TdR in 6 cm ∅ dishes and incubated 16 h before treatment. After a 30 min. treatment in the dark with chemicals, at above final concentrations, cells were washed and pulse-labelled 5 min. with medium containing 5 µCi/ml H-Tdr.
Results are expressed as the percentage of the TCA-precipitable radioactivities of the treated samples to "control" samples at indicated time.

TABLE II : Effect of direct mutagens on the rate of DNA replication of T. pyriformis.

Time after treatment	U.V. 900 erg/mm^2	MMS 11mM	4-NQO 10^{-6}M
0	100	100	109
15 min.	77	65	130
30 min.	55	43	77
60 min.	19	34	56
90 min.	18	59	85

Protozoa at 10^5 cells/ml were treated 10 min. with the above mutagens, washed and aliquoted at 3-5.10^4 cells/ml in the presence of H-TdR 5 µCi/ml at 28°C.
At different times, the labelling was stopped by addition of ice-cold TCA 5 % final. After 30 min. at 0°C, TCA precipitable radioactivity was pelleted, washed twice and counted. Values are % of "control" at indicated time.

TABLE III : Effects of CAF 10^{-3}M and HU 10^{-2}M on DNA replication of T.pyriformis.

Time after treatment	+CAF	-CAF	+HU	-HU
0	116	82	-	-
15 min	91	132	70	-
30 "	100	132	49	26
60 "	74	102	41	54
90 "	79	96	36	86

+ : treatment during labelling
- : labelling after treatment
values are % of control at indicated times.
Experimental conditions are the same as in TABLE II.

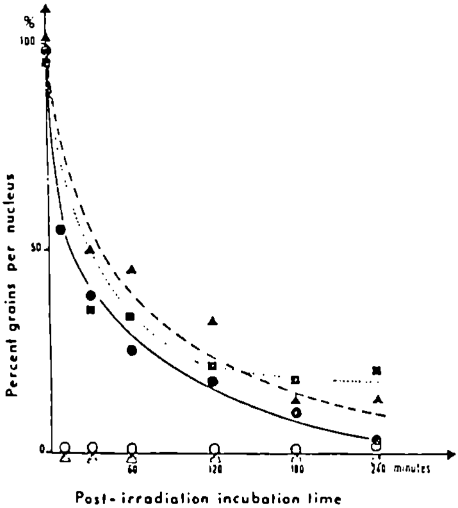

Figure 7 Influence of quinacrine and chloroquine on the elimination of photoproducts from UV-irradiated *T. pyriformis*.
○--○ unirradiated control; △—△ unirradiated control + 10 μM quinacrine; □—□ unirradiated control + 20 μM chloroquine; ● - ● irradiated cells; ▲—▲ irradiated cells + 10 μM quinacrine, ■ – ■ irradiated cells + 20 μM chloroquine.

Contractor: University of Sussex

Contract n^{o}: 182-77-1 ENV UK

Project leaders: R.J. Cole and C.F. Arlett

Title of project: Development and comparison of methods for assessment of potentially mutagenic and carcinogenic chemicals using haemopoietic and lymphoid cell cloning techniques.

Objectives

We set out to develop reliable measures of genetic damage by mutagens and carcinogens appropriate to patterns of human exposure. Priority was given to *in vitro* quantification of genetic damage after *in vivo* exposure of experimental animals, with particular attention to transplacental effects. Results were compared with those obtained from standard and improved techniques using mammalian cultured cell line systems (mouse lymphoma L5178Y).

(A) Application of the micronucleus test to transplacental exposure to chromosome damaging agents.

A kinetic model of erythropoiesis in mouse maternal bone marrow and fetal liver has been developed to provide a reliable basis for comparisons of frequencies of chromosome breakage in maternal and fetal tissues after exposure of pregnant animals. The validity of the model was tested by exposing animals (i/p injection) to Methylmethanesulphonate and Mitomycin C, and the relationships between frequencies of micronucleated polychromatic erythrocytes, proportion of damaged target cells, and exposure times established. The effects of directly acting, rapidly absorbed mutagens are most efficiently detected 24 hrs after exposure for maternal bone marrow, and 18 hrs after for fetal liver. However, optimal effects of other agents may be delayed (e.g. Benzo (a) Pyrene maternal bone marrow 48 hrs, fetal liver 30 hrs). Detailed time courses are important to establish dose response relationships for marginally effective substances. Dose response relationships have been established for Methylmethanesulphonate, Procarbazine hydrochloride, and Benzo (a) Pyrene.

Substance	D1% Bone marrow*	D1% Fetal liver*
MMS	25 mg/kg	25 mg/kg
Procarbazine hydrochloride	< 10 mg/kg	< 10 mg/kg
Benzo (a) Pyrene	150 mg/kg	> 300 mg/kg

* D1% dose to give an increase of 10 micronucleated erythrocytes / 1000 cells scored.

Publications

Cole, R.J., Taylor, N.A., Cole, J., Arlett, C.F. 1979. Transplacental effects of chemical mutagens detected by the micronucleus test. Nature 277: 317.

Cole, J., Cole, R.J., Taylor, N.A., Arlett, C.F. 1978. Detection of prenatal chromosome damage by the micronucleus test. Presented at European Environmental Mutagen Society Annual Conference, Dublin. July 1978.

(B) Detection of genetic damage in haemopoietic progenitor cells.

Granulocyte-macrophage progenitor cells which can multiply and differentiate in clonal culture occur in fetal liver, bone marrow and peripheral blood of rodents and man. We have continued investigations of modifications and improvements of this culture system to facilitate detection of genetic lesions, e.g. gene mutation, chromosome aberrations, sister chromatid exchanges. Improvements in culture media and growth promoting factors ('colony stimulating activity') have led to yields and cloning efficiencies adequate for mutation assays: one adult mouse femoral marrow yields 4×10^5 colonies, and 1 mouse fetal liver (16 day gestation) 2×10^5 colonies.CFUc can be concentrated on bouyant density gradients (e.g. lymphoprep). The frequency of spontaneously variant CFUc in bone marrow has been measured by detecting 6-thioguanine resistant ($HGPRT^-$) and ouabain resistant (changed Na/K dependent ATPase) colonies. The 'spontaneous' frequency of 6-thioguanine resistant CFUc was found to be 2×10^{-5}, and ouabain resistant 6.5×10^{-7}.

The standard CFUc cloning technique,(colonies in semi-solid agar) limits the potential of this system. We have therefore developed techniques which allow measurement of colonies attached to substrata (e.g. tissue culture plastic, glass). This improves the efficiency of the system for mutation assays, and the 'attached cells' are particularly useful for cytogenetic analysis. We have found this technique is appropriate for the determination of frequencies of sister chromatid exchanges after *in vivo* exposure (maternal and transplacental) to chemical mutagens, when BUdR incorporation occurs during the first 2 cell cycles *in vitro*.

(C) Mouse lymphoma cells

L5178Y mouse lymphoma cells have been cultured *in vitro* in suspension and cloned in agar by standard procedures (Cole, J. & Arlett, C.F., Mut. Res. 34 (1976) 507-526).

1. Development of alternative selective systems.

Our major objective was to provide a selective system which might be substituted for ouabain resistance in the modified fluctuation test. The main requirement for this experimental design is early expression of the mutant phenotype. Vinblastine and α amanitin proved to be unsatisfactory selective agents, but cytosine arabinoside (araC) was examined in greater detail. Although ara-C was completely toxic at high cell density and the spontaneous mutation frequency was low ($< 1 \times 10^{-7}$) the time required for the expression of newly arisen mutants was long (96-144h) and thus this system has not proved useful for the modified fluctuation test. However, the use of ara-C as one of a battery of selective agents has revealed a degree of specificity of response to different mutagens which may enable them to be assigned to groupings such as "EMS-like", "γ-like" etc.

2. Characterization of Thioguanine resistant variants.

The long term stability and HGPRT activity of 19 spontaneous and induced TG resistant L5178Y clones has been examined. All clones remained resistant to thioguanine throughout the culture period (> 200 days). No clone was able to grow in medium containing hypoxanthine, amethopterin and thymidine (HAT medium) suggesting that all had low levels of HGPRT. This was confirmed by uptake of ^3H hypoxanthine and gel electrophoresis in which HGPRT activity was shown to be < 5% of W.T. cells. Thus, all TG^{Res} clones we have examined appear to be stable mutants at the HGPRT locus. One clone appears to be capable of spontaneous and induced reversion to HAT resistance. The nature of these revertants and the use of HAT as a selective agent for back mutations is being investigated.

3. Comparative testing.

(i) Our work on methyl methanesulphonate has been completed.

(ii) Natulan and Diethynitrosamine. Both these compounds gave negative results when tested in the traditional experimental design (without exogenous activation) using 4 selective agents (TG, OUA, TdR and ara-C) but proved positive in the modified fluctuation test in the absence of metabolic activation. This confirms the sensitivity of this experimental design, and suggests that L5178Y cells have some ability to convert inactive promutagens to reactive electrophilic forms.

Publications

Cole, J., Arlett, C.F. 1978. Methyl methanesulphonate mutagenesis in L5178Y mouse lymphoma cells. Mutation Research 50: 111.

Rogers, A.M., Arlett, C.F., Burns, V.W. Induction of 1-β-d-arabinofuranosyl cytosine resistant variants in L5178Y mouse lymphoma cells. Studies with ethyl methanesulphonate, 2 acetylaminofluorene and ethidium bromide. Submitted to Mutation Research (1979).

Rogers, A.M. Mutagen detection in cultured mouse lymphoma cells. Presented at 2nd meeting U.K. Branch European Environmental Mutagen Society. York, 1978.

Conclusions

The micronucleus test can be used with prenatal blood cells to assess risks associated with transplacental exposure. The system responds to both directly acting mutagens, and those requiring metabolic activation. Since prenatal erythrocytes develop in the fetal liver, the system may be sensitive to agents which are negative in adult bone marrow, due to its inherently low capacity for metabolic activation.

Committed granulocytic stem cells (CFUc) can be cultured, so that they provide 3 potential in vitro end-points for assessing genetic damage after in vivo exposure to mutagens. These are gene mutations (at HGPRT, and N/K ATPase loci) visible chromosome aberrations, and sister chromatid exchanges. Such techniques are applicable to both pre- and post-natal exposure. Mouse lymphoma cells, used in a fluctuation test, provide a very sensitive system for detecting gene mutations, but only one locus (Na/K dependent ATPase) has so far been found satisfactory for this test. Differential responses of other loci to chemical mutagens, in the standard mutagenesis assay, may allow some classification of molecular changes underlying expression of particular mutations in the L5178Y system.

Contractor:	The Middlesex Hospital Medical School
Contract n^{o}:	184-77-1 ENV UK
Project Leader:	Dr. S. Neale
Title of Project:	Development of rapid, economical screening tests to detect carcinogenic or mutagenic compounds of environmental significance

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Objective of the research

Many of the increasingly large numbers of chemicals introduced into the environment every year are now known to be mutagenic in micro-organisms, insects and mammals. Among mutagens tested for carcinogenicity in mammals the great majority have given positive results. The ultimate aim of a screening method for detection of chemical mutagens is to ascertain the hazard to man of the chemical *per se*, or its metabolic derivatives, present in foodstuffs or the environment.

Mutations in bacteria can be used as markers in rapid and cheap assays to detect mutagenic activity but it is necessary to supply a source of mammalian metabolic activation to detect those compounds normally converted to mutagenic metabolites within mammals. Most bacteria die rapidly if injected intravenously into animals but complement-resistant strains survive and can subsequently be recovered several days later. The purpose of this project is to develop a screening test based on whole animal metabolic activation of the test compound by measuring mutation induction in complement-resistant bacteria sequestered within a host animal. The data obtained can be directly compared with chromosome aberrations induced in circulating lymphocytes or with the observed tumour response in the same host species. Concurrently we hope to increase the speed and sensitivity of bacterial mutation assays by developing a fluorescent method to detect the presence of bacterial mutants.

Materials and Methods

Wistar or BD rats (♂, 150 gm, 6-8 weeks) and Tuck TO mice (♀, 22 gm, 7 weeks) were used. Where required the animals were maintained 7 days on 0.1% phenobarbitone water or 3 days on a low protein diet prior to the experiment.

Atrazine (C.E.C. supply), diethylnitrosamine (Eastman Kodak) and methyl methanesulphonate (T. Schuchardt) were used as part of the C.E.C. on-going project together with various other known carcinogens obtained from commercial sources.

Broth-grown, complement-resistant Escherichia coli K12, D494 (met$^-$ pro$^-$ pan$^-$ thi$^-$, serotype 08) were injected intravenously into rats or mice (2 or 1 x 10^9 cells respectively/animal). The required dose of test chemical was administered by intravenous, subcutaneous or intraperitoneal injection or, in the case of Atrazine, by gastric intubation. Animals were killed between 30 min and 72 h after the bacterial injection and the livers removed, washed and homogenized in pairs in 1.5 volumes of sterile saline. Crude homogenate was plated on agar containing ampicillin (6 µg/ml) or naladixic acid (5 µg/ml) to determine resistant mutants while the total number of survivors was determined by plating suitable dilutions of homogenate onto minimal agar.

Results

The potent carcinogens dimethyl- and diethylnitrosamine require mammalian metabolic activation before showing mutagenic activity. Using E.coli D494 and mice the minimum single doses of dimethylnitrosamine or diethylnitrosamine which could be detected were 0.05 mg/kg and 5 mg/kg respectively A linear dose response curve was observed for dimethylnitrosamine up to 2 mg/kg for 30 min exposures and up to 7 mg/kg for longer exposures, this was in agreement with measurements of the time required for metabolism of these doses in mice.

Evidence was obtained that doses of 10 mg/kg and above caused a slight reduction in the level of mutagenic response and this was in agreement with the known inhibitory effect of high doses of dimethylnitrosamine on its own metabolism in the liver. Metabolism of nitrosamines in rats or mice was increased by phenobarbitone pre-treatment and a concomitant increase was observed in the mutagenic response to high doses of dimethylnitrosamine (30 mg/kg) or diethylnitrosamine (100 mg/kg); conversely low-protein diet reduced the rate of metabolism and caused a reduced mutagenic response to these dose levels.

Linear dose response curves were obtained with both mice and rats for six carcinogenic nitroso compounds of which three were mutagenic <u>per se</u> (<u>N</u>-methylnitrosourea, <u>N</u>-ethylnitrosourea and <u>N</u>-methyl-<u>N</u>-nitro-<u>N</u>-nitrosoguanidine) and the others required mammalian metabolic activation (dimethylnitrosamine, diethylnitrosamine and nitrosomorpholine). Comparison of dose response curves for the direct acting nitroso compounds suggested the best correlation was obtained when dose was calculated as mg/ml blood volume rather than as mg/kg body wt. The mutation dose-response curves obtained using rats and mice and dose-response curves for bacteria exposed to the same six compounds in a buffered suspension, with liver homogenate for metabolic activation, were compared with published data for tumour induction in rodents. Only the mutation results for bacteria exposed within the intact animal gave the same relative order of potencies as that found in the tumour induction data.

Some positive results have been obtained in rats and mice with the herbicide Atrazine (100-600 mg/kg) if the compound was administered by gastric intubation, but not following administration by intraperitoneal injection. These results support the suggestion that Atrazine is activated by nitrosation in the stomach. However, during several repetitions of these experiments the results have proved inconsistent and require further investigation; the inconsistency may reflect the method of administration and the relative insolubility of this compound.

Methyl methanesulphonate is toxic to both bacteria and rodents. A low, but positive, level of induced mutations was recorded in bacteria exposed for 30 min in mice given intravenous injections of 50-250 mg/kg.

Conclusions and additional comments

The results obtained so far demonstrate that this method can be used with both direct-acting mutagens and with compounds requiring metabolic activation. The relative mutagenic potencies of a small group of nitroso compounds tested in this way correlate well with their relative carcinogenic potencies. The method enables compounds requiring activation in the stomach to be tested and should have potential as a test system for volatile compounds. Considerable refinement of the method is still needed, in particular it is essential to increase the battery of genetic markers within the bacterium so as to detect all classes of base-change and frameshift mutagens.

Publications and oral communications

A.K. Solt and S. Neale. Induction of bacterial mutants in rodents treated with N-nitroso compounds. 8th Annual Meeting of the European Environmental Mutagen Society, Dublin, 1978.

A.K. Solt and S. Neale. Bacterial mutagenesis in mice treated with low doses of carcinogens. 3rd Annual Meeting of the U.K. Environmental Mutagen Society, Bath, 1979.

Contractor : Department of Genetics, University of Edinburgh
Contract n° : 194-77-1 ENV UK
Project leaders : Ch. Auerbach, B. Kilbey and P.T. Shukla
Title of project : Development of screening methodology - research on basic problems in the assessment of mutagenic hazards from environmental chemicals

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We have concentrated on the first of our proposals; genetical means for estimating the proportion of small deletions among point mutations induced in chemically treated Drosophila ♂♂. We have used 3 criteria. (1) The ratio between the frequencies of visible sex-linked recessives in treated spermatozoa when scored (a) as mutant ♀♀ in a specific-locus test, (b) as mutant ♂♂ in the progeny of attached-X ♀♀. In (b) deletions will be lost through lethality, so that the ratio a/b should increase with the proportion of deletions among visibles. (2) The ratio of sex-linked lethals in spermatozoa to that in spermatogonia. Because of germinal selection against lethals in the latter stage, this ratio should increase with the proportion of deletions among lethals. This is not expected to be the case for viable visible mutations, which hardly ever are due to deletions. (3) The ratio of lethals to viable visibles in spermatozoa; this ratio, too, should increase with the proportion of deletions.

We have applied these criteria to three substances: DEB (diepoxybutane) which produces many lethals and chromosome breaks in Drosophila; HA (hydroxylamine), which produces few lethals and no chromosome breaks; DEN (diethylnitrosamine), which produces many lethals but no chromosome breaks. DEB would be expected to produce many deletions, HA few or none. DEN, while producing many lethals, would not be expected to do this <u>via</u> deletions. Table 1 shows that our results bore out our expectation. DEB yielded high ratios in Tests 1, 2 and 3. HA yielded low ratios in all three. DEN resembled HA on criteria 1 and 2, but produced a very high ratio of lethals to visibles. These first results were followed up in various ways.

<u>DEN</u> Vogel and Leigh (1975) have reported that spermatids are more sensitive than spermatozoa to DEN. We therefore repeated the first two tests, using spermatids as post-meiotic stage. The results were essentially the same as for spermatozoa, with ratios (1) and (2) both < 1. We also considered that the data on spermatogonia might be made less reliable by the presence of large clusters. When we scored spermatogonial lethals separately in the progeny of 100 treated ♂♂, we found that the lethals (11.5% overall) were distributed over the individual ♂♂ in a Poisson series, with no ♂ yielding more than 3.

<u>DEB</u> We considered the possibility that the low frequency of spermatogonial letha might be due, not to germinal selection against deletions, but to a generally low sensitivity of spermatogonia to DEB. The data on viable visibles, which speak against this assumption, were too small to fully disprove it. We therefore compared the frequencies of both sex-linked and autosomal recessive lethals in

spermatozoa and spermatogonia; autosomal lethals, because of the diploidy of the autosomes in spermatogonia, would not be subject to germinal selection. Thus the ratio of autosomal to sex-linked lethals in spermatogonia should increase with the degree of germinal selection. In our experiment, this ratio was 23% to 11% in spermatozoa, as would be expected from the relative lengths of the first and second chromosomes. In spermatogonia, it was 19% to 2%. This is evidence for strong germinal selection against DEB-induced lethals.

HA We were struck by the fact that most HA-induced visibles arose as mosaics. This suggested to us that HA produces mainly one-strand mutations, which would escape detection in an ordinary test for sex-linked lethals but could be detected by continuation of the test to F_3. For this purpose, we sampled one ♀ each from a large number of F_2 vials that had not yielded a lethal, and scored her progeny for ♂♂ with the treated chromosome. Assuming that a one-strand lethal results in a gonad that carries the lethal in about half its germ cells, each of these ♀♀ has a 50% chance of being heterozygous for the lethal. If the lethal is present in less than half the ovary, this chance becomes correspondingly smaller. It will therefore be a conservative estimate, if we double the observed lethal frequency in F_3 to obtain the frequency of delayed lethals. The data are shown in Table 2. In both experiments, lethal frequencies in F_2 were so low that, on our standard, we should have classified HA at best as a very weak, at worst as a doubtful mutagen. In contrast, in both experiments, lethal frequencies in F_3 were clearly outside the range of spontaneous mutations. Since the deleterious effect of a lethal will be the same whether it has been detected at once or after a delay, we suggest that, in routine testing of environmental chemicals, a negative or doubtful result in F_2 should always be followed by a test in F_3.

Mitomycin-C We used this substance for studying another type of delayed effect which plays a part in mutagen testing: the storage effect on chemically induced chromosome breakage. This effect occurs when treated spermatozoa are stored in the seminal receptacles of untreated ♀♀; it may increase the frequency of rearrangements 10-fold or more. When the relative sensitivities of germ cell stages to chemical chromosome breakage are determined by brood pattern analysis, the storage effect may confuse the issue. Mitomycin-C has been found not to produce translocations in male germ cells past the spermatogonial stage (Schewe et al. 1971). We tested whether this was due to special conditions favouring its action in spermatogonia, or to the fact that, in brood pattern analysis, spermatogonia are sampled 12 days or more after treatment. We compared the frequencies of translocations between the two large autosomes in 3 series;

spermatozoa, sampled on the first day of the experiment (second day after treatment); spermatogonia sampled on the 12th and 25th days of the experiment; and spermatozoa stored in untreated ♀♀ for 12 or 25 days. The results for the 25-day samples are shown in Table 3; those for 12 days were intermediate. It will be seen that 25 days of storage had produced the same frequency of translocations in spermatozoa as had been obtained from spermatogonia sampled after 25 days. Coincidence cannot be excluded, and we intend to test this. In any case, when brood pattern analysis seems to implicate an early germ cell stage as particularly sensitive to chromosome breakage, one should consider that this may be due to passage of time rather than preferential sensitivity.

Conclusions (1) The results of tests with three selected chemicals agreed with what would be expected from the criteria we used for estimating the frequencies of small deletions among recessive lethals. (2) Experiments with hydroxylamine showed that a chemical which produces mainly mosaics may appear a very weak mutagen when tested in F_2, but a strong one when tested in F_3. (3) Mitomycin-C, which previously had appeared to produce no chromosome rearrang ments in spermatozoa, was shown to do so after storage of treated sperm for 12 or 25 days.

Literature references

Schewe, M.J., D.T. Suzuki and U. Erasmus, 1971. Mutation Res., 12: 255-267.

Vogel, E. and B. Leigh, 1975. Mutation Res., 29: 383-390.

References to publications and oral communications

Shukla, P.T. and C. Auerbach. The delayed mutagenic action of hydroxylamine in Drosophila (submitted to Mutation Research).

Shukla, P.T. and C. Auerbach. The brood-pattern of mitomycin-C induced transloc ations in Drosophila: the effect of time (submitted to Genetics).

Auerbach, C. The role of Drosophila in mutagen testing. Talk in Brighton to a meeting of Inveresk Research International in July 1978. Now in press in: "Topics in Toxicology 3. Mutagenesis in sub-mammalian systems", MTP Press Ltd.

Shukla, P.T. and C. Auerbach. Written Report for CONTACT group on the first 6 months of research (in two parts), March and July, 1978.

Auerbach, C. Oral report on research at E.E.C. meeting in Brussels, July, 1978.

Table 1

Diagnostic criteria for estimating the proportion of deletions among point mutations. Applied to diepoxybutane, hydroxylamine and diethylnitrosamine.

	DEB	HA	DEN
1) Sex-linked visibles in spermatozoa			
(a) scored in ♀♀ / (b) scored in ♂♂	2-3	<1	1
% lethals among (a)	75	0	25
2) Freqn. in sp-zoa / Freqn. in sp-gonia			
sex-linked lethals	2-3	<1	<1
sex-linked visibles	1	<1	1
3) Freqn. lethals / Freqn.viable visibles			
in sp-zoa	35	10	50
in sp-gonia	15	11	110

Table 2

Immediate and delayed (complete and mosaic) lethals in the progeny of HA-treated males.

Expt.	Lethals scored in	n.	Lethals n	Lethals %	% delayed * lethals
I	F_2	950	4	0.4	
	F_3	766	11	1.4	2.8
II	F_2	1175	6	0.5	
	F_3	1049	12	1.1	2.2

* Minimal estimates, see text.

Table 3

Delayed chromosome breakage by mitomycin C

Expt.	Method	Day	Treated stage	Sex-linked lethals	II-III translocations		
				%	n	tr	%
I		1	sp-zoa	5.0	600	0	0
	broods	25-27	sp-gonia	2.0	560	7	1.2
	storage	25	sp-zoa	6.0	175	2	1.1
II		1	sp-zoa	6.0	600	0	0
	storage	25	sp-zoa	5.7	375	5	1.2

Contractor : University of Dublin, Trinity College

Contract n^o: 169-77-1 ENV EIR

Project Leader: Professor George Dawson

Title of Project : Mutagenesis test systems in bacteria and mice

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The project is in two parts: one aimed at improving the predictive value of bacterial systems for the detection of carcinogens as mutagens, the other examines further the predictive value of the spot-test in mice and the conditions which maximise its sensitivity. Each will be reported separately.

Development of the Bacterial Assay for Screening Environmental Pollutants.

Project Leader: Dr S. Thompson

Objectives of the research. The reliability of the most widely used system, the Salmonella histidine back-mutation system developed by B. Ames and colleagues, has been variously estimated at 70-95%. Hence an estimated 5-30% of carcinogens may escape detection by the histidine system. The key questions to which this present research has addressed itself are: (a) Are there other bacterial systems, which may have as well as the present reliability of the histidine system, additional potential for the detection of carcinogens as mutagens? (b) Can the histidine system itself be used in an extended series of related tests so as to allow it to detect additional carcinogens as mutagens? To answer the first question, five totally distinct bacterial forward mutagenesis systems have been investigated and their potential compared to the histidine system. To answer the second question, carcinogens negative in the histidine system, anticancer and other agents have been subjected to the DNA repair test, the host-mediated assay and the urine metabolite test, using the histidine back-mutation system, in order to evaluate the usefulness of such extended tests.

Materials and methods. In investigating purine analogue resistance and chromate resistance forward mutagenesis sytems, Salmonella typhirmurium strains were employed; in investigating L-arabinose resistance, lactose resistance and the galR$^S \rightarrow$ galR$^-$ in the multipurpose Mohn strain 343/113, E. coli K12 strains were used. Bacteria previously grown in liquid culture were plated on a mixture of mineral salts and agar supplemented with one or more of the sugars, glucose, lactose, arabinose, galactose or glycerol usually at 0.2% and any required amino acids and vitamins at 0.002 and 0.0002% W/V respectively. The purine analogues were used at 20 µg/ml of 8-azaguanine and 500 µg/ml of each of 2,6 diaminopurine and 6-mercaptopurine. The amino acid analogue, 5-methyl tryptophan was used at 1 µg/ml. For chromate resistance, sulphate free media were supplemented with L-djenkolic acid at 0.15mM and sodium chromate at 0.3mM. Induction and preparation of liver homogenate and other *in vitro* techniques with the histidine back-mutation system were essentially as set out in *Mutation Res. 31* 347,1975. Pure microsomes were prepared as detailed in *Biochem. Biophys. Res. Comm. 47* 611, 1972. The host-mediated assay was essentially as in *Soc. Exp. Biol. Med. 139* 831, 1969 and urine metabolite tests and urine metabolite extraction techniques were similar to those set out in *Proc. Nat. Acad. Sci. 71* 737, 1974 and *Nature 249* 850, 1974 respectively.

Results. In investigating alternatives to the histidine back-mutation system, we had previously shown that the galactose resistance forward mutagenesis system and in addition it can detect thiourea, thioacetamide, amitrole and succinic anhydride, carcinogens undetectable in the histidine system. The spontaneous rate of mutation of the system is relatively high, however, and it is greatly enhanced in the presence of standard liver homogenate preparations. We therefore investigated several other systems. All the systems investigated (conferring resistance to purine analogues, sodium chromate, arabinose and lactose, and the $galR^S \rightarrow galR^-$, in strain 343/311) responded to base-substituting mutagens and the general frameshift mutagen ICR-191. The system exploiting 8-azaguanine resistance and the $galR \rightarrow galR^-$ system also responded to 9-aminoacridine. In contrast to the previously investigated galactose resistance system and the histidine tester strains derived from hisD3052, however, none of the new systems, including 5-methyl tryptophan resistance, $galR^S \rightarrow galR^-$ or the $nad^- \rightarrow nad^+$ and $lys^- \rightarrow lys^+$ in strain 343/311 detected 2-nitrosoflourene. Moreover the presence of standard homogenate preparations considerably enhances the spontaneous mutation rate. Similarly the presence of a plasmid, pKM101, which permits the detection of certain classes of mutagens and increases the sensitivity of detection of others, also enhances the spontaneous forward mutation rates.

In investigating mutagenicity of chemicals negative in the standard *in vitro* mutation test using the histidine back-mutation system, chemicals non-mutagenic *in vitro* are then tested in the mouse-mediated assay and the rat urine metabolite test. Among nine triaminotriaryl methane dyes, three, al hydrochloride derivatives, were negative in *in vitro* tests. No mutagenicity was detected in the host-mediated assay, however. Similarly neither of two synthetic phenazine derivatives, Clofazimine and its quinoneimine analogue (both shown by others to have antimitotic activity, being capable of binding to DNA—especially GC rich DNA, and inhibiting transcription) were mutagenic *in vitro*. Tests of DNA repair were negative and tests in the host-mediated assay and the urine metabolite tests on Clofazimine did not result in the detection of mutagenicity.

Conclusions and additional comments. Our investigations on forward mutagenesi systems clearly show that many of the systems are defective in that they lack one or both of G repeat and CG repeat targets, essential to the detection of many groups of carcinogens which act as frameshift mutagens. One system, the galactose resistance system has all the targets present in the histidine back-mutation testers and is capable of detecting some false-negatives. It will be investigated further. The extension of the use of histidine tester strains to tests with the host-mediated assay and the urine metabolite test, have so far, taking into account previous data, only detected two chemicals, 6-chloropurine and the carcinogen 4-dimethylaminoazobenzene as mutagenic. Other carcinogens negative *in vitro* including Natulan, thiourea, urethane and amitrole have remained negative in these *in vivo* tests.

The Somatic Mutation Test (spot test) in Mice.

Project Leader: G Dawson

Objectives of the research. Current research indicates that reliable predictions of the mutagenicity of particular chemicals in man will only be achieved by using a combination of test systems. To evaluate the reliability of the spot test its response to two classes of compounds was scored: those chosen for the comparative test programme to compare its sensitivity to other test systems, and those carcinogens which are not detected as mutagens by the Ames bacterial system. Mitomycin C, diethylnitrosamine and atrazine were in t first class, and urethane, thioacetamide, thiourea and succinic anhydride in t second. Preliminary experiments were carried out with saccharin which is know to be carcinogenic at high doses to discover whether it can also be shown to b mutagenic in a mammal. In the context of these experiments particular attenti was given to understanding further the test procedures, especially the relationship of response to dose, and the time of injection.

Materials and methods. Female mice of the T strain (non-agouti, aa; pink-eye, pp; brown, bb; dilute, dd; chinchilla, $c^{ch}c^{ch}$; spotted, ss; short-ear, sese) were mated to males of the HT strain (aa; leaden, lnln; pearl, pepe; pallid, papa, fuzzy, fzfz; brachypody, bpbp). Females were treated with the test chemical either by stomach intubation (orally) or by intraperitoneal injection (IP). Treatments were carried out either on one day or several successive days during pregnancy. Offspring were examined at 21 days of age for alterations in coat colour. Coloured spots which are presumably due to somatic mutation were distinguished from white spots which may be due to cell killing.

Mitomycin C (Kyowa Hakko Kogyo), diethylnitrosamine (Fluka A.G.) and atrazine, compounds in the EEC comparative test programme, were provided by Professor Ehling, Dr Vogel and Professor Loprieno respectively. All other compounds were from Sigma Chemical Company.

Analysis of log-likelihood-ratios was performed on the relative frequencies of offspring with non-white spots. The calculations were performed by computer with Nelder's GLIM program

Results. The results with each of the compounds can be summarised as follows.

Mitomycin C is strongly mutagenic in this test. For example an injection of 10 mg/kg on the 8th or 10th day of pregnancy resulted in more than 28% of the offspring having coloured spots. The controls in this, and other experiments, yield about 1% of offspring with such spots. For DENA, the results are not statistically significant. With atrazine the data are not extensive; further experiments are planned.

Both urethane and thioacetamide give positive results. For example three successive treatments of 1250 mg/kg of urethane on days 7, 8 and 9 yield about 10% of offspring with spots. Injection of 100 mg/kg of thioacetamide on day 7 yields about 5%. Extensive data for thiourea indicate that it is not mutagenic in this system, while preliminary data for succinic anhydride are inconclusive.

Preliminary data with saccharin are significantly positive (P=.01). For example injection of 750 mg/kg on day 10 resulted in more than 11% of offspring with coloured spots.

The response to dose for all compounds was consistent with a linear relationship except for saccharin. For example the injection of 7500 mg/kg yielded less than one third the frequency induced by 750 mg/kg. The data is not consistent with a linear relationship (P=.003).

For all the compounds the effects of injecting on days 7, 8, 9 and 10 of pregnancy have been compared. No significant differences were found for benzo (a) pyrene (previous data) urethane and thioacetamide. Maximum sensitivity to MMS was from injections on day 10 (previous data), to Natulan on day 9 (previous data) and to mitomycin C on day 8 or 9.

Conclusions and additional comments. With the exception of atrazine the experiments on the compounds in the comparative test programme have been completed. MMS and Natulan are positive (previous data), as is mitomycin C; DENA is not clearly shown to be mutagenic. Of four carcinogens which are negative in the Ames system two, urethane and thiocetamide, are mutagenic in the spot test. It would seem from these, and other, results that the spot test is likely to prove a strong candidate for inclusion in a combination of tests which together would have high value in predicting the mutagenic and carcinogenic hazards of chemicals to man.

It has been established that the spot test normally shown a linear relationship between response and dose: a chemical can be most effectively tested by using the highest dose which its toxicity allows. An exception is saccharin for which an intermediate dose induced the strongest response.

For some, but not all, compounds the response varies with the day of injection. The detection of a positive response is therefore enhance if experiments are not limited to injecting on a single day.

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TOPIC 13 : ASBESTOS AND OTHER FIBROUS MATERIAL

Contractor: Med. Institut für Lufthygiene und Silikoseforschung
an der Universität Düsseldorf

Contract N^{o}: 289-76-1o ENVD, Project 2

Project Leader: Prof. Dr. med. H.-W. Schlipköter
Dr. K. H. Friedrichs

Title of project: Determination of fibrous particles in ambient air and in human lungdust

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Objective of the research

The association between asbestos exposure and the induction of malignant mesothelial tumors was shown in both retrospective and prospective studies. Until now it is not yet clarified, whether the disease is asbestos related only, or whether other types of fibers may be equally dangerous. Further-more it is unknown, whether asbestos - and/or fiber-concentration in ambient air is in a range high enough to cause a serious risk to health of the general population. With respect to later intended measurements in the atmospheric air it was necessary to find out more information about biological relevant fibers at first. It was believed to be a useful way to analyze the lung dust of persons, which had different dust- (and fiber-) exposures during their lifetime. Subsequently the data of lung dust analysis with those to be anticipated in the atmospheric air were compared.

Methods and results of lung dust analysis

The usual methods of analyzing lung dust samples are light microscopic techniques, preferably phasecontrast-microscopy (PM) (in the medium magnification-range of 4oo - 8oo x). The same method is used by pathologists when deciding, whether or not a mesothelioma was induced by occupational asbestos-exposure. Because of the known limitations of lightmicroscopic techniques a Scanning Electron Microscope (SEM) fitted with an energy dispersive x-ray spectrometer (EDX) was used additionally with normal magnification of 1o ooo x (and also with 2ooo- and

5ooo-x resp. in some cases). Furthermore the use of a normal Transmission Electron Microscope (TEM) was intended in order to obtain electron diffraction data (SAED) of the fibrous particles and elemental analysis to be performed. (Because of technical difficulties this part of the study could not be started until a few months ago, and results should be reported later).

The analyses which followed are based on a total of 8o cases, which were classified by histological diagnosis into four different groups:

N = normal lungs without any provable occupational asbestos-exposure

SM= spontaneous mesothelioma case (i.e. without occupational asbestos-exposure)

BM= mesothelioma case with provable occupational asbestos exposure

A = asbestosis case (where asbestosis was not the reason of death in any case but an accompanying BM).

With respect to reproducable results a lot of "rules" had to be given to the evaluator prior to analysis (i.e. a fiber was defined as a particle having parallel long edges and an aspect ratio of ≥5:1). The results of the counting analysis with the aid of SEM and PM are presented in figure 1. The size distribution of fibers was determined.electronmicroscopically with the help of microfotos. The results, presented in figure 2, indicate that there is no essential difference in the diameter-distribution. On the other hand a definite difference of the fiber-length-distribution was found, the difference of length being due to the occupational asbestos exposure.

The EDXA in combination with the SEM has some limitations (depending upon the method as well as the special microscope type used) in comparison to TEM-capabilities. On the other hand the elemental analysis with the TEM suggests that predominantly amphibole-asbestos fibers are to be found in human lung dusts (regarding the indication that the chemical components do not correspond to the expected spectrum derived from the "standard"). The EDXA-results are supported by the morphological point of view.

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Conf. "Occupational Exposures to fibrous and particulate dust and their extension into the environment S.O.E.H. (Dec. 1977), Washington (Vortrag).

Methods and results of measurements in ambient air

Prior to the measurements the suitability of different sampling instruments (e.g. thermalprecipitator, Andersen-impactor, electro-precipitator) was examined. It was then decided to use a sampling device with a Nucleporefilter (o,4 /um pore-diameter). Measurements were carried out in Düsseldorf and in Krahm (Bergisches Land, which is about 8o km apart from the nearest city). The measuring period lasted from Jan.75 - July 76 in Düsseldorf, but only a few months at the rural location. The results are given in figures 3 and 4. The fiber concentration in Düsseldorf was found to be in a range between $1o^4$ and $1o^5$ fibers per m^3, about 24% of the fibers being asbestos and 1% glass or mineral wool. In Krahm the fiber concentration was found to be in the range of $1o^3$ f/m^3 (asbestos content ~ 4%). The measurement of the length- and diameter-distribution suggested, that most of the fibers were shorter than 5o /um and thinner than 2 /um resp.

Conclusions

In this part of the study the determination of fiber concentration and the measurement of size distribution of fibers in human lung dust and in ambient air was performed by means of SEM. It seems to be necessary to find out further information about the chemical composition of the many different fibertypes in ambient air, which only could be obtained from SAED- and EDXA-analyses with the aid of an analytical TEM.

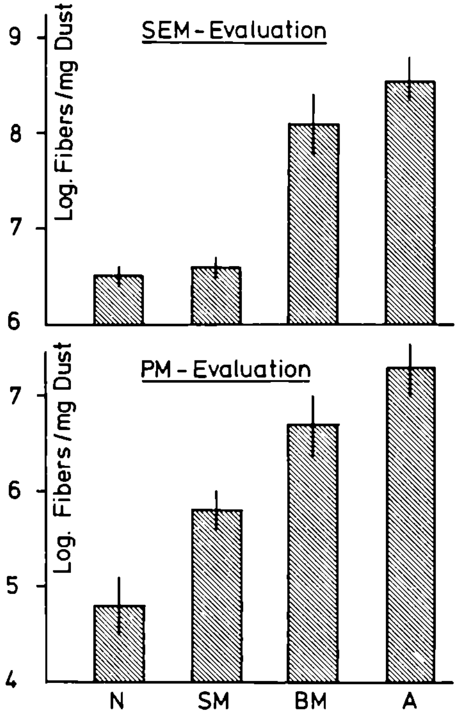

Figure 1: Geometric mean and standard-deviation of fibernumber per mg lung dust in four different groups
N = normal lungs, SM = spontaneous mesothelioma cases, BM = mesothelioma cases caused by occupational asbestos exposure, A = asbestosis cases (with/without BM)

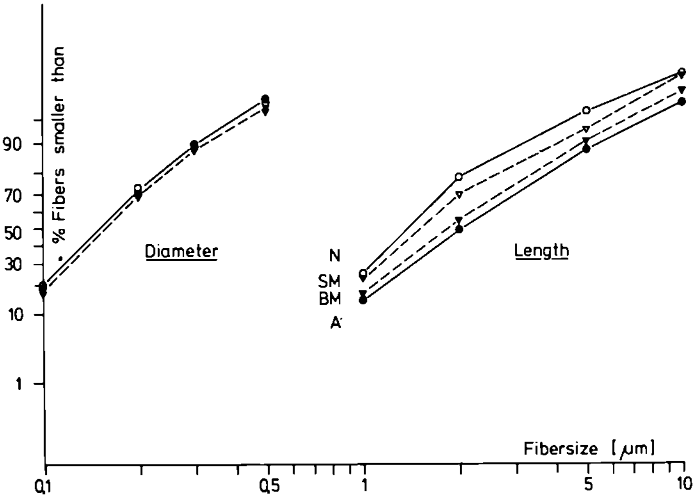

Figure 2: Size-distribution of fibers in human lung dusts SEM-analysis, magnification 1o ooo x.

--- normal lungs and SM-cases (without occupational asbestos-exposure)

___ BM- and asbestosis-cases (with occupational asbestos exposure)

Month	Fiber-Concentration (P/m^3)		
	Asbestos	Glass	Total
Jan	22614	6581	66072
Feb	16075	696	65050
March	14206	-	45753
April*			
May	10418	276	50780
June	12003	463	48868
July	8612	501	40509
Aug*			
Sept	253	88	9035
Oct	31416	679	120545
Nov	7665	286	50504
Dec	9382	139	31017
Mean	13264	1079	54813
Jan	35480	196	130039
Feb*			
March	12899	553	77526
April	9436	126	39825
May	21054	88	74260
June	7006	-	26598
Mean	17175	241	69650

* No data available

Figure 3: Fiber-concentration in Düsseldorf (Jan.1975-July 1976) SEM-evaluation of NPF-samples, magnification 5o ooo x.

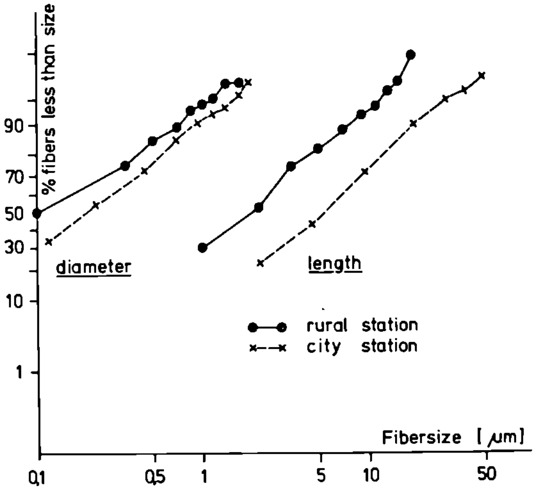

Figure 4: Size distribution of fibers in ambient air (City station in Düsseldorf, rural station in Krahm, Bergisches Land), SEM-evaluation of NPF-samples.

Contractor : Justus Liebig University, D-6300 Giessen

Contract n° 298-78-1 ENV D

Project Leader : Prof. Dr. H.-J. Woitowitz, Institute of Occupation
and Social Medicine of the University

Title of project : Epidemiological investigations on the fibrogenic
risks after exposure to asbestos cement fine dust
on building sites

OBJECTIVE OF THE RESEARCH

Each year in the Federal Republic of Germany between 100.000 and 120.000 tons of asbestos are converted into about 1.5 million tons of asbestos cement products. These products can be broken down as follows : asbestos cement sheets (approximately 80%), asbestos cement pipes (15%) and shaped parts (4%). The objective of the research is to determine both the incidence of dust during handling asbestos cement products on building sites and the possible health hazard resulting therefrom. A considerable proportion of the asbestos cement sheets is handled on building sites by the roofing companies established in the Federal Republic, which number some 6.000 and which have a total labour force of about 50.000. This relatively large group of persons subjected to asbestos fine dust exposure levels which cover a wide area ranging from exposure at the working place to environmental exposure seems particularly suitable for working out the relationship between the dose rate and, in the first instance, the fibrogenic consequences of inhaling asbestos fine dust. As far as the status of knowledge is concerned, it is assumed that, where a fibrogenic asbestos risk exists, one must also reckon, at all times, with a carcinogenic asbestos risk. In any further assessment of the tumour risk, the cross-section study should be enlarged to take the form of a longitudinal study.

MATERIALS AND METHODS

Initially, this report will confine itself to dust measurements in respect of roof coverings incorporating asbestos cement sheets.
Four portable and four static samplers were available for dust-measuring purposes (Table 1).

Table 1

In addition, a tyndallometer (TM digital) was used to record the time response of the fine-dust concentration. Measurements were taken of the fibre concentration and of the mass concentrations of fine dust as well as total dust. Both a phase-contrast and a scanning-electron microscope were used to count the fibres. Fibres were identified under the optical microscopy by means of the index of refraction and under the scanning-electron microscope with the help of electron microprobe by elemental analysis.

	Type of appliance	Manufacturer	Pore diameter [µm]	Detection of dangerous substance
Stationary	<u>Fibre concentration</u>			
	Nuclepore sampler	Self-produced after SPURNY	0,4$^{a)}$	SEM, MP
	<u>Fine dust</u>			
	VC 25 sampler	Sartorius, Göttingen	8,0$^{b)}$	IR
	TM digital	Leitz,Wetzlar	not applicable	not applicable
	<u>Total dust</u>			
	GC 25 collector	Sartorius, Göttingen	Fibre filter$^{c)}$	not applicable
	Nuclepore collector	Sartorius, Göttingen	0,4$^{a)}$	not applicable
Personal	<u>Fibre concentration</u>			
	Fibre sampler	Casella,London	0,8$^{d)}$	Phaco
	SSG	Self-produced after RIEDIGER	0,4$^{a)\ d)}$	REM, MP
	<u>Fine dust</u>			
	Fine dust sampler	Casella,London	8,0$^{d)}$	IR
	<u>Accumulated dust</u>			
	SSG	Self-produced after RIEDIGER	0,4$^{a)\ d)}$	not applicable

Filter material: a) Polycarbonate; b) Cellulose nitrate;
c) Polystyrene; d) Cellulose acetate.

<u>Table 1</u> : Where the roof is covered with corrugated asbestos cement sheets stationary and personal dust samplers are used to measure the fibre concentration and also the mass concentrations of fine dust and total dust.
MP = microprobe (elemental analysis) ; IR ≙ infrared spectrography ;
Phaco ≙ phase contrast microscopy, suction rates from 0,1 to 2,3 m/sec.

For the purpose of determining the asbestos content of the fine dust by infrared spectrography, after sampling with the VC 25 the fine dust ring was ashed at 430° C. However, comparative measurements by the Staubforschungsinstitut (Dust Research Institute), Bonn, showed in this instance that the concentrations, which according to our figures amounted to between 5 and 10% chrysotile in fine dust, were too low and that in reality they amounted to between 10 and 12%. Comprehensive annealing tests showed that with asbestos cement, unlike pure chrysotile absorption is considerably attenuated even at temperatures below 400°C. The ashing procedure of the filter substance described in the literature is therefore not possible and the analytical process developed in the Dust Research Institute - dissolving the filter substance in acetone - must be employed.

PRELIMINARY RESULTS

Past experience on building sites, the questioning of a number of firms and the preliminary anamneses of construction workers reveal the following :

- The laying of corrugated asbestos cement sheets in the Federal Republic of Germany began on a fairly large scale in about the early 1950s.
- By far the greatest amount of dust is raised when cuts are made in corrugated sheets using a grinding machine.
- Such cuts are made as a matter of routine on the sheet-corners which overlap on the roof.
- Occasionally, smaller sheets ("Berliner Welle") are also delivered to the building sites rough-drilled and rough-cut. In such cases the only actual additional cuts are made for matching-up purposes.
- According to the workers, the duration of exposure during these cutting processes ranges from under 30 minutes to over an hour a day, depending on whether the work is carried out on stacks of sheets or on the individual sheets on the roof.

In the main, the emission of asbestos cement fine dust occurs during the work with the grinding machine. The corners of asbestos cement sheets may be cut away in the stack prior to laying. Alternatively, this cutting work is carried out directly on the roof. The observed cutting periods take up between 3 and 13% of the working time (median : 6%).

Table 2 shows the results of measurements of the fibre concentration and also the mass concentrations of fine dust of asbestos cement for stationary and personal dust sampling during cutting processes with the grinding machine.

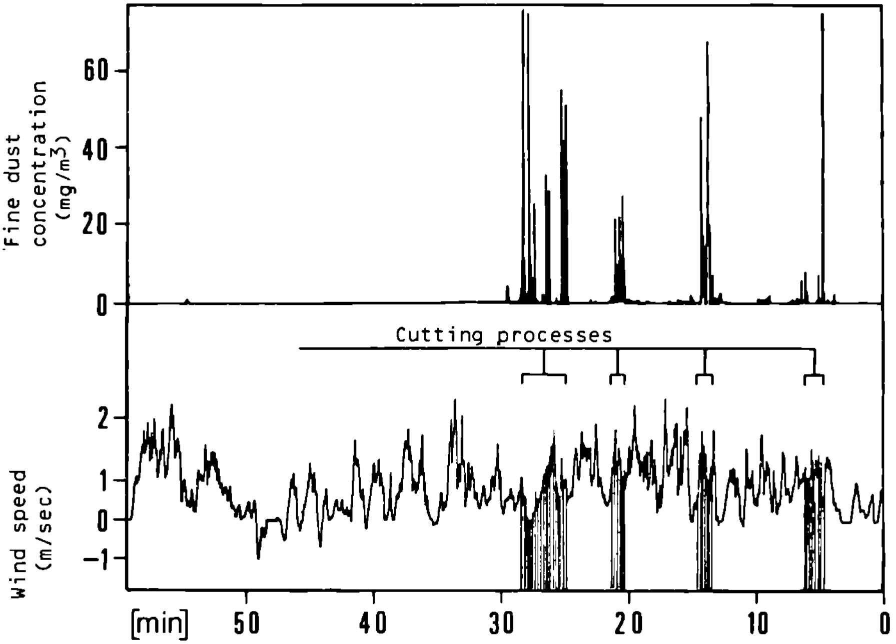

Fig. 1 : Time response of the fine dust mass concentration in the case of a roof covering with asbestos cement sheets at an open cutting location. In order to estimate the fine dust mass concentration, the tyndallometer in the dust tunnel of the Dust Research Institute of the Main Association of the Industrial Employers' Organizations, Bonn (Hauptverband der gewerblichen Berufsgenossenschaften e.V.) was calibrated with asbestos cement dust. While the grinding machine is switched on, the wind signal, which is also logged, is short-circuited approximately every 10 seconds, in order to mark the cutting processes.

	Sample	n	Median	Range
Fibre concentration	Fixed-location (REM)	10	0,94$^{*)}$	0,15 - 1,51$^{*)}$
	Personal (Phaco)	7	0,95$^{*)}$	0,03 - 1,78$^{*)}$
Mass concentration	Fixed-location (VC 25)	11	0,37$^{**)}$	0,1 - 0,78$^{**)}$
	Personal (Casella)	11	1,39$^{**)}$	0,5 - 5,8$^{**)}$

$^{*)}$ Fibres/cm^3 (length > 5 µm) $^{**)}$ mg/m^3 fine dust

Table 2 : Fibre and fine dust mass concentration in the case of roof coverings using corrugated asbestos cement sheets. The personal sampling for the analysis of the fibre concentration was carried out on the roof on workers who were not themselves engaged in cutting operations (bystanders). Stationary measurements in respect of the fine dust mass concentration were taken at fixed cutting locations and personal measurements were taken in the case of workers engaged in cutting operations. In IR spectrography terms the chrysotile content of the fine dust is about 10%.

CONCLUSIONS

On the basis of measurements to date involving stationary sampling both the fibre concentrations and the fine dust mass concentrations amount in the median to about half the Technical Standard Concentration (Technische Richtkonzentration - TRK) = 0.1 mg/m^3 or 2 F/cm^3 (> 5 µm length). On the other hand, in the case of personal dust sampling on workers engaged in cutting operations readings of between 0.5 and 6 times the TRK value were recorded (median : 1.4). It can be concluded from the foregoing that :

1) In the case of peak asbestos cement fine dust exposure on building sites, the results using personal dust samplers show that the actual dust pollution level to which employees are subjected is about three times higher than in the case of using stationary samplers.

2) Admittedly, the peak concentrations only take place for recurring short periods (seconds - minutes) and for a total of between 3 and 13% of the operating time.
Nevertheless, the possibility remains that the TRK value may be exceeded.

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Contractor : BUREAU DE RECHERCHES GEOLOGIQUES ET MINIERES

Contract : 264.77.6 ENV F

Project Leader : Jean Bignon
Centre Hospitalier Intercommunal
Université du Val de Marne
40, Avenue de Verdun
94010 CRETEIL CEDEX

Juan Goni
Service Géologique National
B.R.G.M.
B.P. 6009
45018 ORLEANS CEDEX

Title of project : Cytotoxic effects of asbestos fibres in relation with their physico-chemical properties.

OBJECTIVE OF THE RESEARCH

The study of the toxicity of fibrous minerals necessitates the precise control of the physico-chemical properties of the particles used. Moreover, such a study must take into account the minerals evolution in the biological medium as well as their sorptive properties regarding toxic substances such as benzopyrene or SO_2.

Consequently, the research is comprised of two successive stages : the mineralogical study and the biological study.

For the biological experimentations and for collecting up the determining factors of the toxicity, the minerals were taken according to criteria of morphology, chemical composition and structure.

MINERALOGICAL RESEARCH

Materials and methods

a) Samples : The following are the batches of particles prepared and characterized :

- particles with a fibrous facies and a different chemical composition : chrysotile, amosite, crocidolite, nemalite, glass fibres
- particles having the same chemical composition and different structure : chrysotile and forsterite, quartz and glass fibres
- asbestos fibres of the amphibole group and of different chemistry : amosite and crocidolite.

The asbestos fibres are crushed to a nominal length < 20 µm, the glass fibres < 20 µm, the quartz to a diameter < 5 µm.

b) Preparation of the leached minerals : The dissolution of the asbestos by different biologically interesting acids was followed by photoelectron spectroscopy. Moreover, for a comparison with the crude samples, the amosite, chrysotile and crocidolite underwent a strong leaching by oxalic acid 0.1 N.

c) Physico-chemical characterization : All the following analyses were applied to the crude and leached minerals :

- Granulometric analysis
- Chemical analysis of the major and trace elements
- X-ray examination of the crystallinity and minority phases
- Measurement of the surface area and the infra-porosity.

d) Doping of the minerals : At the present stage of progress of the research, only SO_2 doping of the samples has been carried out. The crude or leached minerals underwent a sweep of weakly concentrated SO_2 in dry or wet medium ; the SO_2 adsorption is followed and recorded by flame photometer detection ; in addition, the desorption is obtained, by sweeping by gas current free of SO_2 ; this makes it possible to find out (among others) the quantities adsorbed from studying the adsorption and desorption curves.

Samples were prepared in the same way, in wet medium for biological experimentation only.

RESULTS

a) Leached minerals : The cinetic study of the behaviour of the main elements of these minerals during leaching gives the following order of stability :

amosite > crocidolite > chrysotile

The magnesium leaching of the chrysotile is progressive and over 85 %. The crocidolite and amosite are much more stable and the Fe and Mg leaching rates quickly attain their level.

After leaching, the minerals are washed and lyophilized.

The amosite and crocidolite keep their crystalline structure after strong leaching ; the surface area goes up from 13 to 26 m2/g for the crocidolite and from 12 to 19 m2/g for the amosite. On the other hand the chrysotile loses its crystalline structure ; the almost complete magnesium leaching increases the surface area considerably and this may reach 300 m2/g compared to 26 m2/g in the crude state.

b) Doping of chrysotile by SO_2 : The adsorption curves obtained on the crude and leached chrysotiles in dry and wet medium lead to the following first conclusions :

1) In a dry medium, the crude chrysotile adsorbs more SO_2 (4,2 mg/g) than the leached chrysotile (1,3 mg/g).
   The increase in the leached chrysotiles's surface area does not, therefore, bring about a higher sorptive capacity ; on the other hand, the disappearance of the brucitic layer and the magnesium must be directly connected to the sorptive properties.

2) The adsorption phenomenon is borne out in wet medium.

3) The leached chrysotile adsorbs less SO_2 in wet than in dry medium, whereas the opposite is noticed for the crude chrysotile.
   The adsorption values in wet medium are :
   - 5,7 mg SO_2/g for the crude chrysotile
   - 0,7 mg SO_2/g for the leached chrysotile.

BIOLOGICAL RESEARCH

Materials and methods

a) Haemolysis : Human red blood cells (RBC) and particles were prepared as described elsewhere (1). RBC were used at a final concentration of 0.5 %. Every minute, the reaction was stopped by addition of glutaraldehyde, then the samples were centrifuged. The degree of haemolysis was obtained by determination of the optical density at 540 nm. To compare the haemolytic activity of minerals, the following parameters were retained : initial velocity of the reaction (v_i), dose to obtain a v_i of 50 %, dose to obtain maximal haemolysis of 70 %. v_i was obtained by fitting the measured haemolysis with theorical curves.

b) In vitro release of enzymes from rabbit alveolar macrophages (AM). In vitro experiments were performed as described in ref. 1, except that alveolar washings were made under reduced pressure. After culture for 2, 4, 6, 8, 18, 20 and 23 hrs with particles the enzymatic activities were determined in the adherent cells and, after centrifugation of the medium in the non adherent cells and in the medium. In addition, a control culture without particles was included. There was a duplicate for each experiment, three experiments were carried out for each particle. The enzymes were assessed as follows : lactate deshydrogenase (LDH) by determination of the rate of oxidation of reduced nicotinamide ademine dinucleotide, β galactosidase by the method of Conchie et al (2).

c) Mesothelial cells : Mesothelial cells from rat parietal pleura have been cultured in various culture media and with different substratum. 5 media differing in their content in amino acids, nucleotides were assessed (Eagle, Hams F 10, Hams F 10 + Vit C, NCTC 109). 4 trade marks of substratum were also used (Falcon, Linbro Costar, Corning).

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(1) Jaurand et al. Environ. Res., 1978, 17, 216.
(2) Conchie et al. Biochem. J., 1959, 71, 318.
(3) Light and Wei. Nature, 1977, 65, 537.
Congrès SFME. Nancy 23-25 Mai 1978.
Jaurand et al. Am. J. Pathol. (in the press)

RESULTS

a) Haemolysis : 3 types of fibres have been studied : chrysotile, crocidolite, nemalite. The effect of leaching with oxalic acid was studied with chrysotile and crocidolite. The effect of SO_2 sorption was studied with chrysotile. Quartz particles were also studied. The results have shown that chrysotile fibres were the most haemolytic particles. The data show that the activity can be ranked as untreated chrysotile ≃ SO_2 chrysotile > leached SO_2 chrysotile ≃ leached chrysotile > nemalite > leached crocidolite > quartz > crocidolite.

b) In vitro enzyme release from rabbit AM : Chrysotile fibres caused a selective release of β galactosidase. Leached chrysotile, unleached acid leached amosite, unleached crocidolite, glass fibers and nemalite caused only a slight increase in the amount of β galactosidase released. The LDH was release only with leached chrysotile, amosite, nemalite and crocidolite.

c) Mesothelial cells : The method used to obtain the growing cells was available to obtain long term cultures, it was as follows : mesothelial cells are isolated by scraping the parietal pleura. The explants so obtained are transfered in linbro cluster dishes containing 2 ml of culture medium NCTC 109, pH 7.2, supplemented with 10 % foetal calf serum, 100 U/ml penicillin, 50 µg/ml streptomycin, 1 % glutamin 200 mM. Subcultures were realized in Costar flasks (25 cm2) after trypsinization as standard method. 6 months old cultures are now still growing.

CONCLUSIONS

From these results, it can be seen that the most haemolytic particles are not the most cytotoxic. This fact can be due to the adsorption of proteins from the medium onto the fibres. However, when both effects were compared, there is a positive correlation between β galactosidase release and haemolysis and a negative correlation between LDH release and haemolysis. The acid leaching decreases the haemolysis by chrysotile and increases the haemolysis by crocidolite as observed by Light and Wei (3). In the case of chrysotile, the sorption of SO_2 modify slightly the haemolytic effect.

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Phagocytosis of chrysotile fibers by pleural mesothelial cells in culture.
Am. J. Pathol. (in the press).

Contractor : Environmental and Medical Sciences Division, AERE,
Harwell, Oxon OX11 ORA, UK.

Contract No. 277-77-10 ENV UK

Project leader : A. Morgan

Title of project : Deposition of inhaled asbestos fibres

Objective of the research

The objectives of this programme are twofold and may be summarised as follows:

1) To study the effect of fibre dimensions on regional deposition of inhaled fibres in experimental animals.

2) To study the effect of fibre dimensions on the clearance of fibres deposited on the alveolar region of the lung on body formation. This aspect of the programme is also being studied in experimental animals but the conclusions are being verified by the examination of human lung tissue obtained at autopsy.

Materials and methods

Radioactive tracer techniques have been exploited in our laboratory to study the regional deposition of inhaled fibrous material in the respiratory tract of the rat (Morgan et al., 1977). While tracer techniques are useful, in that they permit the accurate and non-destructive quantification of very small amounts of fibre in tissue samples, they have limitations. For example, the results are obtained in terms of the mass of fibre present in tissue and generally no information can be deduced as to whether this comprises a few long or many short fibres. If sized material was available, in which all fibres were of the same length, then the mass could be expressed in terms of fibre number but virtually all the materials examined to date (mainly the UICC standard reference samples) contain fibres covering a wide range of length and diameters. Another disadvantage of the tracer technique is that over long periods of time, or even short in the case of chrysotile (Morgan et al., 1971) radioactive constituents of the fibre can dissolve _in vivo_ so that measurement of radioactivity may not always provide a reliable guide to the retained mass of fibres.

To avoid the problems inherent in the tracer technique a method has been developed for measuring both the number concentration and the dimensions of fibres in lung tissue. In this, tissue is digested with hypochlorite (domestic bleach) and the lipids extracted with diethyl ether. An aliquot of the final suspension of fibre is filtered onto a membrane filter (Millipore GS, pore size 0.22 µm) which is washed and dried. A sector of the filter is placed on a microscope slide and cleared with a dimethyl formamide/acetic acid/water mixture (Le Guen, private communication). The cleared filter is mounted under a cover slip with Neutral Mounting Medium. The concentration (fibres mm^{-2} of filter) and the dimensions of uncoated fibres are measured at a magnification of x640 with phase contrast. Among workers using the membrane filter technique there is a concensus that fibres with diameters down to 0.2 µm can be detected. This means that with the thicker amphiboles (amosite and anthrophyllite) most fibres will be detected. With crocidolite a minimum of about 30% will be detected and, in general, chrysotile fibres will not be detected. This technique has been applied to studies of fibres in the lungs of rats, guinea-pigs and also in the human lung.

Results

1. Clearance of fibres from the rat lung and body formation

Using a digestion technique the clearance of anthophyllite fibres from the rat lung has been studied by Morgan et al. (1978) for a period of 6 months following inhalation exposure. This showed that short (< 5 µm) fibres were removed from the lung more efficiently than longer fibres and that fibres exceeding 50 µm in length were unable to be cleared from the lung by this route. More recently, measurements have been made of the length distributions of both uncoated and coated fibres in the rat lung at various times up to 2 years following exposure to anthophyllite asbestos. Combining the results with those obtained in the earlier experiment showed that the frequency of uncoated fibres < 5 µm in length fell from 70-80 per cent immediately following exposure to 19 per cent after 2 years. Fibres in the 5-10 µm range were also cleared but much less efficiently than the shorter.

A typical coated anthophyllite fibre, isolated from rat lung, is shown in Fig.1A. They are quite different in both appearance and colour to coated fibres isolated from the guinea-pig (Fig.1B) and human lungs. The coating is invariably discontinuous and is very pale yellow in colour. All types of amphibole fibre appear to form similar bodies in the rat lung. The uncoated and coated fibres in the lungs of rats killed at various times were classified into six length categories as follows:

< 4.9, 5-9.9, 10-19.9, 20-39.9, 40-79.9 and > 80 µm. The relative frequencies are shown in Fig.2 from which it can be seen that very few coated fibres occurred in the 10-20 µm range and the greatest frequency was invariably in the 40-80 µm category. In general, about 20% of the coated fibres were > 80 µm in length and no uncoated fibres of this length were detected.

It appears that the probability of a fibre becoming coated increases with its length. However, if the absolute frequencies of coated and uncoated fibres per unit area of filter are compared, it is apparent that, although the probability of a fibre > 80 µm in length becoming coated after 2 years residence in the rat lung is 100 per cent, the corresponding value for fibres in the 40-80 µm range is only 7 per cent. For the shorter categories the probability is invariably less than 1 per cent. A paper describing this work is in preparation.

2. Clearance of fibres from the guinea-pig lung and body formation

The analysis of lung tissue from guinea-pigs exposed by inhalation to anthophyllite is proceeding. Preliminary results indicate that short fibres (< 5 µm) are cleared less efficiently than from the rat lung. It has been reported by Vorwald et al. (1951) that the formation of asbestos bodies occurs much less readily in the rat than in the guinea-pig. We have shown that the reason is that much shorter fibres become coated in the guinea-pig lung, confirming the impression given in Fig.1. Virtually all fibres exceeding 40 µm in length become coated and the greatest frequency of coated fibres occurs in the 10-20 and 20-40 µm categories as against 40-80 µm in the rat. Appearance of coated fibres in the guinea-pig lung is much closer to that of bodies isolated from human lung.

3. Clearance of fibres from the human lung and body formation

A study of the concentrations and length distributions of uncoated and coated amphibole-type fibres in samples of human lung taken at autopsy has been made (Morgan and Holmes, in press). The cases included 10 who died with mesothelial tumours, 3 with lung cancer and 8 of other causes. The concentrations of uncoated fibres ranged from 0.1 to 370 x 10^6 g^{-1} dry weight and of coated 0 to 11500 x 10^3 g^{-1} dry weight. These concentrations are similar to those reported by other workers.

The relatively low frequency (mean 19%) of fibres less than 5 µm in length supports the contention that such fibres are cleared quite effectively from the human lung. The only exception was a case with asbestosis in which the frequency of short fibres was 72 per cent. This anomaly is probably a direct consequence of the asbestosis and the inability of fibres of any length to be cleared from fibrotic areas of the lung.

In general, the greatest frequency of fibres was found in the 5-10 µm range indicating that fibres in this category are cleared less efficiently than shorter ones. In some subjects, no uncoated fibres longer than 40 µm were detected but in others, about 15 per cent of the fibres fell into this category. Pooley (1978) reported an upper limit of 40 µm for fibres of crocidolite and amosite in human lung but it is clear from the present work that longer uncoated fibres may be present in some cases.

The length distribution of coated fibres was quite different from that of uncoated. On average, less than 1 per cent of coated fibres were < 10 µm long. In the present study the greatest frequency of coated fibres is found in the range 20-40 µm but on average 10 per cent exceeded 80 µm. The longest coated fibre encountered was 360 µm long.

Conclusions

This work has demonstrated that short (< 5 µm) fibres are cleared more effectively than longer fibres from the rodent and human lung. It has also demonstrated that long fibres tend to become coated more readily than short. The probability of a fibre of a given length becoming coated varies from species to species. It is lowest in the rat, intermediate in man and greatest in the guinea-pig.

Acknowledgement

This work is supported, in part, by the Asbestosis Research Council.

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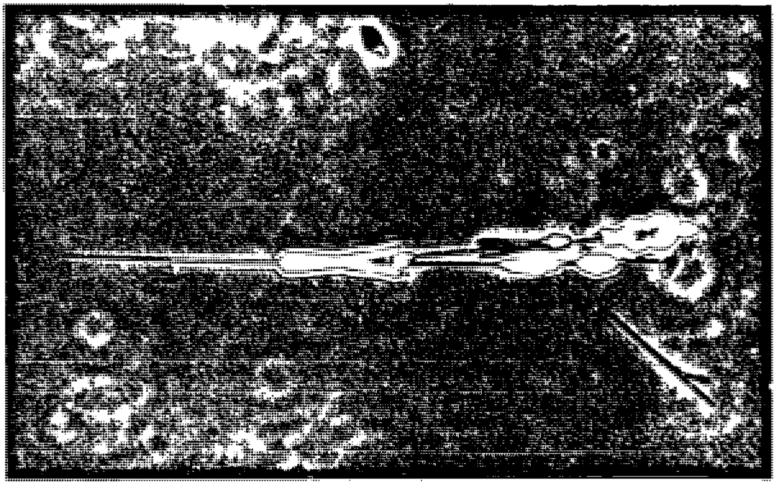

Fig. 1A Coated fibres isolated from the lung of a rat exposed to anthophyllite asbestos (x 820).

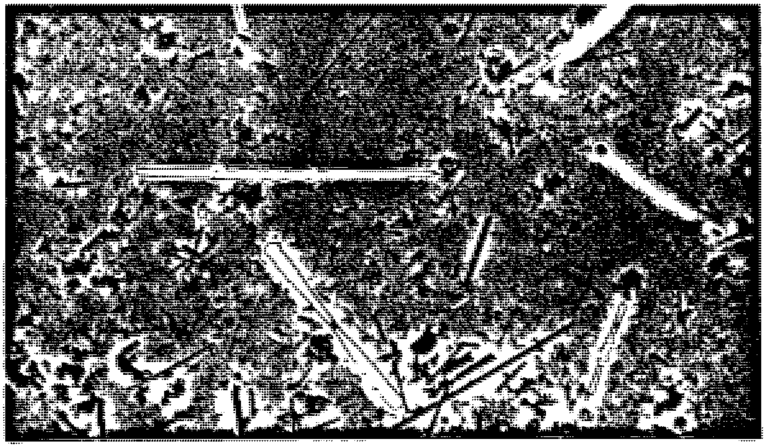

Fig. 1B Coated fibres isolated from the lung of a guinea-pig exposed to anthophyllite asbestos (x 820).

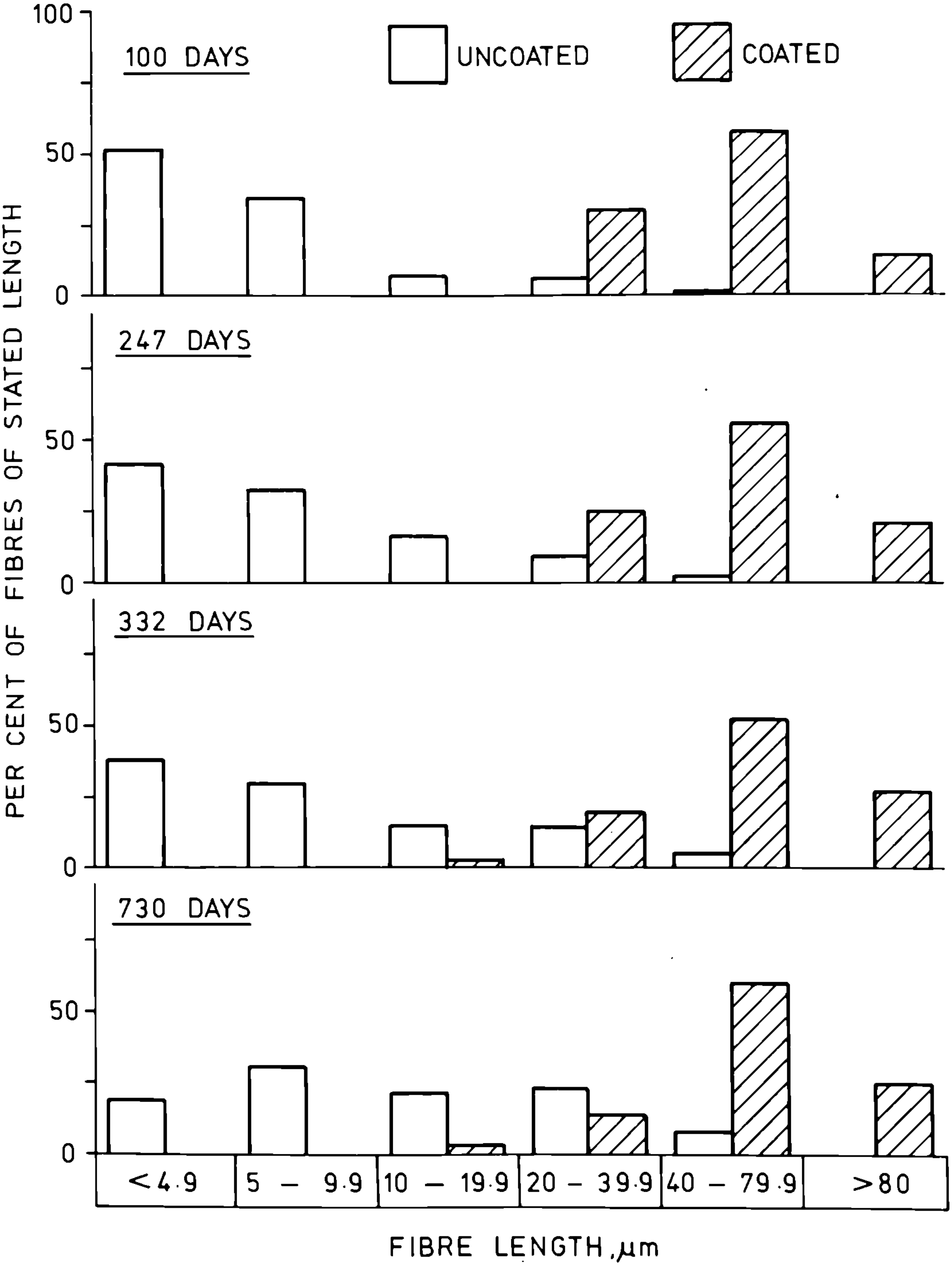

Fig. 2 Length distributions of uncoated and coated anthophyllite fibres in rat lung at various times after inhalation exposure.

Contractor :	International Research & Development Co. Ltd., Newcastle
Contract n° :	281-77-8 ENV UK
Project leaders :	R.W. Gale and J. Armstrong
Title of project :	Improved techniques for detection, measurement and identification of asbestos and other fibrous materials for use in the field of environmental studies

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INTRODUCTION

Present techniques for measuring potential asbestos hazards mainly rely upon the manual counting of asbestos fibres collected by drawing suspect air through membrane filters. The number of fibres counted on the filters, under specified microscopical conditions, is used to determine the fibre density present in the air.

Safe working limits (i.e. Threshold Limit Values) for asbestos are usually expressed in numbers of fibres per ml of suspect air. In the UK these limits are at present 2 fibres per ml of air for white asbestos types and 0.2 fibres per ml of air for blue asbestos.

The procedures for collecting membrane filter samples for analysis are well established and used internationally. However there is great and real concern that the subjectivity and interpretation involved in the present manual counting techniques result in considerable inaccuracy and poor reproducibility of measurement. The development of techniques and equipment which will result in improved accuracy and better reproducibility, without the inherent tedium and subjectivity involved in manual counting, is consequently of considerable importance.

The primary objective of this project has been the development of techniques and instrumentation for the automatic measurement of asbestos fibres present on membrane filter samples. The method is based on the alignment of fibres in a magnetic field, the subsequent illumination of the fibres in a collimated beam of light and the measurement of the variation in light scattered from the aligned fibres as they are rotated in the illuminated field.

BACKGROUND TO THE PROJECT

Dr. Vernon Timbrell of the MRC Pneumoconiosis Unit, Penarth, Wales, has for many years been studying the alignment properties of asbestos fibres in magnetic fields. He has shown that respirable sized fibres, when suspended in a suitable low viscosity liquid and subjected to a suitable magnetic field, align in ways characteristic of their type. This alignment and the quantity of fibres present can be assessed by monitoring the scatter pattern produced when directing a collimated beam of light onto the specimen.

Having recognised at the start of this project the considerable need for techniques and equipment which would eliminate the subjectivity and tedium of manually counting membrane filter samples, and being well aware of the potential application of Dr. Timbrell's basic fibre alignment/light scattering technology, IRD drew up specifications for instrumentation, microscope mounted if possible, based upon fibre alignment/light scattering, and suitable for the automatic measurement of membrane filter samples.

BRIEF SUMMARY OF PROJECT DEVELOPMENT STAGES

In order to produce, under controlled conditions, the large number of membrane filter air samples required, a special 'asbestos environment' chamber was constructed and used to obtain samples of many asbestos types.

Many different techniques were studied for aligning fibres collected on membrane filters - it being necessary to dissolve the surface of the membrane filters in order to release and suspend fibres for alignment in magnetic fields. It was also important not to add any complicated procedures to those employed routinely for rendering filters transparent for manual counting.

For measuring the alignment of fibres, several different light scattering systems were constructed. Using simple systems, the basic parameters required for measuring the amount of fibre present were obtained. These parameters were eventually incorporated in a microscope-based system which was then used to assess fully the practical applicability of the technique. A microprocessor unit was included to perform complex data processing and to present results in the most useful form.

PRESENT STATE OF DEVELOPMENT OF THE ASBESTOS MONITOR

A working model of a microscope-based automatic asbestos monitor has been constructed. The addition of a microprocessor data handling facility has greatly improved the usefulness of the equipment and enabled a large number of samples to be examined.

Techniques have been developed for aligning asbestos fibres collected on membrane filters in easily obtainable magnetic fields.
From exhaustive tests it is concluded that the developed techniques and equipment are capable of measuring the density of asbestos fibres collected from typical work places involving processing or handling asbestos.

DEVELOPMENTS ARISING FROM THE PROJECT

Vickers Instruments, co-sponsors of this project, are now developing this monitoring equipment as a much needed commercial product.

IRD have prepared specifications for the electronics, hardware and sample preparation. The equipment, which should be available on the market by 1980, will include in addition to automatic fibre measurement, a facility for manually counting fibres under regulatory conditions and for full identification of asbestos types from bulk samples.

FUTURE STUDIES AND REQUIREMENTS

The studies and developments carried out under this contract have opened up practical possibilities which will satisfy many of the urgent requirements in the understanding, monitoring and controlling of asbestos. Some of the most significant areas for study form a natural extension to the work already carried out and link magnetic alignment/light scattering of fibres with optical microscopy studies - a logical thing to do since the developed asbestos monitor is microscope mounted, and because optical microscopy is inexpensive, fairly simple to use and is already employed for asbestos monitoring in accordance with regulations.

IRD, following discussions with CEC personnel, are now submitting proposals for continuation of study in these and other important areas.

CONCLUSIONS

Techniques have been devised for aligning asbestos fibres collected from air samples on membrane filters. Equipment has been developed which automatically measures the quantity of fibre present on membrane filters by monitoring the scatter pattern produced when light is incident on the aligned fibres. The equipment has been built onto a conventional microscope and employs many basic microscope features. The incorporation of a microprocessor unit has enabled more automated machine control and data handling, resulting in improved performance and operational ease.

The results of many experiments involving commercial asbestos types and a wide range of fibre concentrations have shown that the equipment and alignment technique can be employed to count asbestos fibres within the ranges usually encountered in samples taken from industrial atmospheres.

Vickers Instruments are now undertaking development of the equipment to a commercial product. The package will consist of:

(i) the microscope-based automatic fibre counter, including facilities for manual counting of fibres and full identification of bulk material; and

(ii) a sample preparation kit consisting of magnet, alignment liquids, slides etc.

This equipment should be available by early 1980.

A copy of a newsletter outlining the equipment is annexed to this report.

Future studies, arising both from this project and from IRD's wider experience in the field of asbestos, are deemed necessary to fully exploit further practical applications of fibre alignment/light scattering, particularly when coupled with optical microscopy. These should enable greater understanding and improved evaluation of the potential health hazards associated with asbestos.

TOPIC 15 : AIR QUALITY

Effects of air pollutants on plants

Contractor : Research Institute for Plant Protection,
Binnenhaven 12, Wageningen, the Netherlands
Contract no: 297-77-8 ENV N
Project leader : Dr. A.C. Posthumus
Co-workers : O. Cleij, G.W.H. Laurens, M.S. Schrijver and
Drs. A.E.G. Tonneijck
Title of project : Research on the influence of different air pollutants, separately and in combinations, on agricultural, horticultural and forestry crops

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Objectives of the research

Several plant species have been fumigated artificially to study the effects of air pollutants in different ways. The observation of morphological symptoms, the establishment of dose-response relationships and the determination of possible, more hidden effects on physiological processes in the plants were the objectives of this research. The aim was also to study the influences of environmental conditions on the effects of air pollutants on plants. Especially to try to relate the results of laboratory studies with the field situations, a start was made to fumigate plants with combinations of air pollutants. It was also the purpose of these combination treatments to detect the possible interactions between the effects of different air pollutants in the total mixture. Results of this research should be applicable in the effect monitoring work with plants for the biological surveillance of air quality. Also in the practice of agriculture and nature management the knowledge of acute and chronic effects of air pollution on plants may be of great importance.

Materials and methods

Fumigation experiments with HF, PAN (peroxyacetyl nitrate), SO_2 and $SO_2 + O_3$ were carried out under experimental conditions in three types of fumigation chambers, all with the same system of a continuous air stream through the chamber, with or without added pollutants.

For fumigations with HF bulbs resp. corms of a sensitive variety of tulips and of a sensitive and a resistant variety of freesias were put in soil and grown up outdoors resp. in a climate room. After 6 weeks the freesias were fumigated with 0.54 2.00 3.75 or 4.80 $\mu g/m^3$ HF for

24 hours in a fumigation chamber for short-term experiments. After the fumigations leaf tips were harvested, quickly frozen in liquid N_2 and freeze-dried, also from control plants in an identical chamber without HF. After 120 days corms of freesias were harvested in the same way. From all dry material enzyme extracts in buffer solutions were prepared and enzyme activities were determined in a standardized way.

To study the response of little stinging nettle to PAN, plants were grown in soil in a climate room. Fumigations were carried out on 5 weeks old plants in a specially designed chamber, during 3 hours in the morning or 3 hours in the afternoon, with 0.125 0.250 0.375 or 0.500 mg/m^3 PAN. Experimental conditions were 74 or 37 W/m^2 of light and 40 or 70% relative air humidity, while temperature was kept between 20 and 25°C. After 1 week the intensity of leaf injury was estimated in classes, and dry matter weights of main shoots, lateral shoots and roots were determined.

Plants of 16 species, grown in a greenhouse, were fumigated for 2 weeks with different concentrations of SO_2. These fumigations were performed on young plants in a special greenhouse for long-term experiments. Control plants were grown during the same time in an identical greenhouse, ventilated with charcoal-filtered air. Except for the one with 200 $\mu g/m^3$ SO_2, all experiments with 400, 600 or 800 $\mu g/m^3$ SO_2 were repeated once.

Young plants of 7 species, grown in a greenhouse, were fumigated with SO_2, O_3 or $SO_2 + O_3$ while control plants were grown in charcoal-filtered air, in order to determine combination effects. These fumigations were carried out for 2 weeks in 4 greenhouses for long-term experiments. SO_2-concentrations varied from 400 $\mu g/m^3$ in the first 2 experiments to 300 $\mu g/m^3$ in the last 2, while the O_3-concentration was always 75 $\mu g/m^3$. Leaf injury was estimated for all plant species, dry matter production and enzyme activities were determined for some ones.

Results

HF did not influence activities of alkaline phosphatase, glucose-6-phosphate dehydrogenase and pyruvate kinase in leaves nor in corms of freesias. Enolase activity was decreased in leaves of both freesia varieties but not in the corms. In leaves peroxidase was activated in some cases, while it was significantly decreased in corms of the resistant variety Rose Marie, but more in those of the sensitive variety Royal Blue with a reduction of at least 50%. Dose-response relationships were not found, probably because of differences in physiological stages of the plants. In one experiment with the sensitive tulip variety Blue Parrot 7.2 µg/m^3 HF also decreased enolase activity in leaves up to 40%, while peroxidase was not affected.

Fumigations of little stinging nettle in the morning at 74 W/m^2 of light and 40% relative air humidity showed that 0.125 mg/m^3 PAN was sufficient to cause leaf injury. In the afternoon at least 0.250 mg/m^3 was required, while for both fumigation periods injury increased with increasing PAN-concentration. An increase in relative air humidity resulted in more injury, while a reduction in light intensity did not influence the effect. Fumigations in the morning with PAN-concentrations exceeding 0.125 mg/m^3 reduced dry matter weights of all plant parts. An increase in shoot/root ratio has always been found. Fumigations in the afternoon were much less effective in this respect, as they were in causing leaf injury.

None of the 16 species fumigated with SO_2 was sensitive to 200 µg/m^3 for 2 weeks. Big mallow, pea and big plantain showed more than 10% leaf area injured after 400 µg/m^3 SO_2 and higher concentrations, while buckwheat, lucerne, red clover and cotyledons of spinach reacted only to 600 and 800 µg/m^3 SO_2. Milfoil, celery, endive, wild chicory, tobacco 'glutinosa', tobacco 'Bel W_3', little plantain, annual meadow grass and little stinging nettle were insensitive to all concentrations used. Measurements of enzyme activities indicated that peroxidase was easily affected by SO_2 in some plant species.

In the fumigations with SO_2, O_3 and SO_2 + O_3 buckwheat appeared to be rather insensitive to all treatments, while lucerne, tobacco 'Bel W_3', little plantain, big plantain, spinach and little stinging nettle showed a synergistic response to SO_2 + O_3 in at least one experiment. For tobacco 'Bel W_3', big plantain and little stinging nettle this synergism could always be found after 7 days of exposure, but sometimes not after 14 days. So, the length of the fumigation period can be an important factor in determining synergistic effects. Among the harvested plants especially big plantain showed a great reduction in dry matter weight after fumigations with SO_2 + O_3. In the same species a very high increase in activity of peroxidase was found after all treatments. Therefore, big plantain has been chosen for further long-term experiments with SO_2 + O_3, the more so, as this species showed a change in growth habit in the combination treatments.

General conclusions

- Environmental conditions during growth and fumigation of plants and physiological stages of the plants themselves interfere with the development of effects of air pollutants on plants.
- Besides effects as leaf injury air pollutants may also cause clear, negative effects on plant growth and dry matter production.
- Changes in enzyme activities due to air pollutants can be measured prior to visible leaf injury.
- Inhibition of enolase activity in leaves of monocotyledonous ornamentals may be a specific effect of HF-exposures.
- Changes in peroxidase activity are probably indirect rather than direct responses, and not specific for air pollutants.
- A good indicator plant for low levels of SO_2 has not yet been found.

Publication

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Oral communications

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Contractor: NATURAL ENVIRONMENT RESEARCH COUNCIL through its INSTITUTE OF TERRESTRIAL ECOLOGY
Contract No: 280-77-1 ENV. UK
Project Leader: Mr. J. N. R. JEFFERS
Project Title: EFFECTS OF AIR POLLUTION BY SULPHUR AND ITS DERIVATIVES ON PLANTS

I. OBJECTIVES of project, with an effective starting date of 1st January 1977:

(a) To measure seasonally and diurnally changing concentrations of SO_2, NO_x and O_3 in the atmosphere immediately above the canopy of a Scots pine forest in a rural locality.

(b) To quantify and characterize acid precipitation falling on a forest.

(c) To compare the amounts of S entering a forest by wet and dry deposition.

(d) To develop techniques for measuring the flux of SO_2 to forests.

(e) Having determined the inputs (a)-(d), begin to follow the fates and effects of sulphur pollutants in a forest ecosystem.

Objectives (a)-(d) are regarded as the first parts of an expanding programme which is concerned with mechanisms and may encompass effects on soils. In addition to being part of the EEC programme, the ITE project is regarded as a component of a loosely integrated but comprehensive national (UK) exercise.

II. MATERIALS AND METHODS

A. Sites: intensive

A laboratory for intensive monitoring was arranged within 6 km^2 of Scots pine (P.47) in Devilla Forest on the north side of the River Forth, central Scotland, an area with a mean winter atmospheric concentration of 30-40 µg SO_2 m^{-3}, which is sometimes augmented by emissions from an oil refinery and two local power stations, and from conurbations in the Forth/Clyde Valley.

Sites: others

Detailed records of wet and dry deposition have been taken at ITE stations at (i) the Bush Estate, near Edinburgh, and (ii) Banchory. west of Aberdeen. Additionally, raingauges are situated at 18 other locations in northern Britain.

B. Instruments (arranged collaboratively with the Warren Spring Laboratory)

Concentrations of gaseous pollutants have been measured at the intensive site at intervals of 10 minutes using Meloy SA 285-SO_2; Meloy OA 350-O_3; Thermo Electron 14-D-NO_x. The data, with others from an array of meteorological instruments, were passed to a logging system for subsequent computer analysis. Precipitation was collected in raingauges.

III. RESULTS

A. Concentrations of SO_2, NO_x and O_3 above a forest canopy

(i) Seasonal variations: amounts of SO_2 and NO_x were maximal during winter, whereas those of O_3 reached a peak in spring (Figure 1).

(ii) Daily variations (ranges of concentrations were significantly greater than those describing seasonal variations; compare Figure 2 with Figure 1). In the absence of "ozone events", concentrations of SO_2 and NO_x were usually positively correlated ($r = +0.82$). At Devilla, with a mean winter SO_2 concentration of 30/40 µg m^{-3}, amounts of SO_2 have exceeded 50 and 100 ppb for 300 and 40 hours respectively, during a period of eighteen months.

Ozone concentrations usually ranged from 10 to 30 ppb. Spring concentrations >40 ppb were associated with arctic air masses whereas photochemical events with concentrations >50 ppb were recorded on c. 20 days in both 1977 and 1978. These concentrations were 'confirmed' by the production of necrotic lesions on leaves of tobacco (part of a scheme co-ordinated by Drs. Bell and Ashmore of Imperial College, London).

B. Nature of precipitation

pH and concentrations of sulphur and magnesium were measured in samples collected at 17-20 localities in northern Britain, measurements of magnesium being used to assess "excess sulphate". Ninety per cent of sulphur in rain in north-west Scotland was of oceanic origin, whereas it formed only 10-30 per cent in south-east Scotland (Figure 3). Precipitation was less acid in the west than in the east, where precipitation chemistry parallels that in southern Norway. Despite relatively large differences in amounts of precipitation and sources of S, the annual inputs of S carried in rain to different parts of northern Britain varied in the range of only 5-15 Kg $ha^{-1}yr^{-1}$.

C. Dry deposition relative to wet deposition

By assessing:

(a) wet deposition of sulphur in raingauges after deducting amounts of SO_2 and SO_4 trapped on the gauges by dry deposition,
(b) dry deposition of SO_2 by applying a deposition velocity (Vg) of $8.0 \times 10^{-3} ms^{-1}$ to atmospheric concentrations at 2 m above ground level, and
(c) dry deposition of particulate SO_4 by applying a VG of 6.0×10^{-4} ms^{-1} for particles in the diameter range 0.1-1.0 µm,

it was found in 1977 and 1978 that dry deposition of SO_2 accounted for c. 21.4 kg S ha^{-1} with c. 11.0 kg S ha^{-1} attributable to wet deposition: dry deposition of particulate SO_4 represented only one hundredth of the total. Dry deposition was relatively more important than wet deposition in winter than summer (Figure 4).

Estimates of dry deposition of SO_2 on snow in February 1978 suggested a Vg of $2.0 \times 10^{-4} ms^{-1}$.

D. Dry deposition to a forest canopy

Estimates of dry deposition already mentioned assumed a Vg to grass swards of $8.0 \times 10^{-3} ms^{-1}$. Attempts are now being made to obtain direct estimates of Vg to forest canopies in different conditions with flux measurements using a very rapid response analyser (0.1 second or better), a method developed at AERE, Harwell.

E. Path of S through a forest canopy

Since late 1978, the amounts and nature of throughfall and stemflow have been monitored. Already stemflow from Scots pine has been found to be more acid pH 3.2 than incident precipitation pH 4.0.

F. Effects of airborne pollutants on trees

Rates of erosion of epicuticular waxes on needles of Scots pine have been studied using a scanning electron microscrope: to minimize variation, attention was restricted to one provenance. On examining needles from two sites with mean winter SO_2 concentrations of 10 and 100 $\mu g\ m^{-3}$, it was found that the waxes eroded more rapidly at the latter site, the effect being greater on the photosynthetically active first-year needles than on the less active second- and third-year needles. Attempts are being made, in controlled conditions, to verify that these effects are attributable to SO_2, also to measure effects on cuticular diffusive resistance to water loss.

Increasing the acidity of simulated precipitation increased the amounts of potassium and calcium leached from tree leaves, ash leaves releasing more than those of birch and pine.

IV. CONCLUSIONS

Although it is necessary to obtain more annual sets of data to support those already obtained for objectives (a), (b) and (c), developmental work in the immediate future will focus on:

(i) the measurement of SO_2 fluxes to forest canopies
(ii) the relation between incident precipitation and the characteristics of of stemflow and throughfall
(iii) the effects on plant growth of small and usually chronic concentrations of 'naturally' occurring mixtures of atmospheric pollutants

The latter will be a logical extension of work already done when observing the erosion of leaf waxes. Essentially, field experiments will be done with and without filtered atmospheres.

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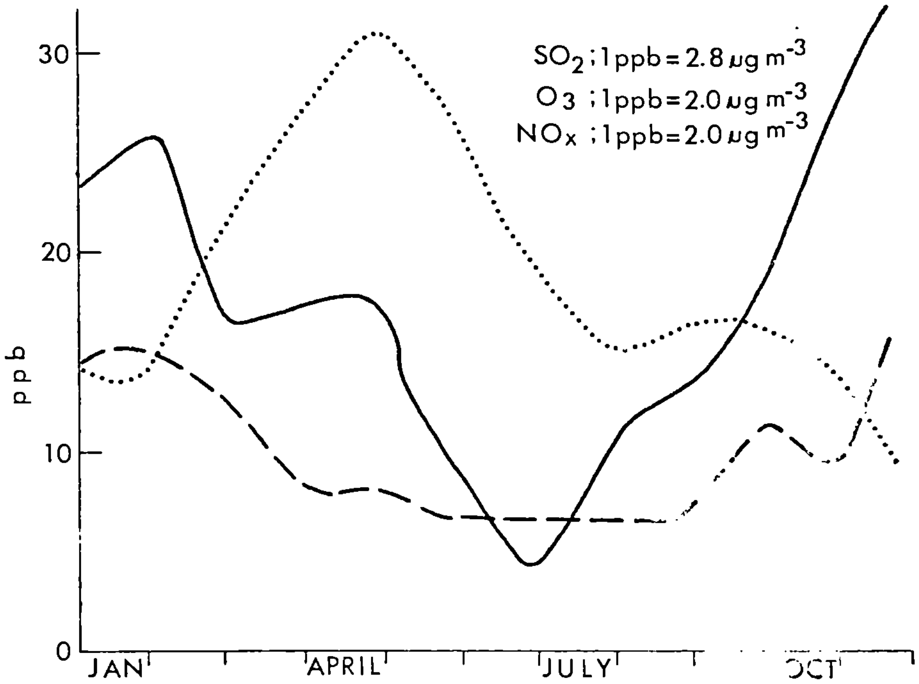

Fig.1. Seasonally changing atmospheric concentrations of SO_2(— —), NO_x (——) and O_3(······) measured 1m above Scots pine canopy at Devilla Forest, Central Scotland . 1978.

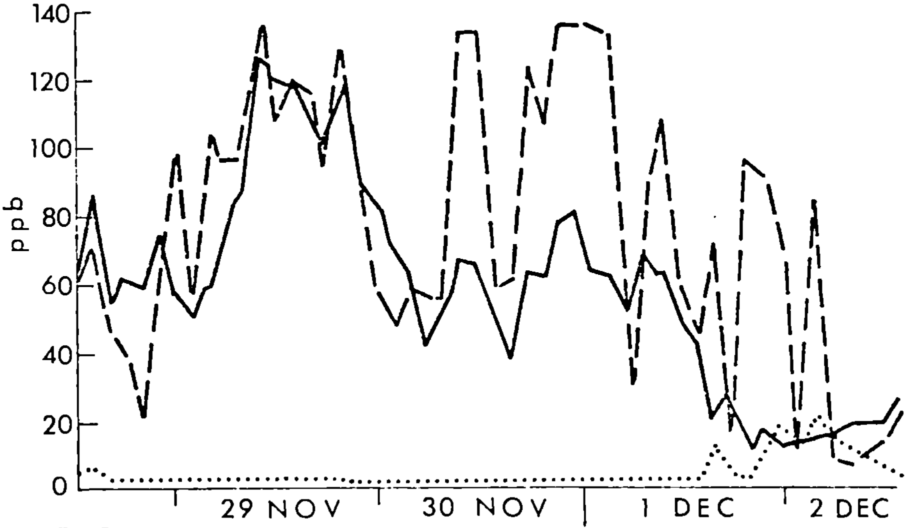

Fig.2. Diurnally varying atmospheric concentrations of SO_2(— —), NO_x(——)and O_3(.....)in the turbulent boundary layer above Scots pine at Devilla Forest, Central Scotland. (29Nov–2Dec,1977).

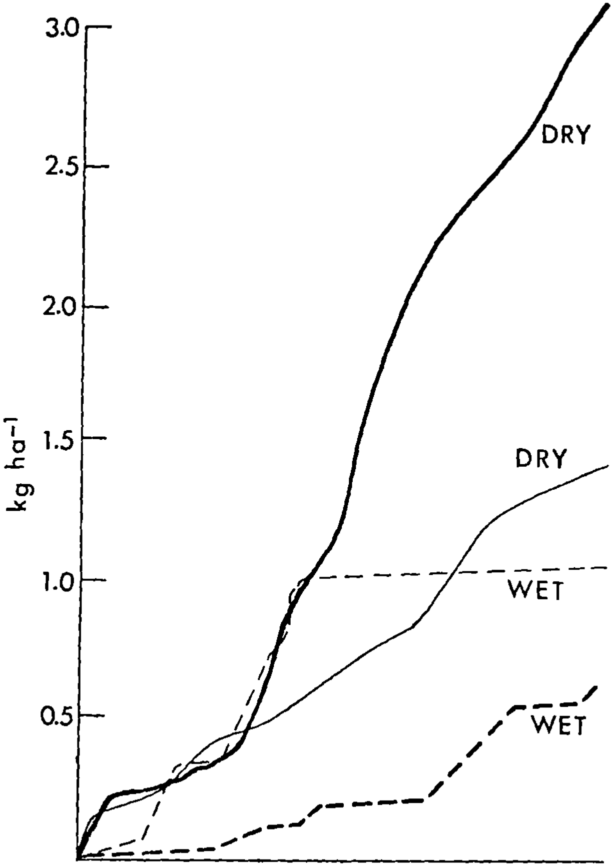

Fig.4. Amounts of sulphur accumulating by wet(– –), and dry(——) deposition in January (▬▬;▬ ▬ ▬) and June (——;– – –), 1977.

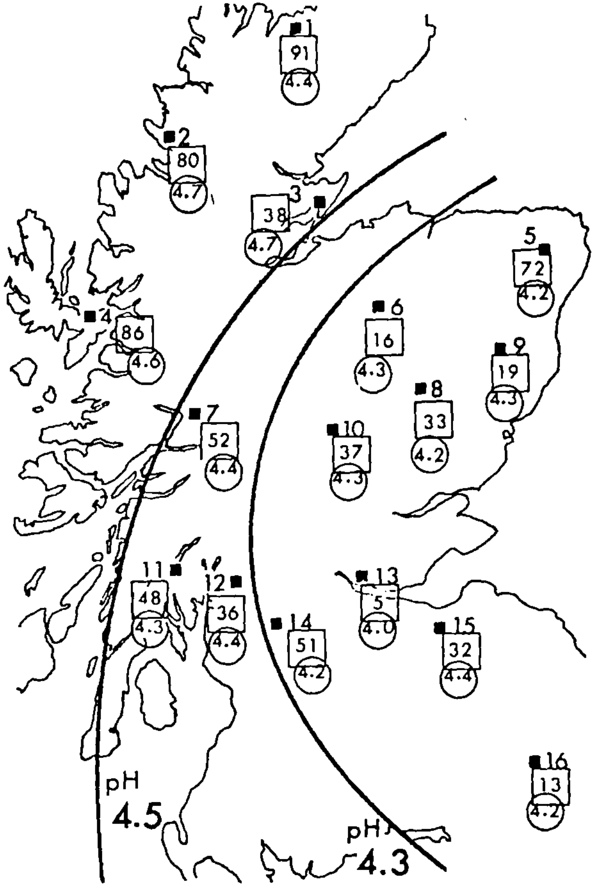

Fig.3. Annual mean pH, ○, of precipitation an the proportion, %, of marine sulphur at 16 sites (■ 1-16), in northern Britain in 1978.

TOPIC 15 : AIR QUALITY

Health effects of air pollutants

Contractors:	Krankenhaus Bethanien, D - 413 Moers Bundesgesundheitsamt, D - 1000 Berlin 33
Contract No.:	145-77-1 ENV D
Project leaders:	Dr. G. von Nieding / Dr. H.M. Wagner
Title of project:	Epidemiological studies of the relationship between air pollution and chronic respiratory disease Part 1: Exposure to inhalative pollutants (dust, SO_2, NO_2, and O_3) in the working area

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Objective of studies

It has been the objective of this part of the epidemiological studies to investigate whether in addition to dust, relevant concentrations of other inhalative pollutants were present in the working area and to find out whether the criteria of evaluation (clinical data, medical history and data on functional analysis) for chronic bronchitis and lung emphysema, respectively, could be related not only to dust but also to the pollutants mentioned.

Material and methods

Data on a total of 1087 male German workers of a metallurgical plant were evaluated. During the periods from 1966 - 1969 and 1973 - 1976 these test persons had been studied in respect of their clinical history and lung function within a study sponsored by Deutsche Forschungs gemeinschaft (DFG) (German Research Association) (1).

Test persons were examined in accordance with the questionnaire for the study of chronic bronchitis and pulmonary emphysema compiled by the High Authority of the European Communities in its 1966 version (2). This questionnaire includes queries on the medical and occupational history, and on smoking habits, present complaints, clinical findings, a description of chest X-ray and electrocardiogram results, and a documentation of the results of lung function analysis.

Additionally, periods of sick-leave during recent years were taken from sickness insurance data and evaluated by defined criteria.

During the period between May 1974 and March 1976, total particulates and fine particulates were recorded separately from 4 measurements performed at each of a total of 54 workplaces of test persons (except for workplaces of office employees) during 12-month periods each, in accordance with a fixed plan of measuring sites.

In February, March, and April 1977 all 54 workplaces were studied additionally for nitrogen oxides, sulphur dioxide, and ozone. Measurements were performed in accordance with the plan of sampling sites used for the particulate measurements. Only where the flow of operations suggested other measuring sites to be meaningful (e.g. in welding plants), deviations from the original plan were made.

Measuring sites and methods

Data were recorded for a total of 54 sites in the working areas 'blast furnace', 'steel works', 'machine plant/maintenance', and 'steel construction'. Following an inspection of the working areas, the measuring sites considered as representative of the former were established within a plan of measuring sites. At each site, a total of 4 measurements was performed in different seasons during a 12-months period. During a later phase, measurements at these sites were performed in the beginning by means of test tubes (DRÄGER) of the most sensitive measuring range for the presence of NO_x ($NO + NO_2$), SO_2, and O_3. This was performed for orienting purposes only. On the basis of the results of these measurements and the consideration of whether any exposure to gaseous pollutants was to be expected from the operation performed at the workplace in question it was decided to perform continuous automatic and manual measurements during an entire shift at 24 of the 54 workplaces studied. However, in contrast to the programme for particulate measurements, these measurements were performed only once and not 4 times per year. The workplaces that had only been subject to checking by means of DRÄGER tubes and not to measurements over an entire shift were rechecked by manual measurement once during the period of study. This did not result in the necessity of automated continuous measurement over an entire shift in any of these cases.

Particulate measurements in the working environment were performed by collecting samples with the VC 25 sampler and subsequent determination of the mass of total particulates on the filters (total particulates) and by the beta radiation absorption method (fine particulates) (3).

SO_2 concentrations were monitored by means of a Total Sulfur Analyzer (BENDIX Corporation). Since the underlying method of analysis was not entirely SO_2-specific, the analytic equipment was completed by an 'H_2S scrubber' (BENDIX). In addition, the SO_2 concentration values were checked by the standardized manual method (WEST and GAEKE, VDI Guideline 2451, sheet 3 (4)).

For continuous monitoring of NO and NO_2, the 'PICOS' apparatus (HARTMANN & BRAUN) developed by BREUER (5) was used. Its operation is based upon coulometric measurement. The apparatus was calibrated and checked before use and at certain intervals during operation by means of the colorimetric SALTZMAN method (6). NO and NO_2 were recorded in two different ways; as NO_2 alone and as $NO + NO_2$ by using an oxidant (DRÄGER).

O_3 concentrations were monitored by the highly specific and technically reliable photoluminescence method (BENDIX). The instrument was calibrated by the neutral buffered potassium iodide method according to VDI Guideline 2468 (7).

Clinical studies, medical history, and lung function analysis

In the study, the following parameters were recorded and used for evaluation.

- Age, body height, and weight
- Smoker/Non-smoker
- Period of work at the present workplace in days
- Symptoms such as cough, phlegm, and dyspnea
- Parameters of lung function analysis to evaluate lung volume

(vital capacity [VC], thoracic gas volume [TGV], respiratory gas exchange (arterial partial oxygen pressure [PaO_2] at rest and during exercise), and breathing mechanism (forced expiratory volume [FEV 1.0] in per cent of the rated value, bronchial X-ray resistance [R_{aw}]

- Total number of days of sick-leave, sick-leave for diseases of the broncho-pulmonary system and episodes due to diseases of the broncho-pulmonary system.

The sick-leave data were taken from sickness insurance records (total number of sick-leave days excluding periods referring to accidents and treatment of such accidents; number of sick leave days due to diseases of the broncho-pulmonary system such as bronchitis, common cold, influenza, pneumonia, pleuritis, or bronchial asthma excluding quinsy, tuberculosis of lung, and the figures for corresponding episodes.

Statistical evaluation and subdivision into groups

The data were stored on magnetic tape and calculated with the aid of a special evaluation programme (8) by means of a laboratory computer (PDP 12).

For the evaluation of effects, the test persons were subdivided int groups with dust exposure only and groups exposed to SO_2, NO_2 and O_3. Although some workplaces with dust exposure only were found, those exposed to noxious gases always exhibited a simultaneous exposure to dust of varying concentration.

The dust-exposed areas were subdivided into those with exposure to fine particulate concentrations of $\leq$ 1.0 mg/m^3 and of $\geq$ 1.0 mg/m^3 (groups 1 and 2).

Workplaces were considered as exposed if the mean pollutant gas concentrations of SO_2 $\geq$ 0.1 ppm, NO_2 $\geq$ 0.1 ppm or O_3 $\geq$ 0.04 ppm were present over the measuring period (mostly a full shift) (groups 3, 4, and 5).

The working areas were also studied for the presence of peak concentrations of the pollutant gases. Concentrations of $\geq$ 1.0 ppm for SO_2, $\geq$ 0.5 ppm for NO_2, and $\geq$ 0.2 ppm for O_3 with corresponding concentration dynamics were taken as the lower limit (groups 6 and 7). For technical reasons, peak particulate concentrations could not be measured.

Another group comprised working areas where there was a combination of particulates and NO_2 + SO_2 in concentrations as mentioned before (group 8).

The groups thus formed were compared with the group of office employees serving as control (group 9).

Results

Pollutant concentrations for groups 1 - 8 (Fig. 1)

Group 1 was exposed to a mean fine particular concentration of 0.43 mg/m^3 while group 2 was exposed to a mean fine particulate concentration of 2.48 mg/m^3.

Also, groups 3 - 8 were always exposed to dust, while the highest fine particulate concentrations of 4.25 and 4.96 mg/m^3, respectively were found for groups 6 and 8. Exposure was comparatively low for the groups comprising the working areas exposed to NO_2, NO_2 peak concentrations and O_3 (groups 4, 5, and 7). The fine particulate concentrations measured varied between 0.94 and 1.83 mg/m^3.

<u>Group 3</u> was exposed to a mean SO_2 concentration of 0.42 ppm (1.08 mg/m^3). This concentration was clearly above the IW 2 value of the German Ambient Air Standard (9) (0.15 ppm/0.4 mg/m^3) and below the MAK +) value (5 ppm/13 mg/m^3). NO_2 could also be demonstrated. Although its mean concentration of 0.3 ppm (0.54 mg/m^3) was low it exceeded the IW 2 value of 0.3 mg/m^3.

The value of 0.54 ppm (1.4 mg/m^3) SO_2 for group 6 that was exposed to peak SO_2 concentrations also exhibited a background burden that exceeded that for the 'pure' SO_2 group 3. Beyond this, SO_2 peaks exceeding 1.0 ppm (2.6 mg/m^3) at least 6 times during one shift were observed at the different workplaces of this group. In general, these peaks did not exceed 2.0 ppm (5.2 mg/m^3) SO_2. However, peaks of 70 ppm (182 mg/m^3) were reached in defined working areas of the blast furnace plant (e.g. at the slag trough). Also in this case, the mean NO_2 concentrations were low (0.06 ppm/0.11 mg/m^3).

<u>Group 4</u> was exposed to NO_2 only (mean concentration 0.16 ppm/0.29 mg/m^3). This value is approaching the IW 2 value of 0.3 mg/m^3 and is far below the MAK value of 5 ppm (9 mg/m^3).

The NO_2 background levels of 0.21 ppm (0.38 mg/m^3) of <u>group 7</u> that was exposed to peak NO_2 concentrations was somewhat higher than that of the 'pure' NO_2 group 4. NO_2 peaks above 0.4 ppm (0.72 mg/m^3) occurred at least 5 times per shift reaching maximal values of 1.8 ppm (3.24 mg/m^3). At the same time a mean SO_2 exposure of 0.22 ppm (0.57 mg/m^3) was present.

As a consequence of the welding operations performed at the O_3-exposed workplaces of <u>group 5</u>, O_3 concentrations always formed peaks that frequently surpassed the present MAK value of 0.1 ppm (0.2 mg/m^3) by a factor of 10. The integrated value over the entire shift revealed a mean concentration of 0.082 ppm (0.164 mg/m^3). Apart from dust exposure, there was a simultaneous low exposure to NO_2 (0.05 ppm/0.09 mg/m^3).

Except for the peak exposure groups, <u>group 8</u> meant the group of workers with the highest exposure to inhalative pollutants. This group also included 3 workplaces from group 6. In addition to a mean fine particulate exposure to 4.96 mg/m^3, mean NO_2 concentrations of 0.15 ppm (0.27 mg/m^3) and 0.69 ppm (1.79 mg/m^3) SO_2, respectively were found. Also, constant re-occurring peak-type SO_2 exposure was observed.

<u>Clinical observations, medical history, and functional analysis</u>

There was no significant difference between the mean values for groups 1 - 9 in respect of age, body height and weight, and smoking habits. Nevertheless, the fact was striking that certain groups, in particular those with elevated pollutant exposure included a higher number

+) MAK = approximate German equivalent for TLV values of smokers. The corresponding percentages were 83 for the control group of office employees and 93 for the groups that had been exposed to peak concentrations of SO_2 and NO_2.

Table 1 gives a synopsis of striking and significant differences between the exposure groups and the control group. Strikingly, the NO_2-exposed groups 4 and 7 did not exhibit any symptoms indicating bronchitis in addition to an elevated number of days of sick-leave being on the whole significant. Reduced FEV in association with a significantly elevated thoracic gas volume may indicate emphysematous changes. Generally, however, these two groups exhibited the least number of significant differences for the parameters studied.

Among the remaining groups, the more significant differences were found in groups 2, 3, 6, and 8 (elevated exposure). In addition to elevated pollutant gas concentrations, also the highest particulate concentrations were found in these groups. Group 5 (O_3 exposure) occupied an intermediate position.

When compared with the clinical data and medical histories, changes revealed by functional analysis were, on the whole, found more rarely. Largely, there was no uniform pattern that could be related to the individual pollutants.

It became evident from groups 8 and 9 in which the percentages of smokers were the same that the differences observed had to be attributed to factors other than low-degree differences in the numbers of smokers. The number of smokers in group 8 was the same as in the control group. Nevertheless, statistically significant and conspicuous changes were found most frequently in that group.

Conclusions

The results of the study revealed that in all exposed groups, there were clearly more symptoms of broncho-pulmonary disease, a higher number of sick-leave days, and impairment found by functional analysis than in the control group of office employees. It should be noted that the general frequency of symptoms such as cough, phlegm, or dyspnea was comparatively high also in the control group. In this respect, influences such as smoking or a high general environmental exposure to inhalative pollutants in the residential areas where test persons lived should be discussed.

Owing to the complexity of working conditions in a metallurgical plant, there have been certain restrictions to direct comparison or comparative toxicological evaluation of the individual groups that had been subject to different pollutant exposure. In addition to the inhalative pollutants, additional strain due to work at high temperatures outdoors or in open drafty halls but also possible differences in the pathogenicity of the inhaled particulates may have to be considered with regard to the development of broncho-pulmonary disease. These may be factors of identical action.

Exposure even to comparatively low concentrations of dust in the working environment appears to be an essential factor in the development of broncho-pulmonary disease. The present studies at least indicate also the presence of an additive effect of particulate and gaseous pollutants. In the opinion of the investigators, not only the concentrations of gaseous pollutants but also those of particulate nature in the working environment should be reduced.

Fig. 1 (for explanation see text)

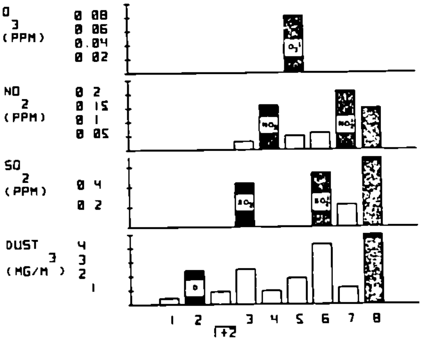

Table 1 (for explanation see text)

	D = 1	D 1	$\overline{SO_2}$	$\overline{NO_2}$	$O_3^{\wedge}$	$SO_2^{\wedge}$	$NO_2^{\wedge}$	$\overline{NO_2}$ $\overline{SO_2}$	CON-TROL
Group	1	2	3	4	5	6	7	8	
Epis. BP	xx	xx	xx	-	xx	-	-	-	
Days BP	-	xx	-	-	-	-	-	xx	
Days tot.	xx	xx	xx	xx	xx	xx	xx	xx	
Dyspnea	x	xx	x	-	-	xx	-	xx	
Phlegm	x	-	xx	-	x	xx	-	x	
Cough	xx	x	xx	-	xx	xx	-	xx	
PO2 exerc.	x	-	x	x	-	xx	x	x	
PO2 rest	-	-	-	-	-	-	-	-	
FEV 1.0	-	-	-	-	-	x	x	x	
Raw	-	-	-	-	x	x	-	-	
TGV	x	x	-	xx	-	-	xx	-	
VC	-	-	-	-	-	-	-	-	
n	414	125	139	83	110	131	58	96	110

x ≙ 1% < P ≤ 5%
xx ≙ P ≤ 1%

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Contractor : Laboratoire de Toxicologie de l'Environnement -
Université de Liège - Professeur D. RONDIA.
Contract n° 162-77-1 ENV B
Project leader : F. DEWIEST, Dr. Sc.
Title of Project : Experimental Study of the Factors Influencing the Availability and Biological Effects of Polynuclear Aromatic Hydrocarbons Adsorbed onto Soot Particles.

Objective of the Research

The soot particles resulting from combustion are rich in surface adsorbed polynuclear aromatic hydrocarbons (PAH) (1,2). The PAH present in these airborne suspended particles are essentially located on the particles having the finest diameter (less than 2 µm) (3,4). As a consequence of inhalation and retention of this particulate matter in the respiratory system, toxicity results from the effects of these PAH. Prior to integration into biochemical processes - carcinogenesis, mutagenesis, teratogenesis - PAH must be desorbed (elution) from their particulate support by the action of physiologic components. In this initial phase the object of the research is to clarify the principal factors controlling the elution process; this study is particularly aimed at the interfacial characteristics of the particles. Human blood plasma is used as the physiologic elution fluid.

Materials and methods

The following forms of particulate matter have been considered : (1) Samples of commercial carbon black (Thermax Floform); (2) Soot samples of incomplete combustion of natural gas and light fuel oil prepared in this laboratory.

After benzenic extraction of the original carbon black (type C_0) a sample is obtained (type C_1) which is easily oxidized by chemical means to modify its initial interfacial characteristics (type C_2). Artificial adsorption of pyrene, benzo(a)anthracene (BaA), 7,12-dimethylbenzo(a)anthracene (DMBA) and benzo(a)pyrene (BaP) is realized on coal types C_1 and C_2, and on previously extracted samples of soot.

Physicochemical characterization of the various samples considered is realized using appropriate techniques for the measurement of colloid interfacial properties (surface BET, electrophoretic mobility, coagulation kinetics and identification and measurement of surface active groups). PAH measurement is based on chromatographic, UV spectrophotometric and spectrofluorimetric techniques.

PAH elution is carried out at 25°C in dilute (20% V/V), buffered(phosphate buffer, pH 6,8) plasmatic medium with an ionic force J = 0.17 fixed by the addition of sodium chloride.

The elution percentages are defined as the ratio between the quantity of PAH desorbed and the quantity initially present.

Results

The principal results of the physicochemical characterization of the studied samples are summarized in the following table :

Physicochemical characterization of particulate samples.

Coal and Soot	A	B	C	D	E	F
C_0	381	$0,27.10^{-10}$	-25,4	-	10,5	0,25
C_1 enriched	45 to	$0,31.10^{-10}$	-33 to -39	-11,4	10,5	0,25
C_2 enriched	514	$2,48.10^{-10}$	-66 to -72	-17	14,5	0,25
Natural Gas Original	343to 641	$2,05.10^{-10}$ to 5.10^{-10}	-43 to -48	-14,8	156 to 200	<0,06
Fuel Oil Original	174	$0,25.10^{-10}$	-59	-16,8	213	<0,06

A : Superficial concentration of organic material (μgm^{-2})
B : Concentration of surface acid and hydrophilic groups (mole cm^{-2})
C and D : Electrokinetic potential (zeta) of double electric layer (mV) measured in isotonic non plasmatic medium (C) and plasmatic medium (D)
E : Specific surface (m^2g^{-1})
F : Particle diameter (μm).

On the basis of the plasmatic elution experiments effected on these various samples it can be concluded :

1. that within the limits of operational precision, the elution percentages of lots C_1 and C_2 artificially PAH enriched are pratically similar (elution percentage after 4 hours : C_1 pyrene 80.5%, C_2 84%; BaA C_1 76.5%, C_2 74.5%; DMBA C_1 77.5%, C_2 75%; BaP C_1 50%, C_2 56%) eventhough the particles clearly differ by the interfacial characteristics (degree of oxidation, nature and number of surface hydrophilic groups, structure of the double ionic layer and size of zeta potential in non-plasmatic media). These observations indicatethat the interactions between the carbon particles and the elutant proteins : (i) are not determined by processes of structuration and water autoprotolysis, nor by specific couplings of the hydrogen bond or polar association variety, (ii) are not controlled by repulsive forces of electrostatic origin; these facts imply that the values of the zeta potential of the

carbon particles must be clearly lower in plasmatic media than the values found in corresponding isotonic aqueous phase. The preceding table confirms this conclusion.

2. that the elution percentages of the original hydrocarbon matter present on the carbon black C_0 and on natural gas soot are very similar (type C_0 46% after 4 hours of elution; natural gaz 40%) despite clear differences in specific surface. In contrast, while their specific surfaces are similar, natural and fuel oil soot liberate the hydrocarbon components in very different proportions in plasmatic media (natural gas 40% after 4 hours of elution; fuel oil 22.5%).

   The polynuclear aromatic hydrocarbons artificially adsorbed on the carbon black are more easily eluted than those originally present on type C_0. The elution of artifically associated PAH occurs following this sequence :

   Pyrene > BaA ∿ DMBA > BaP

   It is seen that those PAH having four nuclei (pyrene, BaA, DMBA) are desorbed more easily than their homologues of greater dimension and molecular weight. The same sequence is observed with samples of fuel oil soot enriched with pyrene and benzo(a)pyrene (pyrene 40%, BaP 13% - Natural gas : Pyrene 42%, BaP 13% after 4 hours of elution).

   It is noted that those PAH artificially adsorbed onto gas and fuel oil soot are eluted with the same speed as opposed to the original hydrocarbon content. All of these facts demonstrate the predominant influence of thermodynamic surface factors which simulteneously control the state and degree of PAH binding with their particulate support as well as the structure of the adsorbed organic film (5).

3. that the PAH elution percentages are independent (i) of their superficial concentration (type C_2 enriched with pyrene : 1572 $\mu g g^{-1}$, 85% elution after 4 hours; 5910 $\mu g g^{-1}$, 88% elution). (ii) of the plasma concentration in the aqueous plasmatic extraction solutions (elution percentages after 4 hours : type C_1 enriched with BaA 72.8% (plasma 20% V/V), 76.2% (plasma 40% V/V); elution percentages after 24 hours : fuel oil soots 30% (plasma 20% V/V), 31% (plasma 40% V/V)) (iii) of the temperature (temperature range : 13-25°C).

Conclusions

The predominant factors which influence the processes of plasmatic elution are those which govern the nature and force of the bonds between the particle surface and the adsorbed PAH.

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Contractor: Medical Research Council
Contract No: 287-77-9 ENV UK
Project leader: Professor P J Lawther
Title of Project: The effects of SO_2, H_2SO_4 and particulate matter on lung function

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Objective

The main objective of the study is to investigate short-term changes in lung function following exposure to mixtures of pollutants liable to be present in polluted urban fogs. Attention was directed initially to sulphur dioxide and the present phase of the project is concerned with sulphuric acid. It was suggested many years ago that this might have been one of the pollutants responsible for acute effects on health observed in the 1930 Meuse Valley and the 1952 London episodes. Substantial concentrations of sulphuric acid were certainly found in some later London fogs, and recent laboratory work at Harwell has indicated that the rate of oxidation of SO_2 in fog droplets, with traces of manganese (from the suspended particulates) acting as a catalyst would be fast enough to account for the observed amounts of acid.

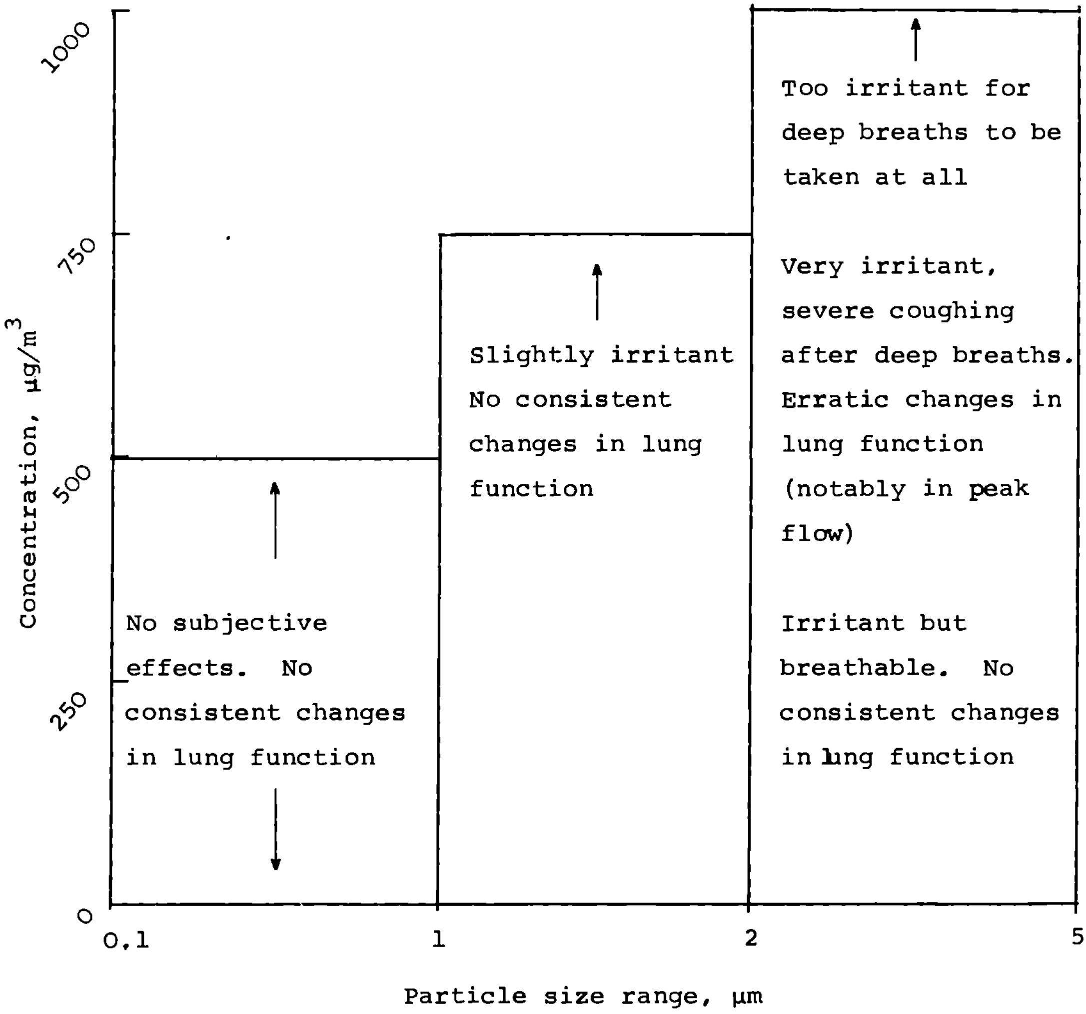
Materials and methods

The experiments are being done in an exposure chamber within the laboratory, using volunteer subjects drawn initially from the Unit staff. Aerosols of sulphuric acid are produced with Lauterbach generators and they are diluted with air at laboratory temperature to produce mixtures at the required concentration and particle size ranges. The amount of acid present is determined by titration of successive samples taken on filter papers. As a guide to particle size distribution, cascade impactor samples are also collected, using indicator-coated slides, but further observations are made with carbon-coated grids in a thermal precipitator, for examination on the electron microscope.

To minimize the total amount of aerosol required, the subjects sit in a small enclosure within the main chamber. Several inhalation procedures have been used, and in the present series a fixed number of deep breaths are taken voluntarily by mouth. To allow transient changes in lung function to be followed with minimum delay, a spirometer equipped with a tape recording system is used, within or close to the chamber. The tapes are read on the laboratory computer, yielding data on FVC and FEV_1, together with average flows over other segments of the spirogram, peak flow, and instantaneous flow at specified fractions of the vital capacity. Every test is preceded by a control experiment, with the subject taking deep breaths of laboratory air. Other lung function tests have also been used, including measurements of peak flow with the simple Wright instrument, and of airway resistance, using a fully automated body plethysmograph.

Results

After much preliminary work, satisfactory techniques have been developed for delivering concentrations of acid in the range 250-1,000 $\mu g/m^3$ either as a fine aerosol (with droplets in the sub-micrometre range, as measured at laboratory temperature and relative humidity), or as a coarser one (with droplets of several µm diameter). The size is controlled by varying the strength of acid in the generators, but as this affects the total output also, there is a finite upper limit to the concentrations of fine particles that can be reached with our present system.



The table above shows, in a qualitative manner, results from a series of pilot studies done to date. The extent of the variation in response between subjects, and even within subjects (when re-tested on successive days) is large, and until more experiments have been done the significance of changes in lung function cannot be assessed properly.

Conclusions

It is clear that in general, sulphuric acid aerosols containing droplets up to about 1μm in diameter are not irritant, even when inhaled deeply by mouth, and no consistent changes in lung function have been observed at concentrations up to 500 $\mu g/m^3$. As the particle size is increased, however, the aerosols become progressively more irritant until, with droplets in the range 2 - 5 μm, at concentrations of 1,000 $\mu g/m^3$ and above, the aerosols cannot be inhaled deeply at all, (although they become tolerable after a few minutes if the subject is able to persist). The former case corresponds in some respects with the situation in polluted urban fogs, but the latter would only occur in certain industrial circumstances (eg in lead-acid battery works).

The interpretation of these findings is probably related to the hygroscopic properties of sulphuric acid. The droplets take up water as they pass through the respiratory tract, so that those that are small enough to penetrate deeply will become dilute before they impact, whereas droplets large enough to impact in the upper respiratory tract will still contain relatively strong acid. The severe coughing produced, even by quite low concentrations of large droplets, could lead to adverse effects on lung function, particularly in patients with existing respiratory impairment. This could be of relevance to the effects of urban fogs, for in some of these it is possible for large acid droplets to be formed, even though the majority of the acid is normally in submicrometre droplets. The more important point to consider however is that in the air sulphuric acid is generally present in droplets containing other dissolved material, or it is absorbed on solid particulate matter, and this is liable to modify its hygroscopic properties. The simulation of such aerosols in the laboratory is a very difficult task, and further evidence of effects of the complete mixture of urban pollutants may have to be sought from actual outdoor exposures.

Publications

The experimental work in the series has proved to be extremely time-consuming, and it will be necessary to continue the work with further subjects before reaching firm conclusions for publication. It is hoped that this can be done in time to present a paper at the forthcoming International Symposium on Inhaled Particles at Cardiff in 1980.

Contractor : National Agency of Environmental Protection
Contract n° : 131-75-10 ENV DK
Project leader : H. Flyger
Title of project : Effects on pollution of a reduction or removal of lead addition to engine fuel

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Abstract

The effect of fuel, lubricant and engine parameters on the emission of polycyclic aromatic hydrocarbons (PAHs) associated with particles were studied by means of a 4-stroke, 4-cylinder, automobile engine operated under steady state conditions in a bench set-up.

Fluorescent polynuclear aromates, carbon monoxide, hydrocarbons and nitrogen oxides in the exhaust were monitored. After dilution and cooling of the exhaust, a fraction was passed through a cyclone battery and a filter, which collected particles of diameters above and below 1 µm, respectively. In two experiments vapour phase PAH was further collected in freeze traps mounted after the filter. The samples were analysed by high performance liquid chromatography with fluorescence detection for 7 PAHs : anthracene, fluoranthene, 1-methylanthracene, pyrene, benzo(a)anthracene, benzo(a)pyrene and benzo(ghi)perylene. More than 90% of the mass of the heavier PAHs (i.a. benzo(a)pyrene) were found on particles with a diameter below 1 µm. For the light PAHs a substantial part was in the vapour phase, but the particle-bonded fraction was predominantly associated with small particles.

The aromatic content, the type of aromatic fraction and the PAH content in the fuel had much affect on PAH emission in comparison to which the lead content of the fuel was of minor importance. The PAH content in unused lubricant was pratically zero and was found to increase proportionally with the time of use, giving rise to an increasing exhaust emmission of PAH. However, the amount absorbed in the lubricant was far greater than the total emission through the exhaust. PAH emissions varied with the air/fuel ratio in a manner similar to particulate matter and to unburned hydrocarbons showing high emission rates at very rich and very lean mixtures. The engine load was found to have much influence on PAH emissions both under steady and under unsteady state conditions.

Other participants in the research :

Jan Ingwersen and Peter Sunn Pedersen, Laboratory for Energetics, Technical University of Denmark.

Torben Nielsen and Elfinn Larsen, Chemistry Department, Risø National Laboratory.

Jes Fenger, Air Pollution Laboratory, National Agency of Environmental protection.

TOPIC 15 : AIR QUALITY

Remote sensing of air pollution, dispersion and modelling

Contractor: Fraunhofer-Institute for Atmospheric Environmental Research
Kreuzeckbahnstrasse 19
D-8100 Garmisch-Partenkirchen

Contract n°: 142-77-1 ENV D

Project Leaders: Dr. Reinhold Reiter and Dr. Walter Carnuth

Title of Project: Absolute Calibrated Double and Three-Frequency Lidar for Remote Aerosol Sensing

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1. Objective of the Research

The main objective of the research was the development of a mobile aerosol lidar system with 347, 530, and 694 nm transmitter wavelengths and calibrating it by comparison with in-situ aerosol data obtained at 750, 1800, and 3000 m altitude. Using different transmitter wavelengths it was expected to get information on both aerosol number concentration and size distribution. Simultaneously, measurements by a stationary lidar system with 347 and 694 nm wavelengths, which had been developed essentially under a previous contract (n° 013-74-1 ENV D), should be continued and the calibration status improved. This system is suitable for stratospheric measurements up to 35 km altitude in the 694 nm wavelength, using the photon counting method. The continuous monitoring of the stratospheric aerosol layer, which was started in 1976, should be continued.

2. Materials and Methods

2.1. The stationary lidar system

A detailed description of the stationary lidar system, which had been completed with a few exceptions at the beginning of the contract, is given in the references. System specifications are the following: ruby laser with frequency doubling, wavelengths 347 and 694 nm with peak output energies .12 and 2.o Joules, respectively, electromechanical chopper for fluorescence rejection, integrating energy monitor with three-digit decimal output, fixed, vertical pointing 52 cm dia. receiving telescope, interference, attenuation and polarization filters, photomultiplier tube for signal detection (EMI 9816 for analog measurements, RCA 8852 for photon counting), signal recording by Biomation 8100 transient recorder or ten-channel photon counter (64-channel counter under development), on-line minicomputer for real-time data handling and automatic control of the whole system.

The energy monitor is a new one, it has been developed last year in our lab for replacement of the former device with 4-bit resolution, which proved to be insufficient for the desired measurement accuracy.

2.2. The mobile lidar system

The mobile lidar system operates with a frequency-doubled neodymium-glass laser (wavelength 530 nm) in addition to a ruby laser used with and without frequency doubling (347 and 694 nm wavelength). The fundamental wavelength of the neodymium laser,1060 nm, is not used up to now because it requires special photodetectors, but preparations have been made for using this wavelength in the future. The backscattered light is collected by a 30 cm telescope, detected by an EMI 9816 tube and recorded by a Biomation 8100 transient recorder. Primary data processing is done by an on-line computer like that of the stationary system, but only semi-automatic operation is possible. Autonomous power supply is available. Transmitter and receiver are mounted on a two-wheel trailer, the electronics in a van. The output energy is measured by a high-resolution monitor of the same type as in the stationary system.

2.3. Equipment for aerosol- and aerological data acquisition

Aerosol size spectra are delivered by five-stage impactors at the institute (750 m) and at 1800 and 3000 m altitude, and Knollenberg optical particle spectrometers at 750 and 1800 m. Aerological data are acquired by different types of radiosondes.

2.4. Evaluation methods

Lidar calibrat~~ion factors~~ are calculated by an iterative method for solving the lidar equation which is of the integral type due to the extinction term. Backscattering cross sections are derived from the measured data using Rayleigh and Mie theories, where the aerosol refractive indices are chosen as functions of rel. humidity according to Hänel's data. The existing tables of Mie functions have been extended considerably for this purpose.
For the two-wavelength calculations (347 and 694 nm, stationary lidar) the aerosol size distribution is approximated by a two-parameter model, consisting of two log-normal distributions with constant center diameters

(.4 and 2 microns) and standard deviations and the total number concentrations as variables. The additional 530 nm wavelength of the mobile lidar is at present used for obtaining two independent particle size distributions. Through systematic variation of the refractive index of the dry aerosol the two distributions are fitted optimally to each other.

3. Results

The beginning of the routine measurements by the mobile lidar has been delayed by the above mentioned extensions until November 78. From the first obtained data the following set of calibration factors $A(\lambda)$ for the three wavelengths have been deduced, valid for photomultiplier high voltages of 1000 V, respectively:

$$A(694\ \mathrm{nm}) = 2.0 \cdot 10^{11}\ \mathrm{mV\ J^{-1}\ m^{3}\ sterad}$$
$$A(530\ \mathrm{nm}) = 2.75 \cdot 10^{11}\ \mathrm{mV\ J^{-1}\ m^{3}\ sterad}$$
$$A(347\ \mathrm{nm}) = 5.6 \cdot 10^{10}\ \mathrm{mV\ J^{-1}\ m^{3}\ sterad}$$

Profiles of size-dependent particle concentrations derived from mobile lidar data are not yet available, but will be presented in the detailed final report. Examples of normalized backscatter profiles obtained by both lidar systems are given in Fig. 1.

By the stationary lidar system numerous routine measurements were carried out during the contract period. After the installation of the new energy monitor the reproducibility seems to be better than before. Last values of calibration factors, again valid for 1000 V photomultiplier high voltage, are

$$A(694\ \mathrm{nm}) = 1.9 \cdot 10^{11}\ \mathrm{mV\ J^{-1}\ m^{3}\ sterad}$$
$$A(347\ \mathrm{nm}) = 7.86 \cdot 10^{10}\ \mathrm{mV\ J^{-1}\ m^{3}\ sterad}$$

The stratospheric aerosol measuring program has been continued since autumn 1976. The results are summarized in Fig. 2, showing isopleths of the particulate backscattering coefficient. For more details see Ref. (8).

4. Conclusions

The experiences gained during several years of work with multi-wavelength lidar systems showed that quantitative remote sensing of aerosols by lidar is possible within the limitations given by the laws of physics. Essential preconditions, however, are an as precise as technically possible measurement of the transmitted and received energies because of the special kind of error propagation through the data evaluation, and a careful calibration of the lidar by means of measurements within atmospheres of known aerosol constitution. An extension of the wavelength range into the infrared is desirable not only because of the resulting extension of the detectable particle size range, but also because of the increased accuracy of the resulting aerosol data with increasing wavelength and with constant initial measurement errors.

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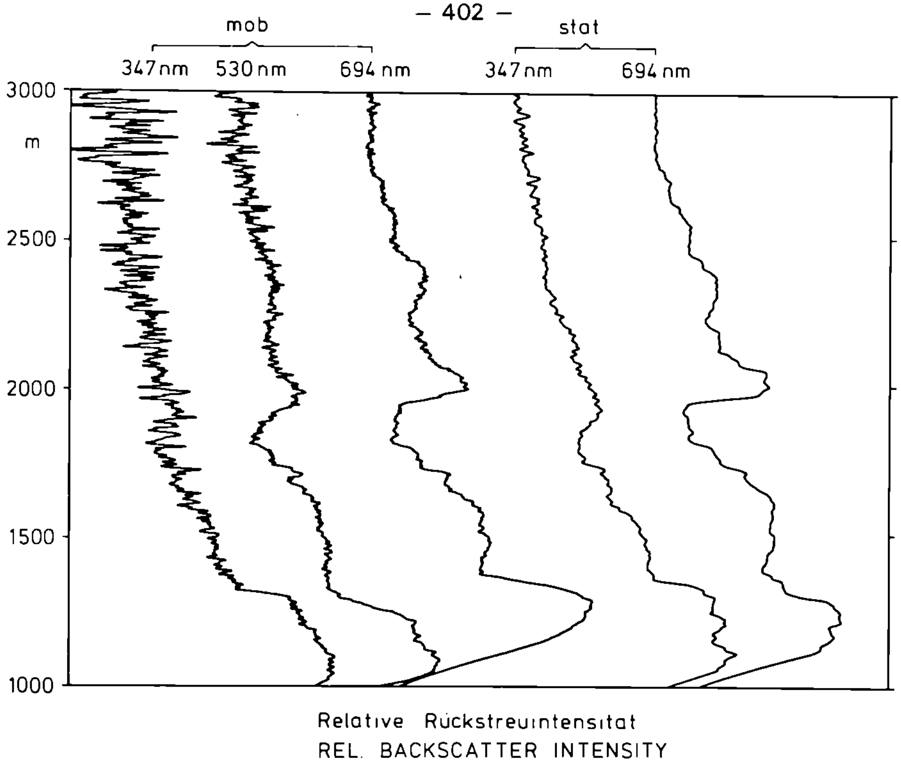

Fig. 1: Range-corrected and energy-normalized backscatter profiles obtained by the stationary and mobile lidar systems in the different transmitter wavelengths.

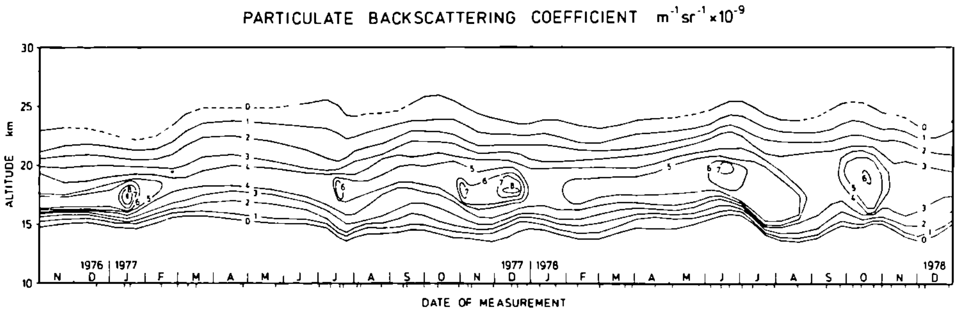

Fig. 2: Stratospheric particulate backscatter coefficient obtained by the stationary lidar.

Contractor : Nuclear Research Center - GRENOBLE

Contract n° : 189-77-ENV-F

Project Leader : M. ROUSSEL

Title of project : Differential infra red absorption long path monitor for SO_2 pollution.

I - ORIGINAL OBJECTIVE

The purpose of this study is to compare the laser differential absorption technique, which gives instantly an average value of the pollution on a given path with the classical methods of pollution analysis which give a time averaged value at one point.

It should be interesting to do this comparison with SO_2 which is the pollutant most commonly monitored by networks. SO_2 cannot be monitored with a natural CO_2 laser, but a CO_2^{18} isotopic laser which has its emission lines shifted towards the shorter wavelengths could be used. The first step of our study will be to build a CO_2^{18} laser in order to see if the absorption coefficients are high enough for monitoring SO_2 with an adequate sensitivity. If it is the case this laser will be settled in our laboratory caravan and will be used for field measurements. If it is not the case, the comparison will be done with a natural CO_2 laser monitoring ethylene.

II - METHOD

The laser system will be used in an industrial site and the results compared with those obtained from a classical network on the same site.

a) Network

The A.S.C.O.P.A.R.G. (Association pour le Contrôle de la Pollution Atmosphérique de la Région Grenobloise) network was settled in january 1979. It includes 5 stations whiche measure, besides the meteorological parameters, the strong acidity, chlorine, and the total hydrocarbons.The last measure is not presently working (1/4/79).

b) Laser method

Taking into account the caracteristics of the network, the comparison can be done by measuring SO_2 (strong acidity) or ethylene (total hydrocarbons). We will first examine the possibility of measuring SO_2.

III - MEASUREMENT OF SO_2

The study of the spectrum available from the literature shows an overlap betwenn the SO_2 absorption spectrum and the CO_2^{18} emission lines.

a) Isotropic laser

A laser with two 1,2 meters long sealed off tubes, filled with a mixture of $C^{12}O_2^{18}$, He, Xe, N_2 (4,4 - 9 - 0,7 - 1,9 mb) has been built. The cavity (5 m long) uses a 5 % transmitting, 5 meters radius of curvature mirror, two 5 meters gold coated folding mirrors and a 150 lines/mm grating.

b) Experimental results

Lines up to R46 (9,0024 μ) have been obtained and absorption coefficients have been measured for lines up to R42 (9,0177 μ).

A 1 m long cell filled with 10 torrs SO_2 in presence of nitrogen at an overall pressure of 1 atm was used.

The following table gives the SO_2 absorption coefficients :

Line	wavelength (microns)	frequency (cm^{-1})	K atm^{-1} x cm^{-1}
R 42	9,01776	1108,92	0,486 ± 0,009
R 40	9,02569	1107,95	0,493 ± 0,01
R 38	9,03379	1106,95	0,305 ± 0,06
R 36	9,04206	1105,94	0,463 ± 0,08
R 34	9,05050	1104,91	0,379 ± 0,07
R 32	9,05912	1103,86	0,360 ± 0,06
R 26	9,08601	1100,59	0,343 ± 0,06
R 22	9,10487	1098,32	0,323 ± 0,07
R 12	9,15508	1092,28	0,268 ± 0,05
P 20	9,35504	1068,94	0,093 ± 0,01
P 22	9,36892	1067,36	0,088 ± 0,02
P 24	9,38301	1065,75	0,064 ± 0,02

- All measurements where done at 295 K
- Errors calculated include only pressure and laser power errors
- R.T. MENZIES (Réf. 6) found 0,53 atm^{-1} x cm^{-1} for the R 40 line

  J. BOSCHER, G. SCHAFER, W. ENGLISCH and W. WIESEMANN (Réf. 7) found 0,41 atm^{-1} x cm^{-1} for this line.

IV - CONCLUSION

SO_2 monitoring

The best absorption coefficient is that of the R 40 line. It should be used as the measure line. The atmospheric absorption due to CO_2 and water vapor (5) indicates that the most interesting line that could be used as reference line is R 12 (9,15508 μ) (it is not affected by CO_2 and absorbed by water vapor nearly in the same manner as the R 40). With those lines, if we measure a 1 % differential transmission the sensitivi-

ty for SO_2 detection is 250 ppb for a 1 km path.
This is not enough for a site where values are about 300 ppb.

On the other hand the sensitivity of the network for total hydrocarbons measurements is 20 ppb. It corresponds for ethylene to a 500m over all path if we measure a variation of 5 % of one of the transmitted laser beams (the minimum variation of transmission that we have been able to measure is 1 %).

Those comparative measures will be done in the vicinity of a factory which uses ethylene as raw material. It is located between two points of the network. Ethylene seems to be the most important hydrocarbon in this region.

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Contractor : BERTIN & Cie - PLAISIR - FRANCE
Unité Micrométéorologie et analyse statistique dynamique
Contract n° : 282-77-1
Project leader : J. Fage
Title of the project : Analysis of air pollution episodes in Paris

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1 - OBJECTIVES

The aim of this program is to obtain further experimental information on high pollution episodes in Paris, mainly associated with important radiative temperature inversions, in order to define a real time alarm system including ground based and teledetection sensors, connected to a computer running in real-time numerical or statistical forecasting schemes.

2 - MATERIAL AND METHODS

2.1. - In situ measurements in Paris

A micro-meteorological station was installed on the roof of the 75 m high "Maison de la Radio" building in Paris. It included an Alcyon three-axis anemometer, two Bertin temperature probes a humidity sensor and a SO_2 Phillips sensor.

- Total sulphur concentration and temperature profiles were obtained on the Eiffel Tower using a Meloy short response time sulphur analyzer and an autonomous Bertin temperature probe.

- Simultaneous wind profiles were obtained by theodolite tracking pilot balloons released from the roof of the "Maison de la Radio" building.

- One point time correlations were calculted at the top of the Eiffel tower between sulphur concentration and temperature, to confirm that the pollution layers are concentrated near the inversion base.

. A prototype of one dimensional Doppler Sodar was installed on the roof of the "Maison de la Radio". The backscattered echo was digitalized as well as the Doppler shift due to the vertical velocity w (z) obtained by a magnified zero crossing technique, applicable to real-time measurements.

2.2. - Field measurements in Jumeauville

Considerable information was acquired on the morning transition period of radiative inversions during a research experiment carried out at Jumeauville (40 km west from Paris) in cooperation with EDF-DER - Mr Méry. Data on the turbulent fluxes of heat and momentum measured by a three-axis sonic anemometer at 18 m were obtained. This instrument was kindly lent by Prof. BERROIR, Laboratoire de Météorologie Dynamique de l'ENS. Simultaneous profiles of temperature and humidity up to a height of 700 m were collected as well as wind profiles (balloon + théodolite) and Vertical Doppler Sodar information (Ref - 6 -).

2.3. - Simplified numerical model of temperature inversion rise

A one-dimensional numerical model of the morning transition period evolution of temperature inversions was developed and tested against the data collected in these campaigns. The original inputs to this model are the turbulent fluxes of heat, humidity and momentum at the top of the surface boundary layer. Initial conditions to this model are the wind and temperature profiles before sunrise.

RESULTS

3.1. - Storage of pollutant inside the inversion layer

Analysis of simultaneous temperature and concentration profiles over Paris gave a sticking confirmation to the hypothesis of a storage of pollutant at the inversion base. This appears as fine sheets of pollutant ~ 40 m thick in which the total sulphur concentration exceeds 2700 $\mu g/m^3$.

As predicted, in many occasions the air below is perfectly clean and the pollution peak associated with the inversion rise is totally impredictable using only ground-based sensors.

The one point time correlations between temperature and concentration are strongly negative when the sensors are located above the pollution sheet and positive when the sensors are located below. This behaviour occurs before the inversion rise and gives reasonable support to the hypothesis that the polluted layer is linked to the inversion base as long as the "pollutant downdraft" mechanism is not developing.

3.2. - Breaking of unstable gravity waves at the inversion base

There is experimental evidence that the entrainment processes at the inversion base are heavily under estimated by the purely convective models of the atmospheric boundary layer. In a similar study carried out in 1975 (Ref - 1-), the existence of unstable gravity waves at the inversion base was evidenced. The instability finds its origin in the strong wind shear at the inversion base. It appears that the most dangerous situations are characterized by an "average critical wind shear" not strong enough to destroy the nigt-time inversion but likely to produce an important wave-induced entrainment during the morning transition period.

Information from the one-dimensional Doppler Sodar prototype was used in some situations to determine the period of such gravity waves.

Considering these features a method for tracking the inversion base was defined, using a digital spatial filtering to smooth the echo curve along each vertical exploration, followed by a temporal filtering at each altitude.

In this way, the "numerical fascimile" obtained is smoothed with respect to both space and time and an automatic research of the echo maximums is possible, opening the way to a real time use of the sodar echo (Ref - 3-).

3.4. - Numerical modeling of the morning transition period

The first efforts were concentrated on the numerical modeling of this period because it is the period in which the "pollution peaks" do appear at the ground. New parameterizations were tested against experimental data to take into account the mechanical entrainment processes associated with the breaking of unstable gravity waves. The ratio $A = \frac{Qh}{Q_o}$ of the turbulent heat flux at the inversion base to the turbulent heat flux at the ground is found to exceed 1 event two hours after sunrise, according to the importance of the mechanical entrainment mechanism.

The maximum of downward heat flux at the inversion base occurs just before the maximum pollution levels at the ground and is associated with a steepening of the temperature gradient inside the inversion. As a passive scalar the sulphur concentration is supposed to have roughly the same behaviour than potential temperature. That is why this model gives a realistic description of pollution peaks at the ground (Ref - 5-).

Further investigation is now dealing with the night-time modeling of the ABL over an urban area and with the storage mechanism occuring during the night.

It is expected that this model will be able to give a reliable numerical prediction of the importance of pollution peaks at 12.00 P.M., in order to reduce the emissions if necessary.

3.5. - Definition on an alarm system

The need for an operational real-time forecast of pollution peaks led to develop two data acquisition systems :

- a three dimensional Doppler Sodar
- a micro-meteorological station including a real-time measurement of the turbulent fluxes of heat, momentum and pollution.

Each of these systems preprocesses the data to send them to a mini-computer whose task is to edit the data and run any statistical or numerical scheme programed in ordinary languages (Ref -4-).

4 - CONCLUSIONS

The main conclusions at this stage may be summarized as :

1) These exists an important storage of pollutant due to radioative temperature inversions, under conditions of weak wind. The pollutant appears in the form of thus high concentration sheets near the inversion base.

2) This storage phenomenon is responsible for pollution peaks at the ground, occuring during the morning transition period, but are not predictable using ground based sensors.

3) The pollution downdraft mechanism is associated mainly with the breaking of unstable gravity waves

4) The importance of this entrainment mechanism is closely depending on the wind shear at the inversion base

5) The digital analysis of the SODAR echo may yield a real time monitoring of the height of the inversion base.

6) Simplified numerical models, using the values of the turbulent fluxes near the ground and a Doppler Sodar description of the wind field as inputs give a reliable prediction of the existence of a pollution peak during the morning.

5 - ASSOCIATED WORK : THREE DIMENSIONAL DOPPLER SODAR

A three dimensional Doppler Sodar was designed and constructed using the experience of the vertical prototype presented before.

Particular attention was given to the acoustic antennas design whose net gain is 27 dB.

An original signal analysis technique was applied, based on a continuous analysis of S/N ratio, leading to the invalidation of some parts of each exploration. A magnified zero crossing technique is then applied (Ref - 5-) to derive the doppler shift on each axis at each altitude and these data are sent to a microcomputer to be averaged and to give the three dimensional wind profile.

The range of this apparatus is between 300 and 1000 m depending on the echo intensity and on the ambient noise. Average range is around 500 m.

The design characteristics allow to meet a good compromise between the real time operation constraints and the versatility that can be asked to such a system for special applications (plume analysis).

This system is now running official tests at Jumeauville under the responsibility of EDF-DER (Chatou).

.../...

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Contractor : Université Pierre et Marie Curie, Paris
Contract n° : 313-78-1 ENV F
Project leader : L. Henry
Title of project : Determination of wavenumber, intensity and broadening parameters of special lines in order to allow detection and concentration measurements in the atmosphere (methane nitrogen derivatives, halocarbons etc.)

Objectives of the research

The general objective of the research is to develop the metrology of gaseous pollutants (and minor constituants) of the atmosphere. This extensive work is needed for two purposes : 1°) choice of the best optical methods for monitoring and 2°) treatment of the informations obtained from the choosen methods.

The choice of methods depends upon the pollutant and is concerned with optical domains, apparatus, proceduces for investigations...The selected methods can be different to develop the analysis of gaseous pollutants in laboratories on samples or near a pollution source, or in the atmosphere at sea level or in the stratosphere.

From field or laboratory optical measurements, data processing will give information on the nature and concentration of gaseous pollutants. A vibration band currently extends over about one micrometer (50 to 200 cm^{-1}). Every band is built with about ten to thousands of vibration rotation lines with different intensities, widths and shapes (both these last data depending upon pressure and temperature). This tremendous amount of data, related by well established theories, can be computed from a very moderate amount of reduced data with the help of small size computers. The aim of the present work is a contribution to determine these basic metrological data for each band of each pollutant of interest. They are line wavenumber, intensity broadening coefficient and the dependence of the last two parameters upon temperature. With such a kind of physical analysis, the very rich spectra of molecules can be fully used to obtain very specific informations with a precision limited only by the quality of field measurements if the metrological data are obtained with a sufficient quality as you try to do.

Materials and methods

The materials currently used for the present research in the Laboratoire de Spectronomie Moléculaire are :

- a large double pass Ebert-Fastie spectrometer monitored by a goniometric interferometer mainly used for intensity and broadening parameter studies and wavenumber measurements from 2 to 6 micrometers
- a long path (12 meters) Fourier Transform spectrometer
- a laser spectrometer for saturated absorption spectroscopy in the 10 µm part of the I.R. spectrum.

Accuracy of measurement needed. The parameters accuracy needed depends upon the width of the spectral lines. At sea level, the broadening parameter γ^{o} has, in most cases, a value from 0.15 to 0.03 cm^{-1} and it must be known with a relative precision of 2%. The wavenumber must be known for isolated lines with a precision of $\pm$ 2 $10^{-3} cm^{-1}$. For higher atmosphere, where the spectral lines are narrower, the precision on wavenumber must be within $\pm$ 0.5 $10^{-3} cm^{-1}$ or better if one could.

Results

We have, as required by the Commission of the European Communities, studied intensities of vibration-rotation lines of important bands for some major pollutants. CO, NO, CH_4 and N_2O were chosen at first. The wavelength of the band center for the four studied bands is at about 5,33 µm (1875 cm^{-1}) for (1-0) of NO ; 4,66 µm for (1-0) of CO (2143 cm^{-1}), 3,3 µm for ν_3 of methane (3000 cm^{-1}) and 4,49 µm for ν_3 of N_2O (2223 cm^{-1}).

This program was well adapted to the facilities of our laboratory two years ago. The expected quality of the determinations was supposed to be unmatched in that part of the infrared spectrum. For the ten micron part of the spectrum, some improvements and equipments were not yet achieved at that time.

Concerning the effect of temperature on line broadening, the suggestions from the European Communities were justified by the need of measurement in much extended temperature conditions including in the upper atmosphere and stratosphere. Only very few studies concerned with the effect of temperature on line widths were until now achieved and big discrepancies in the obtained results occured in literature.

For the four studied bands we have found that the broadening parameter is related to the temperature by the following relation : $\frac{\gamma_2}{\gamma_1} = \left(\frac{T_1}{T_2}\right)^{0.7}$ where T_1 and T_2 are two given temperatures. This result holds for the specific situation where the broadening is due to collision of pollutant molecules with nitrogen and oxygen.

For the different lines associated to different rotational quantum numbers the value of the exponent has different measured values. But at least for the molecules we have studied, the value of the exponent remains between the numbers 0.6 and 0.8.

For methane we have measured also the dependence of the line intensity as a function of the rotational quantum number J for the ν_3 band. This was also a rather time consuming study.

The coefficients associated with the distortion effect of the molecule with the rotation are $C_1 = m \times 3.153.10^{-3} cm^{-1}$ and $C_2 = m^2 \times 8.08.10^{-4} cm^{-1}$ where m = J for the P branch, 0 for the Q branch and J+1 for the R branch.

We have observed that, in every set of lines corresponding to a given rotational state on the vibrational ground state the relative intensities of allowed lines are given by the spin statistic within the precision of our measurements (± 2 %) ; that is to say, the relative intensities in each set, after the distortion effect correction, are like the numbers 2,3, and 5 for individual lines of symmetry type E,F and A respectively.

Concerning the study of halocarbon spectra, we have achieved a preliminary study, but the problem is very different due to the very high density of lines as will be different the method for detection, at least at sea level.

As a conclusion we should like to point out that to go further in the halocarbon studies we must use the long path Fourier Transform spectrometer which is at present at work in our laboratory. For an immediate future it seems useful to look at the spectra not only of methylchloride and methylfluoride but also at some bands of freon 11 and 12, these components having strong bands in the atmospheric window at 10 micrometers (1000 cm^{-1}). Eventually others molecules suggested by the European Communities in accordance with the new increasing needs should be studied in the same domain of the spectrum as well as in the 5 micrometers atmospheric window.

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[a] concerning transparency of the atmosphere

[b] concerning a very useful complementary method to study the halocarbons in the best domain of the infrared - the first work in this way.

Contractor : FIAT ENGINEERING

Contract n° : 155 - 77 - 1 ENV I

Project Leader : Dr. P.P. Gaddo

Title of the project : Urban air pollution from photochemical smog in Turin

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Objective of the research

The project research intends to value the real entity of the photochemical smog phenomena occurring in the urban area of Turin and in the sorrounding zones, related to the promotor agents and transformation products behaviour.

Methods

The research has been carried on during 1977-78 by means of :

- continuous measurements, at ground and elevated levels, of total hydrocarbons, methane, nitrogen oxides and dioxide, ozone and aldehydes;
- continuous measurements of solar radiation;
- gaschromatographic determinations of hydrocarbon species and peroxyacetylnitrate on atmospheric samples taken in the urban centre and in the sorrounding areas.
  Globally, 144 random measurements have been carried out in the early morning and in the maximum solar radiation hours, 134 of which in the urban centre and 14 in the extraurban area.

Results

The detail of the results achieved in the research programme has been presented to the Commission of European Communities by six-monthly progress reports.

Data elaboration aimed at :

- valuate the percentual variation of hydrocarbon species (paraffins, olefins, aromatics) and the daily atmospheric reactivity index variation;
- verify the presence of transformation products (PAN) and the promotor agents pattern.

A whole valuation of medium behaviour of hydrocarbon species shows up, during the utmost solar radiation hours, the paraffins tendency to increase jointly with an olefin and aromatic decrease (see table 1).

Such trends, as the decrease of the atmospheric reactivity index would lead to suppose a recurrent presence of transformation reactions.

On the other hand, only in a modest percent (~20%) of samplings, the PAN presence has been observed, always in limited concentrations (tab.2).

To examine closely these considerations, a more detailed inspection has been carried out, distinguishing days with poor and strong solar radiation conditions.

As evinced in table 3 and 4, the presence of weak solar radiation doesn't seem to induce transformation reactions: this is confirmed by the substantial constancy of the reactivity index of the atmosphere during the day and the absence of PAN.

Otherwhise, in the strong irradiated days, the possibility of transformation reactions setting up appears more evident.

However it's necessary to emphasized that :

- PAN presence has been noticed in some cases in the early morning hours even if the required conditions for its formations were absent,
- in some irradiated days, jointly with an ozone increase and a nitrogen dioxide decrease, the formation of PAN has not been observed.

These considerations confirm both the interpretation complexity of the transformation phenomena, occurring in the atmosphere and the importance to emphasize the study in the extraurban atmosphere, the chemistry of the atmosphere, and the pollutant transport phenomena.

In order to examine closely this latter aspect a part of the research programme has been tended towards the study of the atmosphere behaviour in the urban sorrounding zones with relation to what observed in the urban centre.

The determinations carried out, for a total of 14 samplings, lead to some previous conclusions, that obviously need a more complete statistical confirmation.

Anyway, it has been observed that :

- the extraurban atmosphere, samples in areas not presumably influenced by the urban centre emissions, is as an average, more reactive than the urban atmosphere, because of a more elevated content of aromatic hydrocarbons, probably of natural origin (table 5);
- in some cases in the extrurban atmosphere PAN's presence has been detected in higher concentrations than those present in the urban centre. Appreciable levels of PAN has been recorded also in the early morning (table 6).

These considerations lead to conclude that the extraurban atmosphere reactivity and the photochemical transformations in it occurring are not consequent to the pollutant transport from the urban area.

Conclusions

The results achieved in the research programme seem to point out that in Turin atmosphere transformation reactions of promotor agents and reactive hydrocarbons are taking place , but only in a few cases the PAN formation is observed.

In the extraurban area the PAN presence has been detected in higher concentration than those recorded in the urban area.

Anyway, it is necessary to develop a more detailed study, principally in the extraurban area in order to achieve extra-informations about these phenomena.

For this purpose it has been established to extend the research to 1979-1980, according to the following programme :

- to carry on the continuous and random samplings, according to the criteria adopted in 1977-78, with particular reference to the extraurban area;
- study of the ozone pattern;
- valuation of the nitrate presence in the areas under investigation related to the ozone and nitrogen oxides levels.

Tab. 1

% variation of HC specied and reactivity index in the day.				
	Hydrocarbon species %			reactivity index
	Paraffins	Olefins	Aromatics	
Morning	87.6	4.9	7.5	0.53
Afternoon	88.7	4.5	6.8	0.47

Tab. 2

Determinations of PAN in 1977-78		
Samplings n.	sampling with presence of PAN	Concentrations ppb
198	38	0.5 - 4.2

Tab. 3

HC-PAN pattern in weak radiated days (200 cal/min.cm^2)					
	Hydrocarbon species %			Reactiv. index	PAN ppb
	Paraffins	Olefins	Aromatics		
Morning	89.7	4.2	6.1	0.46	-
Afternoon	89	4.6	6.4	0.48	-

Tab. 4

HC-PAN pattern in strong radiated days (400 cal/min.cm^2)					
	Hydrocarbon species %			Reactiv. index	PAN ppb
	Paraffins	Olefins	Aromatics		
Morning	86	5.5	8.5	0.6	0 ÷ 1.5
Afternoon	88.5	4.3	7.6	0.48	1 ÷ 3.5

Tab. 5

Reactivity index of urban and extraurban atmosphere		
	Urban atmosphere	Extrauban atmosph.
reactivity index	0.6	0.93

Tab. 6

PAN levels recordes in urban and extraurban areas		
	Urban atmosph.ppb	Extraurban atmosp.
Afternoon	0.8 ÷ 3.5	1.1 ÷ 4.1
Morning	0.5 ÷ 1.5	1.9 ÷ 4.2

Contractor: Consiglio Nazionale delle Ricerche C.N.R.
Contract No: 156-77-1 ENV I
Project Leaders: A. Liberti and M. Possanzini
Laboratorio sull'Inquinamento Atmosferico
Title of project: Evaluation of photochemical smog and its prevision in the Rome area

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1. Objective of the research

The research project on photochemical air pollution in the metropolitan are of Rome aimed to evaluate the current levels of oxidants and their precursors and to establish empirical relationships with the main factors involved in oxidant formation, such as ratio of non-methane hydrocarbon to nitrogen oxides concentrations, reactivity of hydrocarbons and meteorological variables.

2. Materials and methods

Data on oxidant and precursor (nitrogen oxides and non methane hydrocarbons) concentrations have been obtained in the Rome area at two monitoring stations operating during the period 15 May - 30 September of the years 1977-1978. The former was located at 20 meters above ground level in an urban zone (C.N.R. Building) with a high traffic, whereas the latter was set up downwind, in a suburban site 13 km east the center of the city.

The oxidant measurements were performed with automatic galvanometric type instruments developed in connection with this investigation[1].Nitrogen oxides were monitored with chemiluminescent analyzers (Monitor Labs. mod 8440). Non methane hydrocarbons (NMHC) were monitored with a continuous analyzer making use of photoionization detector (HNU, mod. PI 201). The total solar radiation was registered by means of a bimetallic actinograph (SIAP, mod. 2800).

3. Results

3.1. Atmospheric levels and variation of oxidants and their precursors

The WHO maximum acceptable value for oxidants (0.1 ppm hourly average) has been exceeded 21% of the days during the last two summers. The highest concentrations (up to 0.2 ppm) have been observed at the suburban site, where the ozone peak occurs in the afternoon one hour later than at the urban station. It should be assumed that the pollutants in the air mass, as they move from the urban complex to areas of low primary emissions, are reacting chemically and that additional ozone is produced in transit counteracting the dispersion effect. The nocturnal oxidant levels are also lower at the urban site where reaction between NO emitted during the evening rush-hour and ozone is an important chemical sink for this compound.

Each of the monitored pollutants has its own characteristic daily cycle. The primary pollutants NO and NMHC emitted directly in the atmosphere do not disappear during night time owing to low inversion and light winds. Their levels begin to rise in the early morning, peaking near 8 a.m. (up to 50 ppb and 1 ppm respectively) as a result of the heavy commuter traffic. Atmospheric instability and traffic decrease (in addition to the photochemical processes) lower these pollutants during the middle of the day, while during the late afternoon they rise again with increased traffic.

The daily pattern of NO_2 is very similar to that of other precursors except that its morning peak (max 60 ppb) occurs later due to the time required for NO to NO_2 conversion. Oxidants (secundary pollutants) are formed in the air by the action of sunlight on the mixture of primary pollutants and oxygen. Thus, oxidant concentrations and insolation exhibit a similar behaviour:they are low at night and their peak occurs near midday.

It has to be noted another aspect of the phenomenon from the weekly trend of the pollutants in the Rome metropolitan area, where automibile emissions are the major source of precursors. On weekends total traffic is considerably reduced; hence morning peaks of NO and NMHC are greatly attenuated on Saturday and virtually eliminated on Sunday. While NO and NMHC show different diurnal patterns on weekdays and weekends, oxidant has a temporal pattern which is almost independent of the day of the week. It means that the variations in emission intensity are counterbalanced, at least on the average, by the meteorological and chemical processes which transform primary pollutants into secondary species. At both stations in Rome, oxidant levels are slightly higher on the weekends than on the working days[2]. At the suburban site between 13:00 and 15:00 h 9% of data range from 0.09 to 0.14 ppm on weekends and from 0.08 to 0.11 ppm on workdays.

3.2 Atmospheric levels of C_2-C_5 hydrocarbons

During Summer 1978 individual hydrocarbons in the C_2-C_5 range, mainly involved in atmospheric photochemical processes, have been detected at the urban site.

The technique used for analyzing light hydrocarbons in air consisted in trapping them by means an adsorbent (Carbopack B, Supelco, Inc.) at low temperature ($-150^{o}C$), heating the adsorbent up to $200^{o}C$ and injecting the desorbed compounds into a chromatographic column packed with Carbopack B coated with Carbowax 2500. Of the 19 compounds detected, 6 were alkanes (ethane, propane, isobutane, butane, isopentane, pentane), and 13 alkenes (ethylene, propylene, isobutene, cis-2-butene, trans-2-butene, 2-methyl-1-butene, 3-methyl-1-butene, 2-methyl-2-butene, 1-pentene, cis-2-pentene, trans-2-pentene) including 1,3-butadiene.

Their total concentration varied from 150 to 300 ppb. It should be noted that the afternoon concentrations are lower than those of the morning for all the species identified. However, due to their high photochemical reactivity, significant variations (from 30 to 50%) were observed for unsaturated hydrocarbons.

The composition of the hydrocarbons sampled from urban air closely approaches to that of auto exhaust gases. Altough alkanes having smaller molecular weight (i.e. ethane and propane) are present in negligible amounts in traffic exhaust, their presence in natural gas as well as in gasoline, coupled with the high volatility, has to be accounted for their occurrence.

3.3 Correlation between oxidants, precursors and meteorological factors

As already known, the day-to-day variations of oxidant concentrations are due to precursors emissions and to meteorological variables, such as solar radiation intensity, temperature and wind speed.

The relationship between ozone, precursors and weather parameters on the basis of 172 valid daily data, has been formulated as regression equation considering a linear model of the form:

$$O_{3max}=b_1T_{max}+b_2V+b_3R_t+b_4\frac{NO_x}{NMHC}+b_5NO+b_6NO_2+b_7NMHC+a$$

where $O_{3\ max}$ = daily maximum 1-hour oxidant concentration (ppb)

T_{max} = daily maximum temperature (°C)

R_t = daily total solar radiation (cal $cm^{-2}day^{-1}$)

$NO, NO_2, NMHC$ = average values of 06:00 - 09:00 h nitrogen oxide and non-methane hydrocarbon concentrations (ppb)

a = constant

From the results of the multiple correlation the following equation is obtained (R=0.88):

$$O_{3max}\ (ppb)=2.79\ T_{max}+4.70\cdot 10^{-2}R_t-1.75V+8.94\cdot 10^{2}NO_x/NMHC\ -$$
$$-2.03\ NO+1.02NO_2-5.6\cdot 10^{-2}NMHC-8$$

It appears that, while the dispersive effect of the wind is manifested by its-negative power, the temperature is the most important parameter because its value can indicate the existence of weather conditions favorable for photochemical smog formation.

Of the chemical variables, nitric oxide only shows a negative correlation. In fact NO is an effective ozone scavenger and very likely the increase in its emissions could decrease the ozone level, according to NO_x/NMHC ratio. This ratio plays an important role in oxidant production. It has been observed that, for absolute NO_x and NMHC concentrations ranging from 0.02 to 0.08 and 0.2 to 1.0 ppm respectively, there should be an optimum precursor ratio for the production of the highest oxidant levels.

Aerometric data collected in several countries indicate that $NMHC/NO_x$ ratios ranging from 10 to 20 as those measured at the urban site of Rome, correspond to maximum ozone level not higher than 0.2 ppm. This is in agreement with our observations.

From the regression equation above reported predictions of daily maximum O_3 concentrations at 95% confidence level, can be made within ± 25 ppb.

4. Conclusions

The results of this investigation indicate that a photochemical oxidant problem exists in the Rome area, even though the highest hourly values never exceeded 0.2 ppm.

The extent to which the photochemical reactions occur and oxidants accumulate is strongly dependent on meteorological factors as well as on ratio of non-methane hydrocarbon to nitrogen oxides concentrations.

Conditions are favorable for these reactions during Summer months, because atmospheric stability at night and in the early morning allows buildup of precursors and temperature together with u.v. radiation during daytime hours is at its seasonal peak.

Daily pattern of oxidants and their precursors in this area suggest that photochemically induced reactions occur in the lower troposphere and products of gasoline combustion are the principal source of oxidant episodes.

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Contractor: Ente Nazionale per l'Energia Elettrica (ENEL)

Contract n°: 228 77 1 ENV I

Project Leader: ing. D. Borgese

Title of project: "Diffusion models of air pollutants near large power plant"

Objective of the research

The object of this research is that of getting information about processes playing the major roles in dilution and transport of airborne pollutants in the Gulf of La Spezia, where a large thermoelectric power plant has been operating for about 15 years and other factories, including an oil refinery and a few ship-yards, are present.

In order to estimate and forecast the transport and dilution of pollution and the amount of their ground level concentrations, a lot of experimental and theoretical studies are at our disposal, but they mainly refer to emissions from sources located in open and flat areas, characterized by definite and reproducible climatological situations. On the contrary, a very small number of studies are available about the dispersive properties of air flowing in sites, like La Spezia Gulf, characterized by surface discontinuity (sea side regions) and by orographic dishomogeneities (complex terrain).

Material and methods

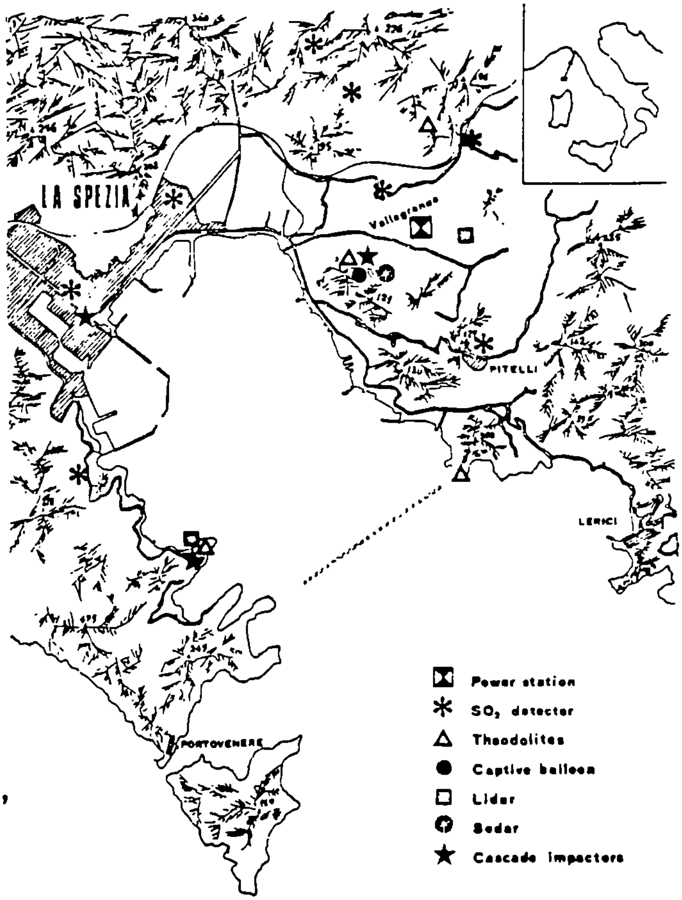
- The site

La Spezia Gulf, on the Northern Thyrrenian Sea approximately 50 km east of Genua, is a typical coastal site of the Ligurian shoreline, i.e. a deep and narrow roadstead bounded by rocky promontories. Its size is one of the most relevant and its shape very characteristics; as the figure shows, the roadstead of this gulf develops along the NW-SE direction and the heights of contours surrounding it ranges from 200 to 600 m. The NW regions is occupied by the urban area of La Spezia ($\simeq 10^5$ inhabitants), while in its northern sector there are the harbour and the shipyards; east of

La Spezia town, there is the oil refinery with chimneys about 100 m high. Near Vallegrande, there is the power station of ENEL, with four sections and four stacks 120, 120, 175, 220 m high respectively.

- Field experiments

Six campaigns, corresponding to the main seasonal weather features in this eastern part of Liguria, were carried out from April 1974 to November 1 1978. Late spring and summer periods were preferred, in that land and sea breezes can easily develop, due to generally established fine weather and high solar heating. The experimental set up employed during each of them consisted in:



a) two systems for tracking aerological balloons, made up by pairs of theodolites. Both pilot and balanced balloons were tracked, in order to measure vertical wind profiles as far as 1500 m and an trajectories respectively.

b) two systems for measuring vertical temperature profiles of air as far as 700 m, the first one made up by a radiothermosonde with accuracy of 0.2 °C towed by a 23 m^3 captive balloon, the second one by free radiothermosondes lifted by pilot balloons.

c) one lidar system to track smoke plumes emitted from the stacks of ENEL power station. The instrument, built by Laser Associated, consisted in a ruby lidar, emitting light pulses 20 ns long with power peak of ≲ 15 MW.

d) an aircraft, with thermosonde, for detecting temperature profiles as far as 1000 m (only operated during last exercise in november 1978).

As long as it was possible, all of measurements (wind, temperature, smoke plume) were carried out simultaneously; unfortunately, in many cases, we had to suspend one kind of sounding or the other, due to lack of permission by Air Force or Navy Authorities.

Results

- Air circulation and broad characteristics of sea and land breezes

The first characteristic of atmospheric circulation in the site is the large and unpredictable distorsion of wind field, both in the horizontal and in vertical planes. There is neither correlation between wind direction and speed at any levels, nor any clear trend in turning of wind vector with height. Neither geostrophic wind (found at about 1500 m), nor any surface wind measurement can be taken as good predictors for air circulation at levels interested by plume diffusion (from 300 to 500 m); just one station, Vezzano, seems to be a little more correlated with winds at such heights.

Irrespective of geostrophic circulation, in fine weather conditions, wind direction below 500 m display a more regular pattern along south and north direction, peculiar of sea and land breezes. Sea brezees top extend as far as 800 ± 200 m in summer, with a first maximum of wind speed at 300 m, a minimum at 500 m and a second maximum at 700 ÷ 900 m. Land breezes top reaches 300 ± 100 m, and wind speed profile shows the same qualitative shape as sea breeze do, with minimum and maxima at lower heights.

A general scheme of land breeze circulation in lower layers (up to 300 m) can be drawn as follows:

- lower layers of nocturnal stable breeze do not get over the barrage of hills in the northern part of gulf, but are canalized into Valle Grande;
- their higher layers, on the contrary, fly over the hilly edge and recombine, over the gulf, with the previously described circulation;
- downwind to hills (northern part of gulf), over the city, a zone of stagnation is created. It determines dust accumulation observed, by night, bet-

ween 100 and 200 m.

- Stability and instability of local flows at La Spezia

The main stability patterns found within the first 600 m of air in the site have been:

- Stable stratification during sea breezes

Radiative inversion

They occur in nighttime, due to nocturnal radiative cooling of ground surface; like the ones in continental regions (Anfossi et al., 1974), their depths Z_i above sea level place themselves around the curve, whose analytical expression is: $Z_i = 70 \sqrt{t}$, where Z_i is measured in metres and t in hours.

The average intensity of this kind of inversions was evaluated to be about $(1.5 \div 2.)\ 10^{-2}$ °K/m, instead of $(3. \div 4.)\ 10^{-2}$ °K/m measured in the Po Valley.

- Stable and unstable stratifications during land breezes

Subsidence inversions

They occurred both in daytime sea breezes and in nighttime land breezes, as result of flow convergence at mesoscale. Their average intensity was $1.8\ 10^{-2}$ °K/m, the height of their bases was widely variable from one run to another, so as not to allow any average value to be defined, and the height of their tops averaged about 550 m; this last value was evaluated from all of runs with inversion top < 700 m, which was the maximum height attained by our captive balloon.

To overcome this limitation, a programme of temperature profiles with aircraft has been started in november 1978.

Convective mixed layers

They form in daytime sea breezes, when the fairly stable air current coming from sea arrives over land. Unstabilization processes, due to heat fluxes and surface roughness, start to operate at that time, and their action deepens more and more, as air goes on inland.

A mixed layer develops in the bottom of the stable flow, and its height H is a function, besides other physical parameters, of downwind distance from coastline.

Van der Hoven, in 1967, proposed an empirical formula for prediction of H values at various downwind distances x from the shoreline, under different conditions of breeze strenght $\bar{u}$ and bulk air stability (the potential temperature difference $\Delta\vartheta$ between sea surface and top of sea breeze).

In applying the Van der Hoven model to the heights H measured by our soundings, we noticed that the bulk stability could not be taken as satisfactory index of the potential energy of air against which thermal vortexes had to work, (also because we did not know the temperature profile above sea), but a better quantity was found to be the potential thermal gradient $\left(\frac{d\vartheta}{dz}\right)$ of the layer of maximum stability, deduced from our inland measured temperature profiles. As a consequence, by introducing the stability parameter $s = \frac{g}{\vartheta} \frac{\Delta\vartheta}{\Delta z}$ (s^{-2}) we modified Van der Hoven formula as follows:

$$H = \alpha \sqrt{\frac{x}{\bar{u}\, s}} \quad (m),$$

where α is a constant to be determined by experiments. In our cases we found 0.05 $m/s^{3/2}$ as an average value for α .

Plume rise

Plume rise is a well understood phenomenon at locations were vertical wind profile is expressible by usual power laws in the first 500 m of PBL.

So, a few suggestions (Slade, 1968; Briggs, 1969; Moore, 1974) have been given about the choice of the wind speed value best representing the average effect of wind on rise in the layer in which plumes diffuse.

This has not been the case for coastal sites, and this makes application of usual formulas somewhat arbitrary and not reliable.

Experimental data have been compared with theoretical expectations of two of the best known models, by Briggs (1969) and Moore (1974) respectively, in which the levels of wind speed evaluation, by power law extrapolation, have been selected following authors' recommendations.

Conclusions

The main characteristics coming out from our experimental campaigns at La Spezia gulf may be as follows:

a) sea breeze depth was about 700 m, as an average value, while land breeze is measured as high as 300 m.

b) the field of motion is very irregular, both in vertical and in horizontal planes, and unpredictable on the basis of surface measurements or geotrophic wind (at about 1500 m).

c) radiative inversions were generally present in nighttime. The time evolution of their depth Z_i may be expressed by the relation $Z_i = 70 \sqrt{t}$, which is the same we found in a flat country like Po Valley, even if their intensity was quite different.

d) the average intensity of the subsidence inversions,sometime present, was greater than 1.8×10^{-2} °K/m with a widely variable heigth.

e) the Van der Hoven model for the heights of the convectively mixed layer during sea breezes was modified as follows: $H = 0.05 \sqrt{X/(U \times S)}$.

f) a fex examples of tracking of smoke plumes by lidar technique show that one can suddivide them in two classes: to the first belong plumes with rising shapes similar to those found in the Po Valley and to the second belong anomalous plumes which present two stages of rising. Tentatively an explanation of this last phenomenon was found, looking at the simultaneous wind profile. Briggs and Moore's models work satisfactorily well for the first class and for the first stage of the second class. For this last group an improvement was found b calculating a mixed Δh_{max}

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Contractor :	CNR - Laboratorio di Ricerca e Tecnologia per lo studio del plasma nello spazio Frascati, Italy
Contract n° :	241-77-1 ENV I
Project leader :	G. Fiocco
Title of project :	Applications and developments of Lidar and Sodar Doppler systems for the study of the dynamics of the atmospheric boundary layer

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SUMMARY

The work on the optical and acoustic radar systems has proceeded along parallel lines.

Comparatively a larger effort has been recently devoted to the acoustic sonde in view of difficulties which we have experienced in procuring a spare tube for the Lidar.

Both systems are presently operational.

The basic unit of the Sodar systems comprising most of the analog functions was developed during 1976. The system was then extensively tested during a campaign at Trino Vercellese. Already during those early experiments the system performed satisfactorily and was able to reveal and follow the evolution of convective and wave phenomena, inversions, fog conditions, etc., with a good signal-to-noise ratio and space-time resolution.

During 1977 the effort on the Sodar was mainly devoted to develop techniques for extracting quantitative data useful for a comparison with other instrumentation. Thus an analog recording system was developed that permits to acquire afterwards the Doppler information. A digital acquisition system was added and the software necessary was also developed.

In the month of July, at La Spezia, a collaborative campaign was carried out with ENEL, to characterize the La Spezia site and SODAR data were recorded continuously for 15 days.

During 1977 the Lidar-Sodar systems were also operated simultaneously for a period of time. Unfortunately the Argon plasma tube of the Spectra Physics 170/3 laser collapsed after a short while.

As regards the Lidar, the new plasma tube has been installed and the system has been operational at the end of 1978, at full power. Various improvements had in the meanwhile been carried out: the receiving optics were redesigned, the transmitting and receiving beams were made coaxial, and the speed of the photon counting electronics was increased.

Also the chopper system has been modified and spurious echoes originating from the chopper were suppressed.

In 1978 a considerable number of Sodar records were analysed, and Doppler analyses were carried out on records of particular interest.

For the Doppler analysis, the received echo is sampled at intervals which are fixed by the reference frequency of the transmitted signal.

For each emitted pulse and for each height interval of approximately 40 meter a complete power spectrum analysis is carried out with the aid of the Fast Fourier transform.

The spectral shift of the returns is a measure of the vertical velocity w (z).

The analysis of a convective plume observed at

La Spezia in July is shown in the report: vertical ascending velocities up to 2 m s^{-1} were measured in the height interval between 350 and 450 m in the plume. In the intermediate zone between the plumes descending velocities of smaller intensity were obtained.

Other cases were also analysed, showing the capacity of the system to carry out systematic measurements of w (z) with high time resolution.

The analytical procedure requires at present the use of an off-line computer and is relatively time-consuming.

In our experience it appears, however to be largely superior, in terms of resolution and signal ~~s~~ to noise, to the use of analog type spectrym analysers.

Work is going on in the development of a real time digital Doppler analyser.

We mention that at the end of 1978 the Sodar system has been installed on the ship Salernum for participation in the GARP program in the Indian Ocean.

Contractor: TECNECO, S. Ippolito (PS), Italy
Contract n° 291-77-1 ENV I
Project Leader: Prof. O. Vittori and Dott. F. Gianni
Title of project: Remote Sensor for air pollution monitoring system

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1. Objective of the research

The objective of the research is to study the characteristics of an air pollution monitoring system using remote sensors based on the mask correlation spectroscopy.

The research has been carried out in three campaigns during which SO_2 concentration data have been measured by point sensors, as well as by the longrange TELETEC.
The purpose of the surveys was to collect time series of SO_2 concentration by two types of instruments and to compare them, using statistical analyses.
The first campaign was held at La Spezia, on July 1977, in collaboration with ENEL/CRTN; the second was held at Piombino on March 197 and the third at Milano, on January-February 1979.

2. 1st Campaign - La Spezia - 29.VI.77 - 7.VII. 77

The site and the period for the survey were chosen to make the campaign together with ENEL/CRTN (Contract N° 228-77-1 ENV. I). Meteorological parameters were measured in detail and SO_2 measurements were made by the ENEL network. In addition to the 8 sensors of the network, further 4 point instruments, as well as the Teletec with 2 lamps, were assembled by Tecneco.

The Teletec was put near Isola, 194 mt. above sea level. Two optical paths were decided, as closer as possible to the point sensors. The lamps was placed at Punta Pezzino at sea level (optical path n. 2, 7300 mt long) and at Baccano 100 mt. a.s.l. (optical path n.1 5400 mt long).

The measurements by the Teletec were made on the days 1st and from 4th to 7th July, from 9 am to about 6 p m. The sampling time was about 1 minute and the frequency 10 + 12 measurements/hour.
In the period of the campaign only low levels of SO_2 concentration (few ppb) have been found by the point sensors and by the Teletec, over the whole area.

Some point sensors have measured, only in few hours of 1st and 4th July, values higher than the background. The Teletec too has found mean concentrations higher than few ppb, and the values are plotted in Fig. 1. Because of the shortness of the period of significant me surements, it has not been possible to make any statistical analysi over the time series, nor to compare the two methods of measurement based on point and remote sensors.

3. 2nd Campaign. Piombino (6th - 12th March 1978)

The site of Piombino was chosen to carry out the 2nd campaign because of the particular configuration of the urban area. Within a rather small zone urban and industrial areas are present and a network, three instruments of which are in the urban area, works.
The measurements have been performed on the days from 8th to 12th March. During this period the winds blew mainly from N, NW and NE, except on several hours of the 8th, on which the wind blew also from W and SW, and several hours of the 10th, on which the provenance was also from E, S and SW. Fig. 2 shows the wind roses on the days, 8, 9, 10, 11 and 12.
During the period of the survey the major part of the concentration data, measured by the point sensors and by the Teletec, had values lower than 30 ppb, which can be thought to be the background value of concentration.

Measurements significatively higher than this background value have been found only on 10th March, between 5 a.m. and 1 p.m., in situations of the wind different from those found on the other days.
This case beeing the single to be occurred, it has not been possible to make any statistical analysis with the time series, and the values obtained by the Teletec and the point sensors can only be compared.
Fig. 2 shows also the plot of the data of the 10th March.

4. 3rd Campaign - Milano - January-February 1979

The third campaign has been carried out in the urban area of Milano, in the period 29th January - 5th February 1979.

In Milano a network of several point sensors by the Laboratorio Provinciale di Igiene e Profilassi (L.P.I.P.) works, five of which are placed around the zone chosen for the survey.

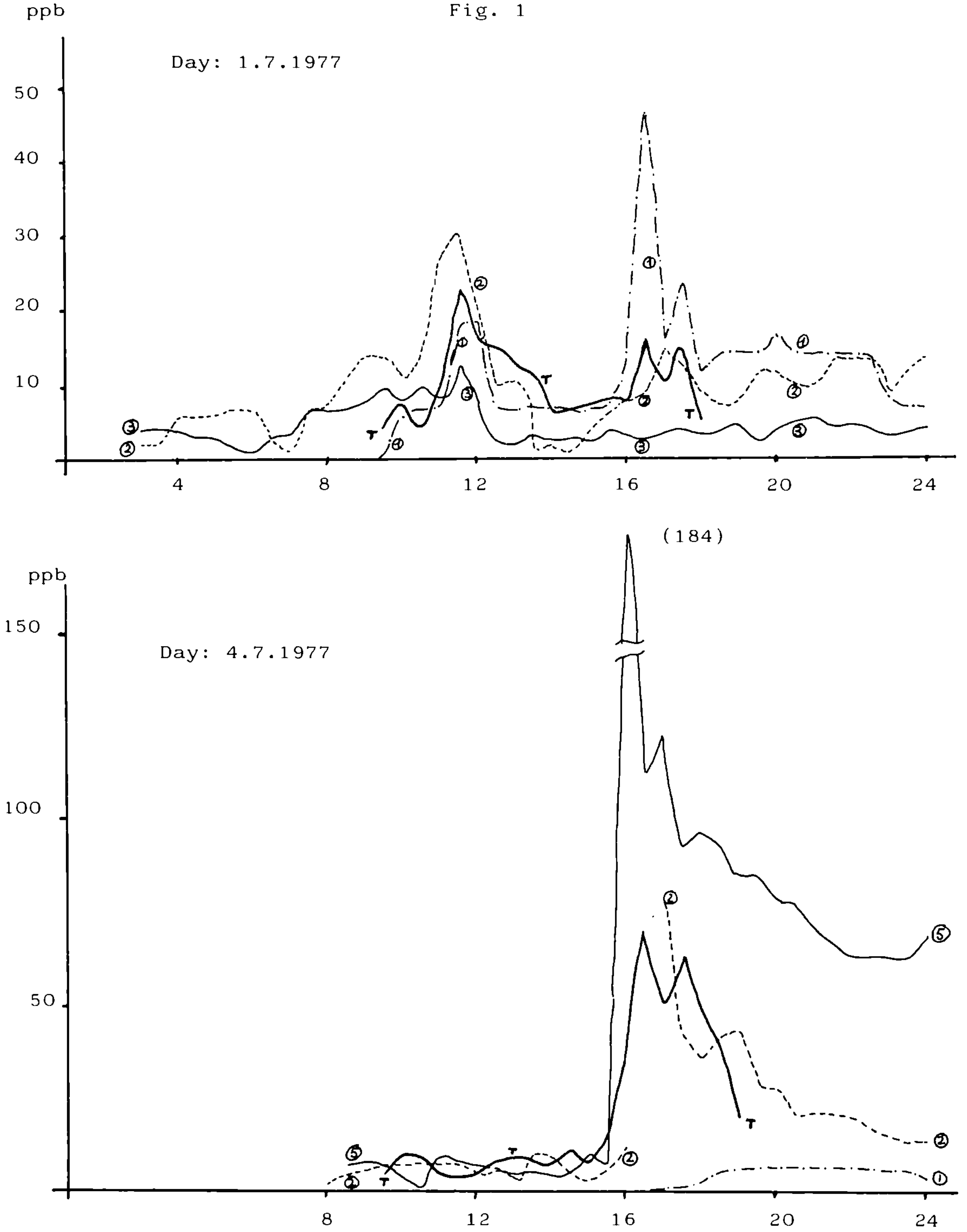
The Teletec has been placed at the 6th floor of the L.P.I.P. offices and a point sensor has been assembled by Tecneco near the Teletec.
At ground level a point sensor of the network is placed.
The lamp has been placed at the 22th floor of the city-hall. Three point sensors have been assembled in the same building: at the 4th, the 14th and the 22th floor. A survey to measure the possible difference in air pollution at different levels from the ground was carried out in the winter 1978.

In particular, SO_2 concentrations were measured from December 1977 to March 1978 at ground level and at the 6th floor of the L.P.I.P. Building.
A very good agreement has been found between the measurements at the ground level and at 25 mt of height; the results of this survey will be described in a "ad hoc" paper that will be published.

On the basis of these results it has been considered the feasibility to compare the measurement by the Teletec at roofs heights with those by the point sensors, either at ground level or at different heights.
The results of this campaign and the processing of the data of vertical profiles of SO_2 will be discussed in future reports.
Up to now it seems that the Teletec has supplied informations on the air pollution on an urban area by which it will be tried to describe the state of the air quality following a new method based on the long path measurements.

In this campaign the Teletec has worked automatically and continuously, from the 30th January to 5th February. During this period significant measurements have been taken, even if particular unfavourable meteorological situations have made not well utilisable some data.

Fig. 1

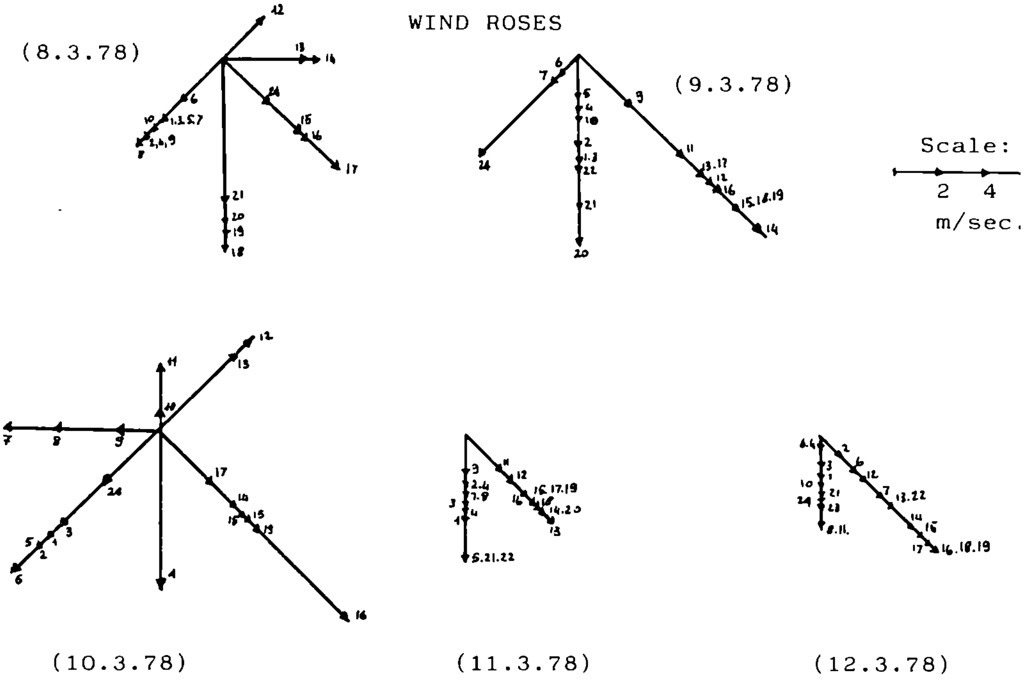


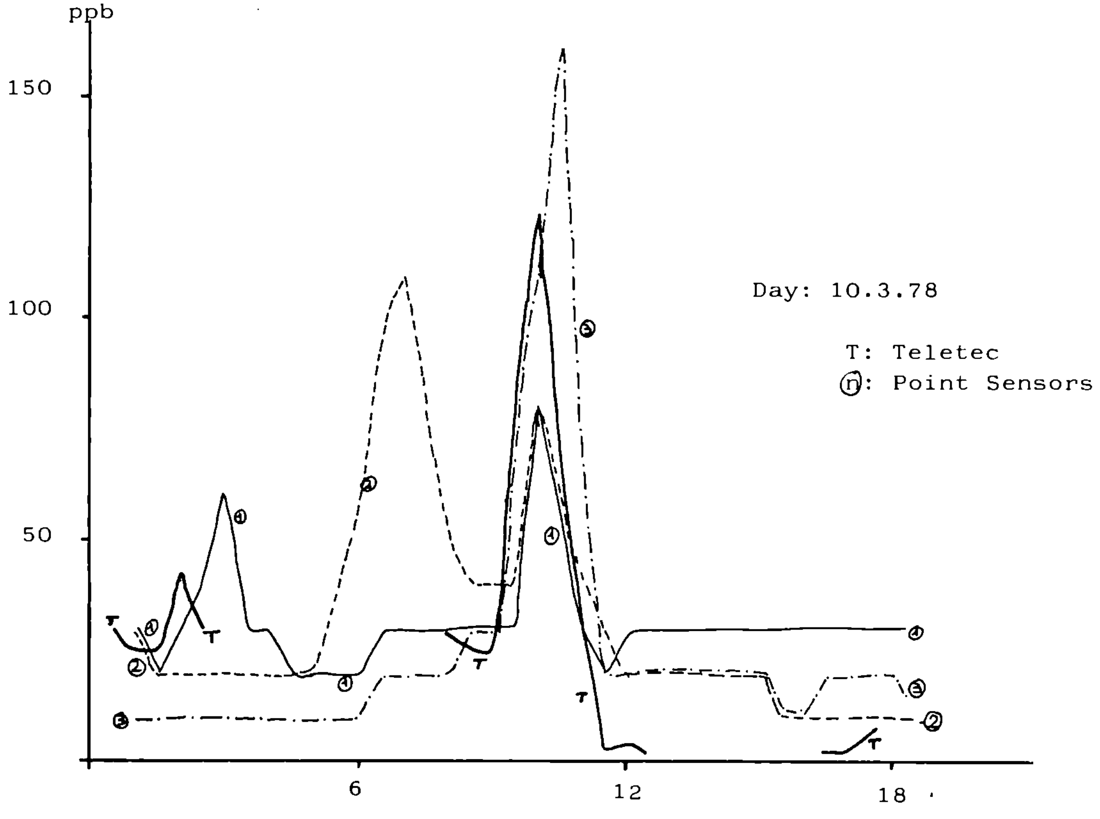
T: Teletec

ⓝ: Enel point sensor

Fig. 2

WIND ROSES





Contractor: Institute of Applied Physics TNO-TH, Stieltjesweg 1, 2628 CK DELFT
Contract No. 200-77- 1 ENV N
Project Leader: ir. H.J. Raterink
Title of project: Investigation of remote heterodyne detection techniques
to measure air pollutants (phase 3)

The objective of the research program in phase 3 is a further investigation of the application of heterodyne detection techniques for remote measurement of nature and concentrations of air pollutants. Phase 1 and phase 2 of this investigation were carried out under contract No. 107-74-11-ENV.N. Phase 3 is the first phase of the 2nd Environmental Research Program.

In phase 3 an advanced experimental set-up has been developed, using e.g. a new HgCdTe detector with a large electrical bandwidth as mixer and a tunable CO_2-laser type 500 A, manufactured by Apollo Inc. (USA) as local oscillator. To obtain a faster and more direct processing of the measurements, an electronic filterbank and a programmable calculator HP9830, coupled to the system through a HP6408 multiprogrammer, have been applied behind the detector. The electronic filterbank, initially developed by the Astronomical Institute of the University of Utrecht, consists of 8 channels with a 20 MHz channel width each, for measuring simultaneously a band of 160 MHz in the total bandwidth of 1200 MHz. This total bandwidth is scanned by using a second mixer together with a second local oscillator, which is stepped through the spectrum at frequency intervals of 160 MHz; one of the side bands is suppressed by adequate filtering.

The complete experimental set-up is given in figure 1.

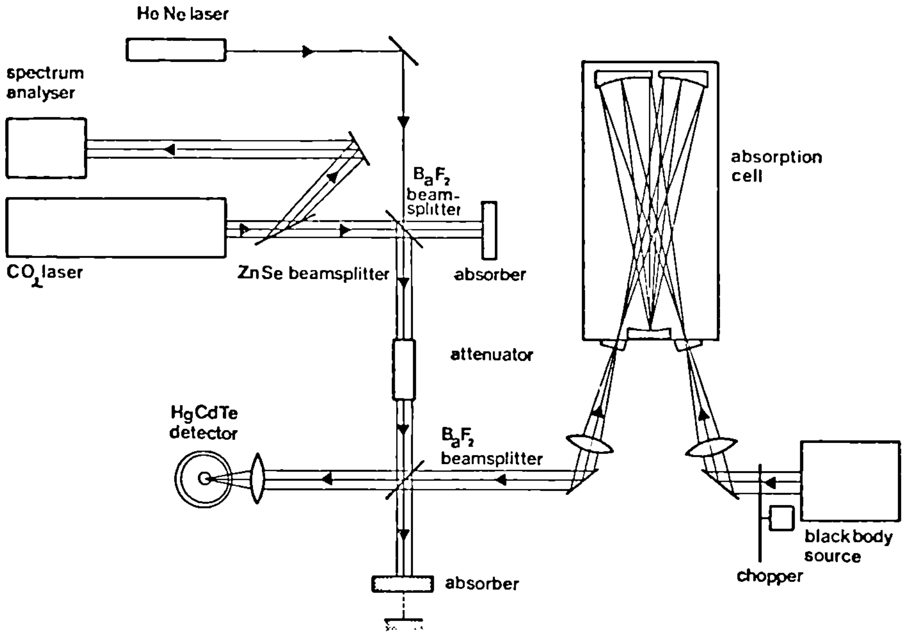
With the described set-up an extensive measuring program has been carried out. The following gases have been measured at different pressures and with or without mixtures of N_2 up to atmospheric pressure in the variable path absorption cell: C_2H_4, NH_3, SO_2, Freon12, Vinylchloride, CO_2 and N_2O. For all measurements the transmission of the empty cell was measured first in the total bandwidth of 1200 MHz. From 50 measurements of each measuring point the standard deviation is determined and stored. The measurements are then repeated with the gas under study in the measuring cell. The transmission is calculated for each measuring point from these two values and plotted together with the standard deviations of the 100 procent transmission curve and other relevant data.

Some transmission profiles of the gases NH_3 and SO_2 are presented in figure 2. The transmission profiles of SO_2, using the CO_2-laserline 9.2396 μm (R26), have been measured for the first time, so far we know.

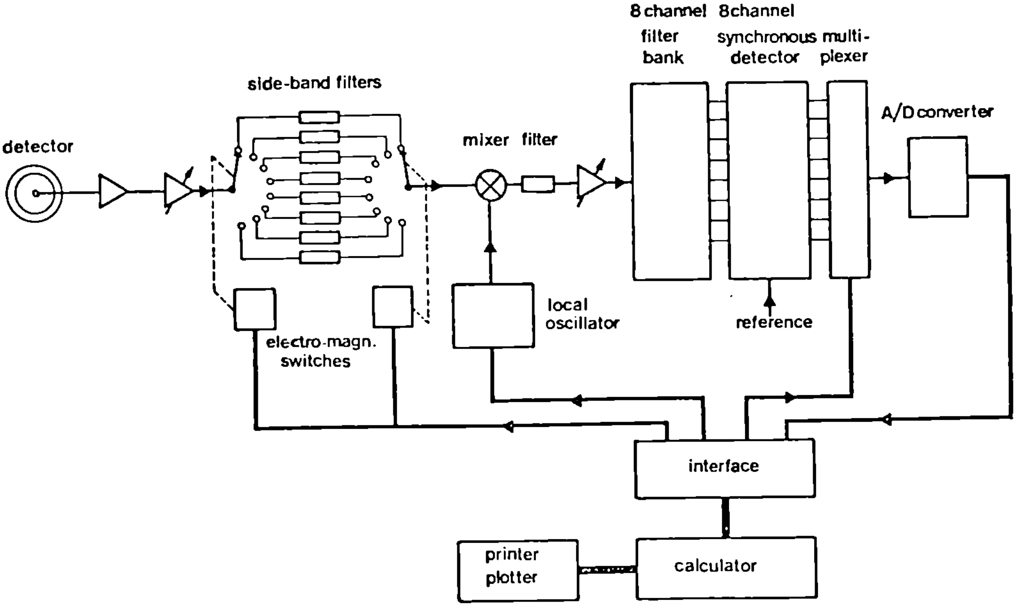
The absorption profiles have been analysed, taking the double side band detection into account. It means that if for a single absorption profile a maximum absorption percentage of 50 is plotted, a 100% absorption really occurs. The interpretation is more complicated, if the absorption measurement contains more absorption peaks in the bandwidth of 1200 MHz and if wings of absorption profiles of other gases occur also in this bandwidth. The occurrence of pressure broadening is clearly shown, which contains basic information for all absorption measuring techniques.

From the absorption profiles important parameters as absorption coëfficients and width at half max. have been derived. The center frequencies of several absorption lines of C_2H_4 have been measured with a higher accuracy than given in literature. The sensitivity (ppb-km) has been determined for the measured gases, using the data of the absorption profiles for pressures up to atmospheric pressure. From the pressure broadened profiles it can be decided which part of the total bandwidth behind the detector can be used for accurate measurement of the concentration of the pollutant. Knowledge of the occurrence of parts (wings) of absorption profiles of other gases in this total bandwidth must be available to make this selection for a practical system (active or passive). The results of all calculations are summarized in table 1. The sensitivity (ppb-km) has been calculated for a 20 MHz bandwidth region close to the wavelength of the applied local oscillator.

In the investigation, carried out in this first phase of the 2nd Environmental Research Program an experimental heterodyne detection system is developed, with which important information is obtained concerning the sensitivity of detecting air pollutants, using an active or passive laser system. The calculated absorption coefficients of the gases NH_3, C_2H_4, SO_2 Freon 12, Vinylchloride, CO_2 and N_2O with and without mixtures of N_2 up to atmospheric pressure, are compared with data given in litterature. The absorption profile of SO_2 has been measured, using the R26 line of the tunable CO_2-laser as local oscillator. The influence of pressure broadening is shown clearly and has been taken into account to determine the sensitivity (ppb-km) using a certain part of the total bandwidth behind the detector. The absorption profiles contain already interesting information about the presence of wings from other absorption lines; however to produce more quantitative information concerning these interference effects, additional measurements must be carried out. A more detailed report, containing all technical details and measuring results is in progress.

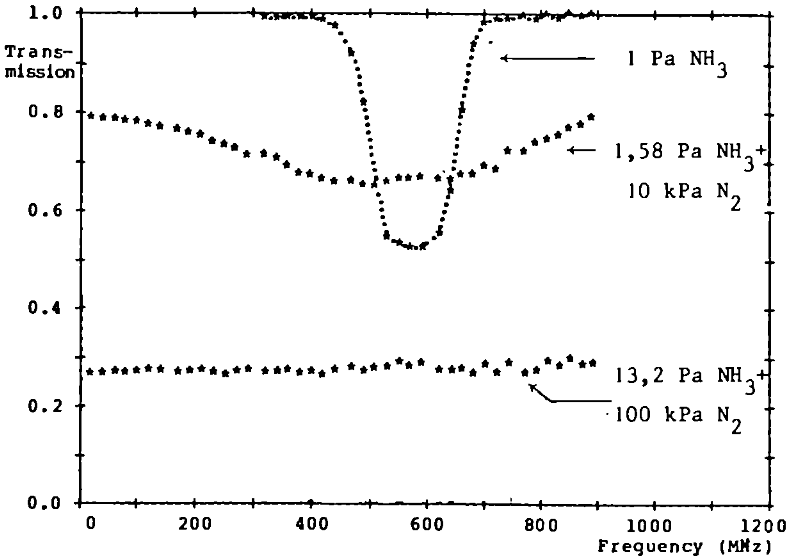


Optical System

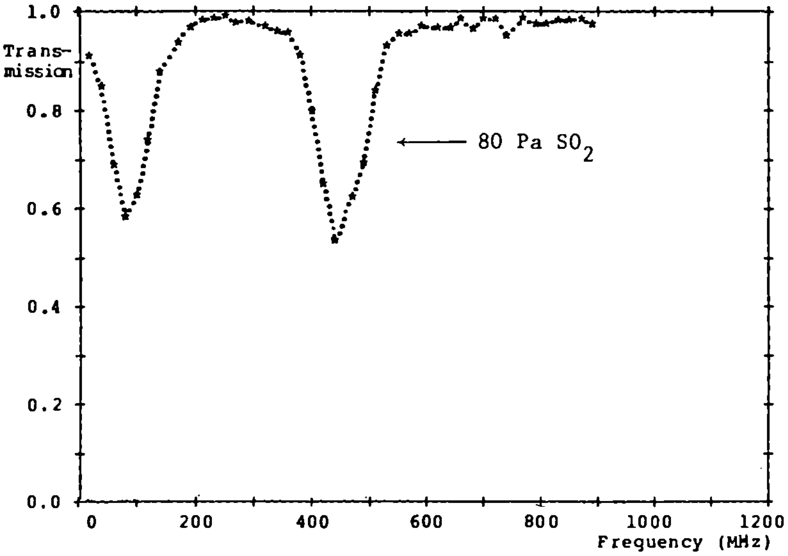


Electronic block diagram

Figure 1



Sample gas: NH_3
Band: $00^{o}1$-$10^{o}0$ Transition: R (6) Wavelength: 10,349 μm



Sample gas: SO_2
Band: $00^{o}1$-$02^{o}0$ Transition: R (26) Wavelength: 9,240 μm

Figure 2

Gas	NH_3	C_2H_4	SO_2		Freon12	Vinyl-chloride
Laser transition	R6	P14	R26	R30	P36	P22
Laser wavenumber in cm^{-1}	966.250	949.479	1082.296	1084.635	929.017	942.383
Gas wavenumber in cm^{-1}						
- litterature	966.269	949.454	1082.281	1084.666	-	-
- measured	966.2696	949.4951	1082.2814	-	-	-
Pressure broadening coëfficient in MHz/Torr$^+$						
- litterature	3.5	-	4.3	-	-	-
- measured	4.7	> 2.6	5.7	-	-	> 2.6
Abs. coëfficient in $(atm.cm)^{-1}$						
- litterature	30.0	33.0	-	0.11	45.0	8.8
- measured	28.8	35.5	-	0.13	43.9	9.0
Sensitivity in ppb.km						
- litterature	1.1	1.0	-	-	-	20
- measured	3.0	2.8	-	820	2.3	10.1

$^+$ 1 Torr = 133 Pa

Table 1

Contractor:	The University of Hull (UK)
Contract n°	141-77-1 ENV UK
Project Leaders:	Dr. B.J. Rye and Dr. E.L. Thomas
Title of Project:	An Infrared DAS System for the Remote Sensing of Air Pollution

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Introduction: The long term aim of this project is to develop an infrared, differential absorption by scattering (DAS) system based on a pulsed, multi-atmosphere, CO_2 laser transmitter and a heterodyne receiver, using a narrow bandgap, CW, diode laser as the local oscillator. Since both the above lasers can be continuously tuned over most of the 9.1 to 11.3 micron spectral range, it is expected that the remote sensing system will be capable of monitoring at least 8 to 10 pollutants with a similar range, range resolution and sensitivity to those currently obtained using U.V./visible, DAS systems and photomultipliers. The main advantages of an infrared, DAS system over an U.V./visible DAS system are that it will be eye safe, can be operated during daylight hours and is affected less by changes in the weather.

The objective of the present work is to test the feasibility of such a tunable, infrared DAS system using lasers which are readily available. Construction is nearing completion on a monitoring system designed to detect ozone (O_3) which uses a line-tunable, hybrid transversely excited, atmospheric pressure (TEA), CO_2 laser transmitter and a low pressure, CW, CO_2 laser as the local oscillator in the heterodyne receiver. The monitoring system is shown diagramatically in figure 1 and will be described in more detail below.

1. The transmitter laser: A hybrid, TEA, CO_2 laser was chosen as the transmitter. This laser consists of a pulsed, TEA section and a CW, section in the same resonator. The resonator is mounted on a steel girder frame-work and is mechanically decoupled from the pulsed section. Since it would be too expensive to passively stabilise a two metre resonator using invar rods, only active stabilisation has been used. However, the active stabilisation of the resonator length has been sub-divided in such a way that the long-term and short-term instabilities are countered separately. Long-term changes in the resonator length are off-set by heating a metal bar whereas the short-term changes can be accommodated by applying a voltage to a piezoelectric cylinder. It is convenient to stabilise the resonator length in this way since much lower voltages are required to drive the peizoelectric cylinder than would be the case if all the instabilities were countered using the piezoelectric cylinder alone.

A hybrid, TEA, CO_2 laser has several advantages over a simple TEA, CO_2 laser. Without hybridisation, the output frequency of the TEA laser would chirp at about 7 MHz.μsec^{-1}. For a 1μsec pulse, the chirp would represent a significant proportion of the detector bandwidth and would lead to a serious loss of signal. The chirp rate of the hybrid laser is about 100 KHz.μsec^{-1}. The CW section in the hybrid laser also offers a convenient method of measuring the relative frequencies of the transmitted pulse and the local oscillator. The CW laser can either be peak stabilised in the same manner as the local oscillator or it can be mixed with the local oscillator and the beat frequency minimised as shown in Figure 1. Finally, hybridisation leads to a longer more symmetric pulse.

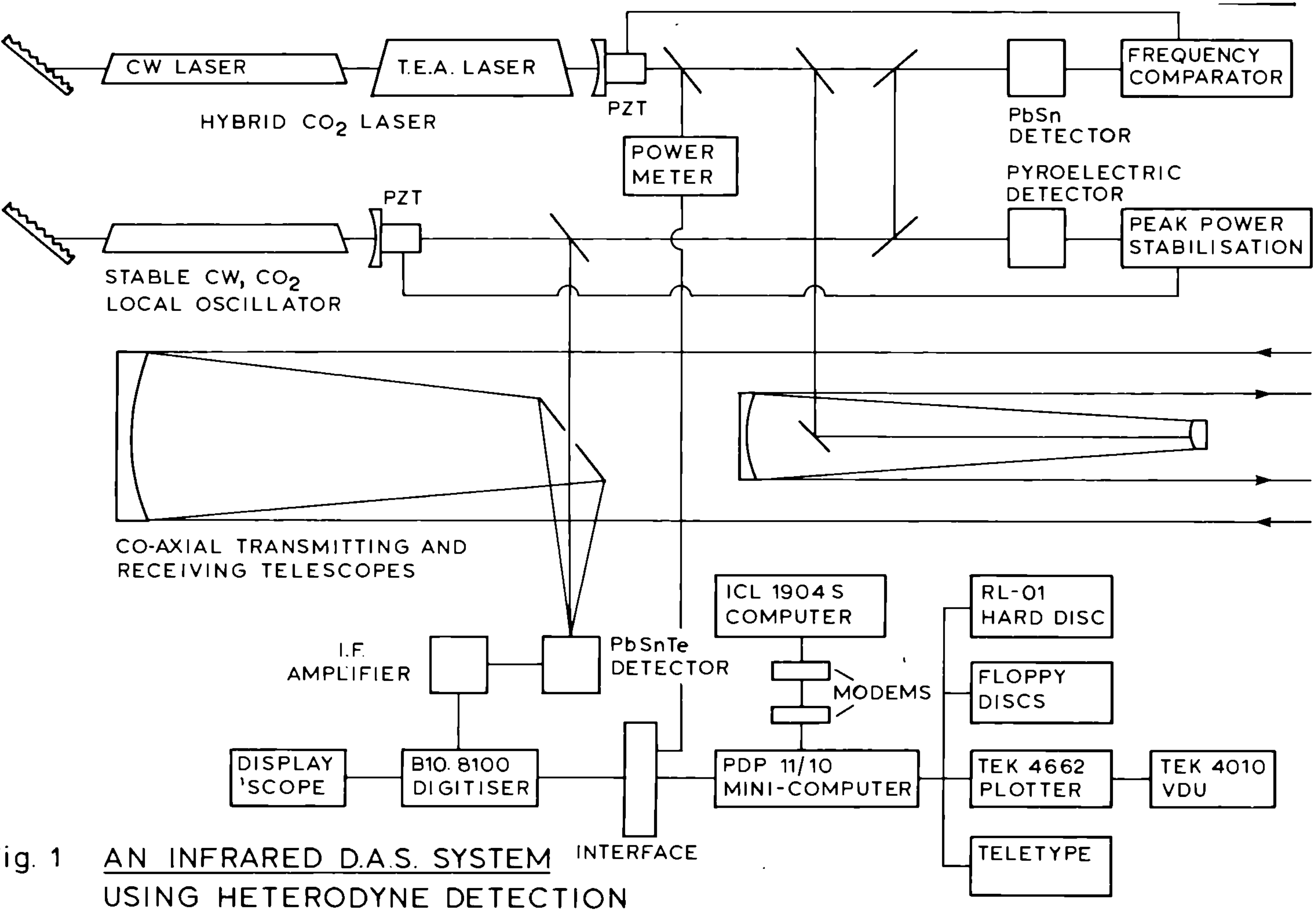

Fig. 1 AN INFRARED D.A.S. SYSTEM USING HETERODYNE DETECTION

The hybrid laser is capable of delivering 300mJ in a 1μsec pulse at repetition rates of up to 100Hz. The chirp rate, bandwidth and frequency stability (both within a pulse and from pulse to pulse) are being investigated.

2. The local oscillator: The local oscillator is a frequency stabilised, low pressure, CW, CO_2 laser. The resonator is passively stabilised using three invar bars and actively stabilised using a piezoelectric cylinder. At a power level of 200mW and using only passive stabilisation, the local oscillator can maintain a clean TEM_{oo} mode for more than one hour with an amplitude stability of 0.25 per cent. When this laser is locked to an absorption or a fluorescence band of CO_2, it should be capable of a short term frequency stability of about 1 part in 10^{10}. However, since we are only seeking a frequency stability equal to the reciprocal of the pulse duration over a ranging operation (i.e. ∿ 1 MHz), peak power stabilisation will be sufficient. The laser can easily provide enough local oscillator power on most of the lines in the 9.4 and 10.6 micron bands.

3. The telescope design: The telescope originally designed for this project has still not been delivered (approximately 18 months late). Consequently, it was decided to construct a second pair of coaxial telescopes, one for the transmitter and the larger one for the receiver, which were based on the best mirrors which could be obtained from stock. The primary mirrors chosen were a 30cm, f/6 parabola and a 15cm f/8 parabola. Both mirrors were intended for use by amateur astronomers and were manufactured by Grubb-Parsons. The mirrors were figured to λ/10 (in the visible) and were front surface aluminised.

3a. A computer program to design the transmitter optics: There are two versions of this program. The first version of the program assumes that the parameters of the primary mirror in the transmitting telescope have been fixed (e.g., by the availability of the mirror from stock) and that the laser beam can be matched into the transmitter telescope by varying the focal lengths and positions of two lasers in an auxillary beam expansion telescope. An interactive, BASIC language program has been used to solve the propagation equations of the laser beam through the beam expansion and transmitting telescopes. It has been assumed that the laser is operating in a TEM_{oo} mode and the beam is Gaussian.

The second version of the program has been prepared for future use and is designed to meet fixed output requirements. For a given beam waist radius and range, the program will suggest ideal values of the primary mirror diameter and focal length, secondary mirror diameter and focal length and the focal lengths and positions of the matching optics. Material damage and eye safety considerations are built into both versions of the program.

3b. The telescope system hardware: The chosen telescope system is coaxial and based on the mirrors described above. The transmitting telescope is a 15cm diameter, f/8 Cassegrain system. The receiving telescope is thus designed around a 15cm diameter obscuration. For this reason, a Newtonian configuration using a 30cm diameter, f/6 primary is used. By using a large Newtonian flat (as allowed by the 15cm obscuration already present due to the transmitting telescope), the overall dimensions of the transmitting system have been reduced to approximately 2.5 metres. Using the first version of the BASIC design programs described above, the length of the optical train was found to have a convenient value when the focal lengths of the lenses in the beam expansion telescope were 8 metres for the expander and 3 metres for the condenser. The laser transmitting optics are shown in Figure 2.

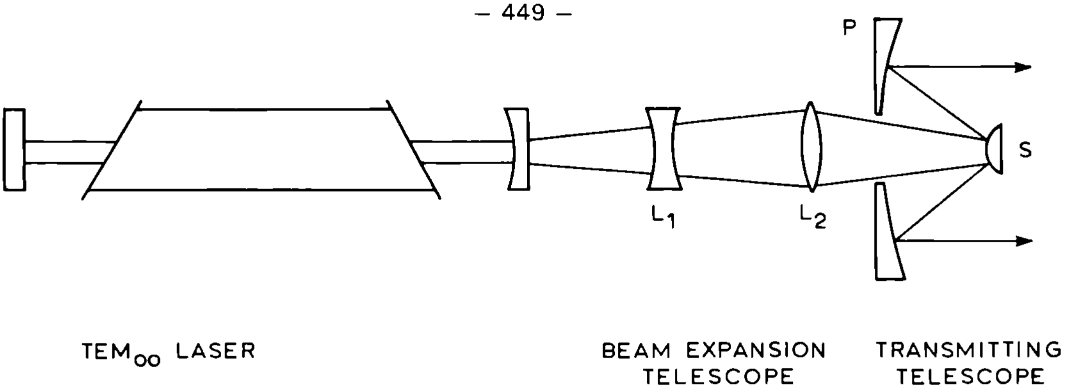

Fig. 2. LASER TRANSMITTING OPTICS.

The prime function of the receiving telescope is to image the cross-section of the scattering volume onto the detector. For detector diameters at the lower end of the range available (∿ 50µm), the angle subtended at the receiver by the cross-section of the scattering volume is approximately 144 micro-radians (∿ 29 arc.secs). Consequently the field-of-view and detector parameters introduce alignment constraints in the arc second region. Other system design considerations were:

(i) protection of coated optics from a dusty environment,
(ii) variations in operating temperature,
(iii) vibrations due to the location of the equipment and associated experimental equipment,
(iv) size and floor-loading constraints in the available space,
(v) maintainability of the system.

By compressing the two coaxial telescopes in series to 2.5m overall, it became possible to house the entire system in a single, rolled carbon steel tube of adequate strength to maintain optical alignment whilst remaining quite portable. The tube can be completely sealed when not in use so as to minimise maintenance of the optical surfaces.

4. The data processing system: The signal acquisition and data processing system shown in Figure 1 is now complete. The signal is acquired via a Biomation 8100 Transient Digitiser. Preliminary data processing is carried out in the PDP11/10 which is backed by an RL-01 hard disc and run under Version 3 of the RT-11 foreground/background operating system. The mini-computer is connected to a larger computer (an ICL 19045) by modems and the line is run by software which enables the minicomputer system to emulate an ICL 7020 terminal. The line to the larger computer makes available a more powerful machine and enables the remote sensing data to be outputted and/or stored on the peripherals of the larger machine.

The hard disc is a recent addition to the system and was found to be necessary because all the operating system software could not be accommodated on one floppy disc. The system software includes Version 3 of RT-11, Plot 10 packages for the VDU and plotter, a 7020 emulator and extended Fortran IV.

By using a signal generator to simulate the return signals expected, the data processing system has been tested and found to be capable of operating at laser pulse repetition frequencies of about 70Hz, (which is well within the capabilities of the hybrid laser).

5. Theoretical aspects of signal acquisition from incoherent laser backscattering: The basic principles of signal acquisition in incoherent backscatter laser radar have been reviewed placing emphasis on the use of the DAS technique for pollution monitoring in the infrared spectral region and the capabilities of heterodyne detection. The conclusions indicate that whereas greater sensitivity is obtainable at short range using direct detection, the presence of a high level of background noise in the infrared ensures that the sensitivity falls rapidly with range. For heterodyning, on the other hand, the sensitivity is essentially independent of range out to a limiting value which, in general, is larger than that attainable using photomultiplier detectors in the visible or near ultraviolet spectrum. The better transmission of the atmosphere at infrared wavelengths is mainly responsible for the improved performance.

Optimum sensitivity can be obtained in a heterodyne system by employing pulses which are as short as is consistent with the absorption linewidth of the transition under observation, using an array of detectors and a signal processing network of matching bandwidth. The pulse energy density in such a system would appear to be well within the currently recommended, maximum exposure levels.

An analysis of the geometrical parameters of importance in DAS systems (but neglecting atmospheric scintillation) has been carried out. Expressions have been obtained relating the heterodyne system antenna gain and the two components of the heterodyne efficiency (associated with the amplitude profile and wavefront curvature of the local oscillator respectively) for arbitrary range, transmitter aperture function and receiver antenna geometry. The analysis assumes circular symmetry in the optics. Computation of these quantities has been carried out with a view to producing a parametric, graphical display which will facilitate design calculations.

6. Progress to date: The construction work (apart from some relatively minor modifications) for the first phase of this project has been completed. Efforts are now concentrated on obtaining some preliminary measurements using direct detection and a topographic target. This will enable us to confirm the alignment of the transmitting and receiving telescopes. In parallel with these measurements, the local oscillator laser and the detector/preamplifier package are being made ready for using in the heterodyne system.

Looking into the future and the further development of this DAS system, work has also been started on

(a) a scanning telescope (to take data from any direction)
(b) a short pulse ($\leq$ 5 nanoseconds) CO_2 laser to be used with a matching fast detection system (to relax the stability requirements on the lasers and to improve the statistics of signal acquisition),
and (c) a tunable, multi-atmosphere CO_2 laser (to enable us to detect more pollutants).

7. Publications and oral communications:

1. F.J. Bryant, B.J. Rye and E.L. Thomas, "Pollution Monitoring Work at Hull", Report to the Remote Sensing Working Group, NPL, Teddington, U.K., January 1977.

2. C.J. Koh, H.D. Pandey, B.J. Rye, E.L. Thomas and L.W. Winchester Jr., 'An Infrared DAS Remote Sensing System using Heterodyne Detection', Conference on 'Lasers in Chemistry', Royal Institution, London, May 1977.

3. C.J. Koh, H.D. Pandey, B.J. Rye, E.L. Thomas and L.W. Winchester Jr., 'An Infrared DAS Remote Sensing System using Heterodyne Detection', 8th International Laser Radar Conference, Drexel University, Philadelphia, Pa., U.S.A., June 1977.

4. E.L. Thomas, 'Laser Techniques for Pollution Monitoring', 3rd National Quantum Electronics Conf., Southampton, September 1977.

5. B.J. Rye and E.L. Thomas, 'Pollution Monitoring Work at Hull', Second Report to the Remote Sensing Working Group, Leatherhead, U.K., November 1977.

6. B.J. Rye and E.L. Thomas, 'An Infrared DAS System for the Remote Sensing of Air Pollution', Meeting of the EEC Contact Group, Leatherhead, 9-10 March, 1978.

7. B.A. Greene, J.L. Pinto, B.J. Rye and E.L. Thomas, 'Heterodyne Receivers in Atmospheric Backscatter DIAL Systems', IOP Meeting on 'Atmospheric Sensing with Lasers', London, 9-10 October, 1978.

8. E.L. Thomas, 'Infrared Techniques for Atmospheric Pollution Monitoring', SIRA Meeting on 'Industrial Applications of Infrared Technology', London, 7-8 November, 1978.

9. B.J. Rye, 'DIAL System Sensitivity with Heterodyne Reception', Applied Optics 17, 3862-4 (1978).

Contractor : National Physical Laboratory, Teddington, UK
Contract n° : 193-77-1 ENV UK
Project leaders : J.R. Gott and I.H. Curtis
Title of project : Differential absorption system to monitor air pollution using tunable diode lasers

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Objectives

To establish a long-path monitor for measurement of ambient concentrations of carbon monoxide and other pollutants in the open atmosphere using a tunable diode laser.

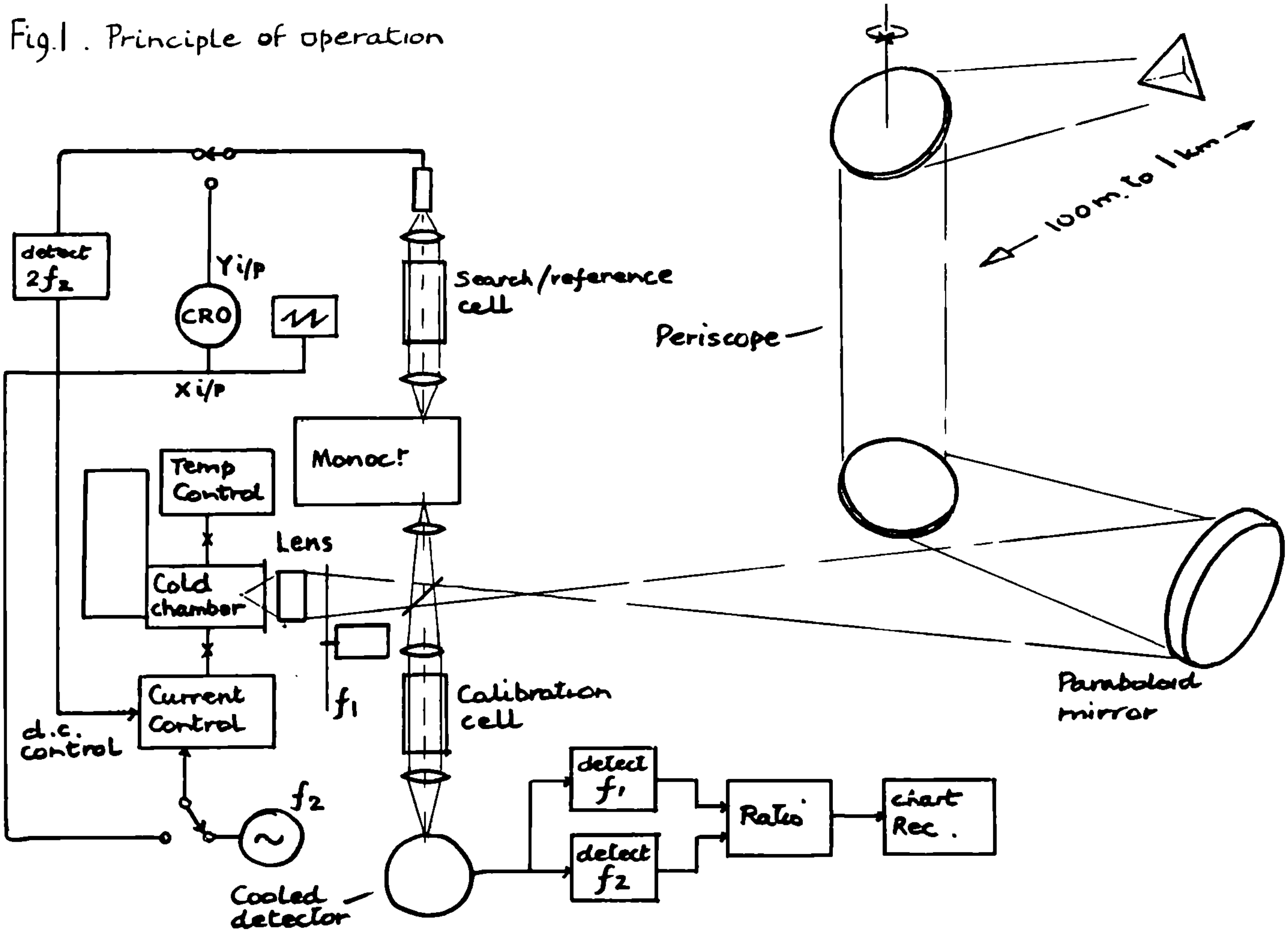
To investigate in detail those aspects of the method which bear upon the accuracy and validity of measurement, emphasizing potential sources of systematic error.

To evaluate the technique in field operation and to determine the extent to which this type of environmental measurement is valid and reliable.

Method

A schematic representation is shown in figure 1. The laser is available commercially from Laser Analytics Inc. The Cryogenic Technology, closed-cycle cooler can, if necessary, accommodate two lasers simultaneously and will maintain indefinitely specific temperatures between 10 and 50 K. The laser material for the initial application is PbSSe emitting at approximately 4.7 μm cw, which is suitable for carbon monoxide. Tuning is achieved by adjusting the diode temperature and the drive current. Stripe-geometry lasers emit with large beam-divergence and this necessitates the use of a special, wide-angle collecting lens. The focussed beam is projected by an off-axis paraboloidal mirror to a cube-corner retroreflector situated at a distance of between 100 m and 1 km, the return beam being focussed, via the beam splitter, on to a cooled detector. Total power available is about 10 mW in up to 20 modes. The frequency range is about 100 cm^{-1} but continuous tuning is possible over bands of order 1 cm^{-1}. The beam divider also provides a reference path for purposes of monitoring and controlling the frequency of the radiation. The output beam is chopped mechanically at about 150 Hz and the wavelength is modulated at around 1 kHz. The entire transmitter-receiver assembly will be mounted in a small van which is intended to serve as a mobile laboratory for field work.

Fig.1 . Principle of operation



Laboratory Measurements

The structure, stability and reproducibility of the mode spectrum of the diode laser are of fundamental importance in evaluating its potential as the basic component in a pollutant monitoring system. Information on these factors is at present inadequately documented, and for this reason it has been necessary to analyse in detail relevant aspects of the output characteristics of the diode laser.

Information has been obtained (main report) for the mode structure and spatial distribution of power from the laser. The relevant CO spectroscopy has been carried out, and the development of real-time spectroscopy techniques has made possible an instantaneous atmospheric spectral display which is necessary for field work.

Operating problems

The operating method to be used for field work (figure 1) incorporates techniques (main report) for minimizing errors arising from atmospheric turbulence and laser frequency drift. Other potential sources of error which have been evaluated include laser noise, calibration problems and laser aging.

Field System

(a) Laboratory-based measurements

The optical assembly is to be mounted on a honeycomb table which will be used initially for laboratory-based measurements and later transferred to the mobile laboratory. The system will shortly be evaluated by measurements on carbon monoxide from a laboratory window over a path length of approximately 200 m. A laser with 70% of the total power in one mode will be used for these measurements, and a 150-mm diameter, off-axis, parabolic mirror will project the beam to a hollow cube-corner retroreflector of inscribed circle 125 mm diameter. The detector is cooled InSb in a dewar of 24-hour holdtime.

A laboratory simulation of long-path measurements has been carried out. The gas cell contained 1% carbon monoxide in one atmosphere of air and the P(4) line at 2127.7 cm^{-1} was used. Noise on the record was equivalent to about 150 in 10^9 over a 300-m range, assuming effective ratioing. Since two orders of magnitude of improvement are possible by using the cooled detector and a longer time constant, we estimate a sensitivity for field measurements of a few parts in 10^9.

(b) Mobile system

The instrument described above and illustrated in Figure 1 will later be transferred to a small van. The sub-structure for the optical table has been designed so that, in operation, the legs can be lowered to the ground, the optical assembly thus becoming independent of the van. The laser beam will be transmitted via two plane mirrors and a rotatable periscope to give a 360° field of view, thus permitting operation with more than one retro-reflector if desired. It is anticipated that this phase of the project will be field-tested early in 1980.

Conclusions

Evaluation of the diode laser system and associated equipment has been completed.

A laboratory-based monitoring system will shortly be tested by long-path measurements on carbon monoxide in the open atmosphere.

It is anticipated that the mobile system will become operational in 1980. It is estimated that the pollutant detection sensitivity will be a few parts in 10^9.

Associated work: differential-absorption lidar

A lidar system for remote detection and measurement of atmospheric SO_2 and NO_2 is currently being assembled at NPL by Dr P T Woods and Mr B W Jolliffe. The system uses an Electro-Photonics dye laser producing pulse energies of approximately 16 mJ in the visible and 1½ mJ at 300 nm after frequency-doubling by an ADA crystal. Assembly of the optical components is almost complete, and this equipment, together with associated on-line data-processing facilities, will be housed in a van for field test during 1979.

A dye laser has also been used to make high-resolution measurements of SO_2 and NO_2 absorption coefficients as a function of probe-laser line-width, concentration, temperature and buffer gas (N_2) pressure. Significant differences have been found from values previously reported in the literature. This work emphasizes the importance of reliable laboratory measurements for environmental applications if systematic errors in field data are to be avoided.

Work is also proceeding at NPL on the construction of a high-resolution ($0.1\ cm^{-1}$) mid-infrared interferometer for laboratory measurements on molecules of environmental interest.

Contractor : Central Electricity Generating Board
Contract No. : 290-77-1 ENV UK
Project Leader : Dr R H Varey
Title of Project: Development and application of remote sensing for measurement of air-borne materials

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OBJECTIVES

The aim of this program is to construct a mobile differential lidar for the measurement of SO_2, NO_2 and other gases in the atmosphere. Subsequently the instrument will be compared with other similar apparatus such as the Hull University infra-red system (contract 141-77-1 ENV UK) and used in an operational mode to make pollutant measurements.

MATERIALS AND METHODS

The project is being carried out in two stages. The first comprises the production of a non-mobile prototype and the second covers the installation and development of the system in a vehicle to provide a fully mobile facility which can be used in the field to traverse industrial plumes. The main components of the system are a CMX-4 tunable dye laser, a PDP-11/34 computer and a specialised vehicle - all of which have been purchased in the period 1977-78. The prototype system has been assembled and the programme of experiments with it is complete. The major part of the optics and receiver electronics for the mobile facility is being supplied by Ferranti Ltd. Delivery of this system is now nine months late but is expected by mid-March 1979.

RESULTS

The prototype has been used to measure SO_2 concentrations in plumes at Drax power station - during the CEC campaign 1976 - Fawley power station and at a location in central London. It appears that the sensitivity of the system is within a factor three of that predicted theoretically, a range resolved sensitivity of 150 ppb being easily obtained at a range of 1.5 km. At shorter ranges the performance is similarly close to expectations (e.g. at 500 m range approximately 10 ppb is easily detected in a range resolved manner). Expected performance figures are produced in Adrain et. al. 1977. The small discrepancies between expected and measured performance can be accounted for in terms of factors such as optical alignment, photomultiplier efficiency etc. It is expected that the performance will be improved in the final system.

Considerable attention has been paid to the shape of the light pulses emitted by the laser. Pulses at two adjacent wavelengths are required to measure SO_2 and it has been found that although the difference in wavelength is only a few Angstroms there can be consistent differences in the pulse length at the two wavelengths. This introduces errors in the measurements for small ranges, a problem which is being overcome by chopping the tail of the pulses with a pockels cell.

Deconvolution techniques have been used to increase the spatial resolution of the measurements. The resolution available with the 1 μs pulses from the CMX-4 is about 150 m and this can be improved by a factor of two or three, the limit being set by noise in the signals.

CONCLUSIONS

Differential lidar has been shown to have a performance close to that predicted theoretically. During the next six months the fully mobile system will be developed for use in the field and it will be used in the CEC campaign at Turbigo in September 1979. As soon as the Hull University infra-red device is operational a comparison will be made between results obtained with both systems.

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TOPIC 16 : WATER QUALITY

Ecological effects of water pollution

Contractor :	Universität des Saarlandes D 6600 Saarbrücken
Contract no :	242-77-1 ENV D
Project leader :	Prof. Dr. Heinrich Kaltwasser
Title of project :	Ecological effects of pollution by heat, industrial and municipal wastes on microorganisms in a river ecosystem

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Objectives of the research

The effects of industrial, municipal and thermal pollution on activity and diversity of microbial populations were studied by numerical taxonomy and uptake kinetics. The mechanism of adaptation to heavy metals was studied in algal strains isolated from a polluted river. The applicability of fecal indicator bacteria in hygienic control steps was studied in river sediments. Furthermore, the production and consumption of methane, hydrogen, and sulfide by microorganisms were investigated as parameters of sediment/water interaction in order to reveal the effects of canalization on the carbon and sulfure budget of the river Saar.

1. Effects of industrial, municipal and thermal pollution on diversity and activity of bacterial populations

1.1. Materials and methods: To characterize the diversity of bacterial populations, plate counts were determined on 21 different media. A representative number of bacterial strains was isolated and 74 to 93 characters were determined. Using a computer program the strains were grouped by single linkage clustering, and the clusters from different experiments were compared with subsequent computer programs. The activity of heterotrophic microbial populations was determined by the incorporation of ^{14}C-labeled glucose, according to the method of Hobbie & Wright (1965).

1.2. Results: Numerical taxonomy of strains isolated from parts of rivers with different degrees of pollution revealed that the diversity of the bacterial population was inversely related to the degree of pollution. Some clusters were sensitive to environmental pollution, others were resistent and distributed over various habitats. Toxic effects of the milieu resulting from industrial waste discharges were detected during uptake experiments performed with natural microbial populations following transfer to water from other habitats. At different habitats and seasons, the optimum temperature for uptake velocity at saturating substrate concentration was observed between 25 and 30°C in the river Saar. At temperature slightly above this optimum less glucose was taken up, but a higher proportion of it was respired. Temperature optima were lower at low substrate concentrations due to an increase in substrate affinity. Consequently, in river populations the turnover time of glucose was lowest at 15° C. In populations from a cooling tower, however, it was lowest at about 30° C. Both values were found to be close to the mean water temperature at the specific habitat.

1.3. Conclusions and Comments: Numerical taxonomy grouping of bacterial strains turned out to be applicable to monitor biological effects of industrial waste discharges on a river ecosystem. Improvement of this technique may allow to determine water quality criteria as based on the diversity of microbial fresh water communities. So far, temperature increase was generally believed to enhance microbial activity and thus to accelerate self-purication in fresh water systems. Our experiments indicate, however, that bacteria are well adapted to the environmental conditions in the river, e.g. to low substrate concentrations and low temperatures. Increased water temperature may therefore "in situ" lead to decreased microbial activity and impaired self-purification due to decreased substrate affinity.

2. Uptake, accumulation and tolerance limits of heavy metals in pure cultures of representative strains of the algal flora

2.1. Materials and Methods: The algae were cultivated under sterile conditions according to Meisch and Bielig (1975). Heavy metals were determined by atomic absorption spectroscopy in a Perkin-Elmer 420/HGA-74 apparatus following injection into the graphite furnace (Meisch and Reinle, 1977) or after acid treatment in a teflon autoclave.

2.2. Results: The effect of chromium was studied in Chlorella pyrenoidosa and in a wild Chlorella strain, which had been isolated from a Cr-polluted river. In permanent light chromium caused a decrease in chlorophyll content, with chromate more toxic than Cr(III)-glycine. The wild strain was more sensitive towards Cr-compounds, it exhibited, however, a limited uptake of this trace metal. Toxic effects of Cr on cell division were only observed with Chlorella pyrenoidosa. Under a light/dark regime Cr was found to be more toxic for both strains than in permanent light; the wild strain, however, was only slowly effected by Cr(III)-glycine. - Vanadium, essential for growth and chlorophyll synthesis, turned out to be inhibitory to cell division. In permanent light, 10^{-5} g-at V/l (as NH_4VO_3) caused a 5-fold enlargement of the cell volume. Inhibition of nuclear division was accompanied by the appearance of giant nuclei with polyploid sets of chromosomes. In synchronous culture, vanadium caused total inhibition of cell division after 3 periods. Chlorella was found to tolerate up to 150 µg V/g dry weight; higher concentrations caused a decrease in biomass production.

2.3 Conclusions and Comments: Obviously green algae are able to adapt to chromium present in the environment. The effects are further studied with respect to food chains. In the case of vanadium it was shown that a heavy metal which is not regarded highly toxic may be able to influence basic metabolic events in algae, thereby severely disturbing the normal reproduction rate.

3. Activity, distribution and destiny of waste water microorganisms

3.1. Material and Methods: Fecal indicator bacteria were determined in water and sediment samples using standard techniques. Clostridium perfringens was determined by membrane filtration and by Hungate technique. Hydrogenase and CO_2-production was determined according to Zaiß and Kaltwasser (1979).

3.2. Results: In the water, colony counts of indicator bacteria were liable to fluctuations according to municipal sewage discharge characteristics as shown in longitudinal profiles of little creeks and to diurnal rhythms at single sampling stations. In sediment C. perfringens reflected best the hygienic situation. Vertical sediment profiles revealed a penetration into deep layers of all indicator bacteria studied. In laboratory experiments C. perfringens was more resistent to environmental conditions, whereas E. coli did either multiply or rapidly die. Under conditions similar to the river biotopes, metabolic activities of C. perfringens measured as hydrogenase activity and CO_2

production decreased constantly.

3.3. Conclusions and Comments: As drinking water supply has become more dependent on surface waters, control criteria for sediment analysis must be improved. Adsorption and survival of pathogenic and of indicator bacteria in sediments demand to include sediments into the hygienic water control, since resuspension of sediment may be dangerous. Despite of its ubiquitous presence C. perfringens turned out to be most suitable as indicator for fecal pollution in river water and sediments.

4. Significance of microorganisms within the sediment/water interaction: Possible effect of a river canalization

4.1. Materials and Methods: Methanogenesis, methanogenic bacteria, hydrogenase activity, methane oxidation and methane-oxidizing bacteria were determined in the river Saar according to Zaiß et al. (1979) and Zaiß and Kaltwasser (in press), desulfurication and desulfuricants according to Zaiß and Kaltwasser (1979)

4.2. Results: Canalization of the middle part of the river Saar led to a significant increase in methanogenesis, sulfide production and interspecies hydrogen transfer. Annual and vertical sediment profiles of these activities revealed close synthrophic relation between these bacterial groups. Depending on sulfate penetration (which was increased at high-water flows) the electron flow shifted to sulfate or to methane production. Like in the rumen, hydrogen and carbon dioxide were main precursors of methane in the sediments of the river. Microbial methane oxidation turned out to be of minor importance to the carbon and oxigen budget. Methane-oxidizing bacteria and their activities, however, correlated well with methane concentrations determined in the river water.

4.3. Conclusions and Comments: Methanogenesis turned out to play a significant role in the carbon budget of the Saar. About 20 % of the particulate carbon is transformed to methane within the canalized river part. Only 0.2 % of the methane is oxidized in the water, but most of it escapes to the atmosphere. Presence of sulfate increases the oxidation of organic matter under anaerobic condition, leading, however, to the formation of toxic sulfides. Further investigations should elucidate the role of methane-, hydrogen- and sulfide-oxidizing bacteria at the sediment surface.

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Contractor:	Bundesanstalt für Gewässerkunde, Koblenz
Contract No:	276 - 77 - 7 ENV D
Project leader:	Dr. Peter Kothé
Title of project:	Typology of running waters in rural areas

Objective of the research

The project deals specifically with small watercourses in the order of brooks, which are wide-spread in the most diversified regions from the alpine areas to the coastal lowlands. The brooks of the diverse regions differ in their hydrological, hydromorphological and hydrobiological character as much as the areas in which they flow. The differences are more or less complex and involve the entire structure and dynamics of the brooks, as well as their reaction to anthropogenic influences and interferences. As far as these small watercourses are concerned, the validity of observations, data and practical experiences is, therefore, to a high degree confined to special geographical regions.

The purpose of the research project is to develop a typology of brooks which meets the following requirements: This typology should

- determine the regional characteristics of the brooks on an interdisciplinary basis, as far as they result from the natural geographical characteristics of the regions

- quantify the differences between the regional types of brooks, as far as they are scientifically significant and also important from the practical point of view of water management

- give a survey of the variety range and type diversity of brooks in the Federal Republic of Germany

- determine factors and criteria which may contribute to improved utilization and protection of the waters of rural areas and to the development of a more natural hydraulic engineering and maintenance pattern for small watercourses

- establish a common brook classification system allowing differentiation between basic brook types, which can then be further differentiated under specific aspects

- enable, for instance, the hydrologist, the biologist and the water management expert to assign to the same extent any given brook unequivocably and with interdisciplinary validity to a defined type of brook.

Due to this objective, the brook typology to be developed differs necessarily in a number of ways from the stream typologies suggested in the presently available literature. The project endeavours, of course, to take these typologies into account as fâr as possible, but an essential task of the project is the development of an individual typological conception, which meets all the above-mentioned requirements.

Materials and methods

The data encountered in the literature of the various disciplines are too incomplete and inhomogeneous to serve as a basis. In some respects, the literature contains nearly no relevant information. Of course, the project tries to utilize as far as possible the knowledge already available from literature. In addition, it tries to evaluate unpublished data of various other institutes, but our own systematical research is the purpose of the activities.

The research purpose is approached from three sides, so to speak. On one side, there is the evaluation of relevant oro-hydrographical and thematic maps. This map evaluation aims at

- a water-related classification of regions
- a universally applicable sub-division of the brook networks into hydrologically equivalent reaches or brook formations

- the selection of representative brook model areas for the practical stream investigations as such

On the other side, there are the practical stream investigations carried out periodically. These investigations form the largest part of the project. During the surveys, hydrological and hydromorphological, as well as hydrobiological and ecological measurements and observations are undertaken.

On the third side, there are the collection and evaluation of long-term climatological and hydrological data series. These are collected primarily from official meteorological stations and gauging stations. In addition, data series are recorded at our own points of measurement.

The brook model areas mainly used for the practical investigations are located in different parts of the Federal Republic of Germany. They were selected to meet the following requirements: each of them is characteristic and, therefore, representative of one specific geographical region type in respect of its orographical and hydrographical structure, its altitude range, its mean specific discharge, and the hydraulic gradient of the brooks. Each area is homogenous geologically and in its topographical structure, only sparsely populated or entirely unpopulated, and extensively wooded. The brooks are not regulated, and the pollution is so slight that no significant change of the hydrobiocenosis need be expected. There are several official gauging stations, and at least one climatological station.

Results

The project was started in summer, 1977. In view of the broad scope of subject coverage, it was laid out for relatively long running term. Initially, research work concentrated on the development and theoretical foundation of a purpose-oriented typological conception.

On the basis of the available geographical and hydrological knowledge, the data taken from literature, and comparative map evaluations, a brook type system was developed providing for a general differentiation between four basic brook types: alpine brooks, mountain brooks (subalpine), highland brooks, and lowland brooks. Each of them was subdivided into two sub-types which were designated as carbonate brooks and silicate brooks.

During a second working phase, a classification scheme was developed which allows the subdivision of large brook systems into hydrologically and topologically equivalent reaches and hydrotopes. This scheme introduces a general differentiation between three brook formations which, starting from the source, are called formations of the 1st, 2nd and 3rd order.

During the third working phase, the brook model areas that seem suitable for representative practical investigations and for a first, approximative verification of the type system are determined.

A systematical study of these brook model areas was begun in summer, 1978. The investigations were initially of a more extensive and orientational character, with emphasis on hydrochemical and benthos-biological studies. Even though the abnormal and unfavorable weather conditions prevailing in 1978 affected the investigations considerably, the results clearly tend to confirm the suitability of the typological conception.

A first summary of our investigations into the macrozoobenthos is given in Figures 1 and 2. These graphs are based on the analysis of 360 benthic samples taken at 90 representative sampling points. The samples were taken in all brook areas at the same time (same season), using the same method. In taxonomic analysis, always the same degree of differentiation was used. The regional differences in the structure of the benthic population depend on natural differences of the biotops. The results of the chemical analyses of about 180 water samples show that no pollution is involved.

On the basis of the investigations carried out until the end of 1978, a detailed working programme for the next years, subdivided into 12 subject groups, has been worked out.

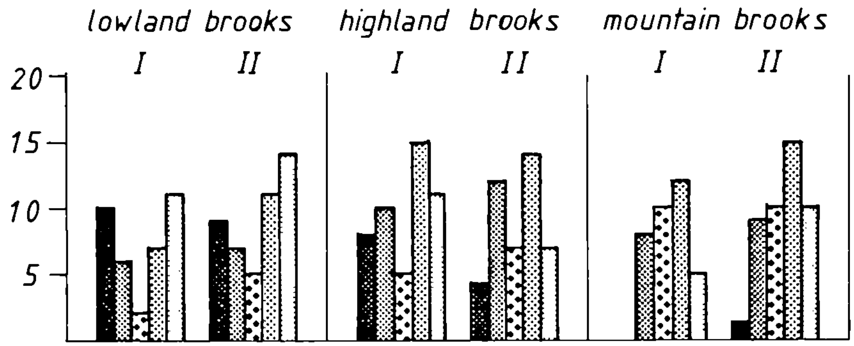

Fig. 1: Number of genera of 5 organismgroups

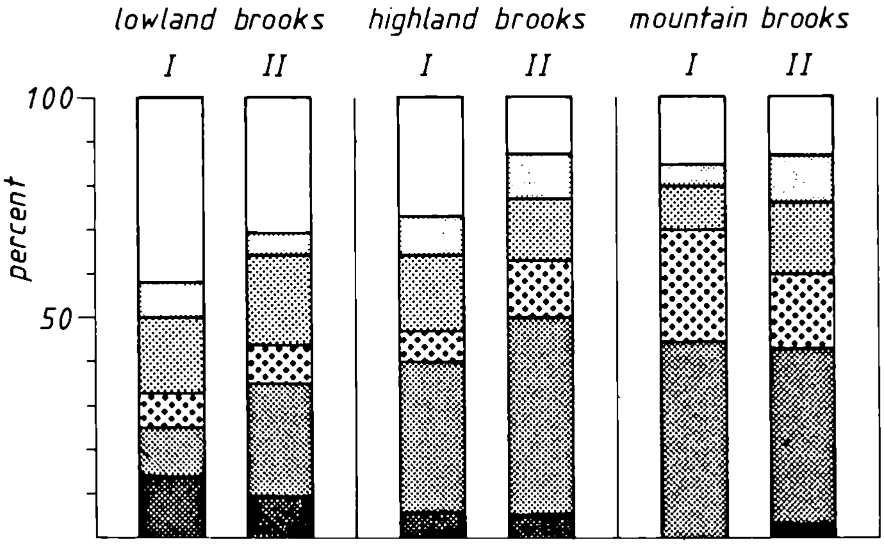

Fig. 2: Quota of individuals of 5 organismgroups

I = carbonate brooks II = silicate brooks

■ = Mollusca ▦ = Plecoptera ▭ = Coleoptera

▩ = Ephemeroptera ░ = Trichoptera □ = other groups

Conclusions and additional comments

The investigation results obtained so far indicate that the typological conception developed here fits the purpose and can be realized. They confirm that the natural differences between the brooks are, actually, so fundamental and varied that they require a more intensive typological coverage for scientific reasons and to meet the needs of water management and water protection. To attain the aims of the project with the present allotment of funds and personnel, a period of three more years is required.

Contractor : Institut Européen d'Ecologie
1, rue des Récollets, METZ

Contract n°: 212-77-1 ENV F

Project Leader : M. PELT

Title of project : I. Hydrobiological studies on Moselle's fluvial ecosystem
II. Ecotoxicological studies
III. Impact analysis

I. HYDROBIOLOGICAL STUDIES ON MOSELLE'S FLUVIAL ECOSYSTEM

A. Physico-chemical and biological characteristics inventory. Sampling problems. Problems to apply biological methods for the assessment of surface water quality.
The study concerns the river MOSELLE between the french frontier and the confluence of the river SARRE with 8 stations and 4 annual prospections. Physico-chemical and biological analyses were made to resolve 2 problems : sampling and application and discussion of biological index'methods.

A.1. Sampling methods

We applied artificial substrate method as one.
For the benthic flora, artificial substrates were tested too. (glass, plastic, wood ...). We have not yet precised if these kinds of substrates are faithfull to give good relations between algal populations and water quality.

For the benthos, the results can be resumed as following :

A new type of composite artificial substrate was developed for the river MOSELLE. Because of its simplicity its easiness of use on field, and its reproductibility, we can use this substrate as sampler in a biological survey of a downstream zone.
The characteristics of use have been specified and allow us to use it for a river.
It is now possible to have a standard sampling which represents the natural population enough to compare a station with another one. It makes easier the actual biological methods application, or the possibility of using other methods.

A.2. Application and discussion of biological index methods

We have made a comparative study of the main biological methods by taking one example in each following group :

- biotic indexes
- diversity indexes
- saprobial methods

For what concerns benthos and algal flora, the results in this situation that is downstream, allow us to make some remarks :
It's difficult to apply some of the technics because we haven't enough data concerning the ecology of the species we have found. (sarobial system).
The methods are not sensible enough to distinguish differences between the studied stations.
It is very difficult to compare the different methods because everyone has its own estimating reference for water quality, with different classing numbers for example.

For a hydrobiological survey of a downstream zone, in conclusion of river, these studies have shown that :

- It was necessary to avoid a standard sampling.
- At present, we use several methods to analyse the biological water quality.
- Only a pluridisciplinary study can give data.

Now, it is possible to develop a new methodology using more quantitative data like :

- algal biomass, primary production
- dynamic of population of one or two dominant species.

B. Conception of new scientific materials

B.1. Development of a semi mobile unit to sample and study continually physico-chemical ratios

This unit has been constructed out of a caravane frame with an available surface of more than 8m2. The front part has been arranged for 2 persons.
For what concerns the energy problems, we have worked from the 3 following data.
Total independence with all functions working must be possible for 3 days minimum.
This time can be prolonged for studies where we need an automatic water sampling, provided that we arrange a maintenance for what concerns the samples (rechurging and cold).
It must be possible to record the physico-chemical ratios during a long period (2 weeks, a month).
So the electric power feed has been provided as necessary. In our caravane, we shall permanently have 12V and 24V current, 220V alternating (P maxi. 600W).
Once this unit achieved, it will be tested in situ from the three following data and then will be the subject of a precise study. The the research team on hydrobiology will have an equipment that will be powerfull enough to investigate the natural environment.

B.2. The Plankton Sampler

The Plankton Sampler presented here collects samples of phyto- and zooplankton under the best possible conditions. As the sample never comes into contact with the mechanical parts of the apparatus it is in excellent condition and its preservation as far as the laboratory is guaranted without need for its transfer from one recipient to another by its being transported in a small case within the very reservoirs where it was collected. Since these reservoirs are transparent, direct macroscopic observation is possible.
As the quantity of water to be filtered off can be programmed in advance, the specimen sample has both a qualitative and a quantitative value.
The apparatus is compact and reliable, and can be put to work under difficult conditions (e.g. speleological researches).
Two easily detachable accessories extend the range of application of the apparatus :

1) a barrel system takes three successive specimens without the apparatus having to be removed from the water : these samplings can, for example, thus be made at three different depths at the same point ;

2) a larger reservoir with a watertight entry makes it possible to take water samples with a view to physico-chemical analysis.

C. Evaluation of the intoxication degree of the main components of the MOSELLE's fluvial ecosystem

Samplings of water, suspended matters, sediments, phyto-plankton, benthic invertebrates and captured fishes are analyzed to determine their zinc and cadmium concentration.
With the obtained results, it was possible to develop the following points :

- It is not at the direct downstream of the industrial area that we have found the maximum values.

- The data concerning the fishes don't appear to be significant for metallic pollution. On the other hand, the benthic invertebrates, especially Bithynia tentaculata seem to be a good biological indicator.

- If we compare the dissolved zinc and cadmium data with biological samples values, the concentration factors are the most important with cadmium.

To complete the data, we have worked on a non-polluted zone. The preliminary results seem to corroborate the choice of benthic invertebrates, especially snails, as good indicators of metallic contamination of aquatic system. For what concerned the fishes, it was difficult to give a good interpretation. We have no information on the age and consequently how much time they are exposed to the toxic pollutant.

In short, if these data are punctual, they show the interest of first studies on their sector of the river MOSELLE.

II. ECOTOXICOLOGICAL STUDIES

A. Ecotoxicological studies on food chains (cadmium, hydrocarbons)

A model of an experimental fresh water food chain has been elaborated to try to point out hazards related to the presence of toxins in the aquatic environment. For this preliminary study, cadmium and benzene have been selected.
In this model, three different species belonging to various trophic levels but to the same aquatic system have been selected, an algae (Chlorella vulgaris), a crustacean (Daphnia magna) and a fish (Leucaspius delineatus). Bioaccumulation (ability of an organism to concentrate toxins from the medium) and biomagnification (possibility for the toxin to be highly concentrated all along the chain) have been estimated.

"Direct ecotoxicity tests" correspond to trials on isolated species exposed to the toxins and "Integrated ecotoxicity tests" to trials in which species having trophic relations are associated.

1. Cadmium

1.1. "Direct ecotoxicity tests"

For Chlorella, the inhibiting concentrations 50 for ten days were about 4.5 mg/l. The lethal concentration (LC_{50} - 24h) for Daphnia has been estimated to 71 µg/l. The LC_{50} for fish are in the range of 5.75 mg/l for 24 h to 3.9 mg/l for 96 h.

All the species are able to concentrate the metal in their tissues, the factors of concentration being about 3000 for the algae, 700 for the crustacean and from 18 to 140 for fish according to time and dose of cadmium.

1.2. "Integrated ecotoxicity tests"

- Two level chains

Chlorella-daphnia system :

The three ways of contamination (aquatic medium, food, combination of two) have been checked. Toxic concentrations in the algae medium were 5, 10 and 15 µg/l of cadmium (as nitrate) and the exposure lasted for 20 days.
The strongest effect is obtained when algae have been intoxicated and the toxin still present in the medium, but the contamination strictly by food is already significant. The metal is, in al cases, concentrated in the organisms.

Daphnia-Fish system :

It is also possible to contaminate fish by intoxicated Daphniae but the most efficient concentration of the cadmium in the medium regarding the transfert is about 11 µg/l, a sufficient number of crustacean remaining alive.

Chlorella-Fish system :

As fish may be fed strictly with algae, it can be significantly contaminated.

- Three levels chains

Algae are intoxicated in batches for 10 days with 10, 50, 100 and 250 µg/l of cadmium in the medium. After centrifugation, the algae are isolated, washed and delivered to 100 Daphniae 24 h old placed in the special compartment hanged in the 21 tanks. After the tenth day, the Daphniae become adult and a parthenogenic birth may occur. Every day, young Daphniae are placed in a new compartment. The contamination rate measured. This lasts for 10 days. Then, all the Daphniae are put back in the aquarium, the compartments removed and fish is introduced. As a scavenger, it eats all the living species. It is killed after 4 days and analytical determinations are done. Each trial (5 models) is repeated 5 times to get an average. Undoubtfully, fish and Daphniae are contaminated by the food chain started with the algae. Mortality, decrease of natality, loss of weight are very significant at the crustacean level and these toxic phenomenon occur already for concentration of cadmium of 50 µg/l. Analytical results are interesting for fish.

2. Benzène

IC_{50} for the algae is about 40 mg/l (5 days) and the solvent is concentrated in the Chlorellae. LD_{50} for Daphniae is about 18 mg/l (5 days) ; reduction of reproduction, loss of weight are evident and the solvent is also concentrated in the tissues. Very small concentrations of phenols may appear. LD_{50} for fish estimated at 15 mg/l for 96h. Phenols (mainly monophenol and catechol) appeared systematically in the medium. They are elaborated by bacteria (Moraxella and Pseudomonas) brought by fish.

The degradation of benzene to phenols is essentially aerobic, fast, at an optimal temperature of about 25°C. Benzaldehyde, Chlorobenzenes, toluence can also be transformed. The phenols may be ysed by the algae and eutrophisation may occur. Although all the organisms involved have the ability to uptake the solvent as long as they are exposed, there is no definite evidence of long term accumulation hazard in the food chain. The reason is that they are all able to release the benzene stored and detoxification mechanisms may occur.

B. A physicological parameter to determine the toxicity of a modification of the aquatic environment. The variation study of the oxygen uptake of fish in spontaneous activity.

The measurement of oxygen uptake, a physiological test, can be easily done with the rain-bleak (able).

Method

We have used the method of aquatic confinement for the short-time tests and of the aquatic semi-confinement for the longer tests. The decrease of the partial pressure of oxygen in the respirometry chamber is controlled by a pO_2 electrode "RADIOMETER" and the pH/Blood Gas Monitor connected with a servograph.

Results and discussion

a) The influence of temperature on fishes after 4 days of fasting

For each temperature, we find that the VO_2 is a linear function. The O_2 uptake is nearly doubled when temperature rises 10°C. We have noticed a semi-logarithmic relation between oxygen uptake and temperature.

b) Fasting and temperature effects

At 10°C, the oxygen uptake decrease is very slight or even inexistant. The decrease, if any, is rather important after one day of fasting. Then, there is a stabilization. At 20°C, the $\overset{o}{V}O_2$ only stabilizes after 4 days. At 30°C, we can notice that the oxygen uptake decreases during 7 days maxi.

c) Nychtemeral rythm effect

At 20°C, the oxygen uptake is raising to a maximum at midnight and to a minimum at midday.

Conclusion

The measurement of the oxygen uptake is a reliable and precise method to appreciate the impact of a temperature increase. The daily variations of the oxygen uptake seem to show that this fish is well adapted to its environment and can be considered as a good representative fish of the second category waters (French Piscicultural classification). This study will enable us to determine afterwards the synergies which could exist between tested molecules in the environment and temperature, nutritional state and nycthemeral rythm.

II. IMPACT ANALYSIS

A. The biological effects of heated water discharge from a thermic power station on the life cycle of a few animals of benthic fauna

We have limited our studies to three representative examples of benthic fauna :

- a Crustacea : Caridina
- two freshwater snails : Bithynia and Physa

The maximal increase of the water temperature is about nine degrees Celsius. So the water temperature of the evacuation channel and the aquatic area is always above ten degrees Celsius at any month of the year.

For comparative studies, fauna samplings are made in the offtake channel, evacuation channel and in aquatic area.

1. Effects on the life cycle of Caridina

The major effects on this animal are in the change of the seasonal moulting cycle and the reproduction cycle. In the temperate water, for instance, like that of the channel from MARNE to RHINE, the moulting cycle is suspended during the winter to start again in spring. This is what we have observed in the offtake channel. But, in the evacuation channel and aquatic area, the animals moult without interruption. We explain this fact because the temperature of the water discharge is above ten degrees all the year long.
For what concerns the reproductive cycle, the reproduction period is shorter, two months instead of four, but it takes place two months earlier. The laying precocity and the harching of the new generation are explained by a fast maturation of gonads under higher temperature conditions. A seasonal variation of sex-ratio is a natural phenomena. The longevity of the male is more important than the female.

2. Effects on the freshwater snail population

Preliminary results that we have obtained concerning the evolution of the Bithynia tentaculata population show an important time change of the reproduction activity depending on whether it concerns the freshwater snail living in temperate water or in heated water. In both cases, there seem to be two or three annual periods of hatching but with an unequal importance.

For what concerns Physa, the results are less precise because the relative scarcene of this species in the intake canal makes the comparaison difficult.

Nevertheless, the data obtained in the discharge canal show the great abundance of this species in the heated water and the existence of two annual generations.

B. Hydrobiological effects produced by the installation of an electronuclear power station on the river MOSELLE near the frontier.

The major problems are :

1) The very low water flow in summer (under 10m3/", some years like 1976 for example).

2) The necessity to limit the heating of the water (under 30°C).

3) The important risk of the destruction of the ecosystem by increasing the level of eutrophisation already disturbed.

4) The necessity for an european river to flow through the frontier with a quality compatible with european norms.

5) The necessity to know the toxicity of some chemical products added to the water.

Actually, the propositions made to resolve each point are the following :

1) The support of the summer's water flow by the construction of an important dam in the upper stream (VOSGES mountains). Its capacity may be of 50M of m3. This water may compensate the water evaporated by the humid atmospherical refrigerants (five for 2x900MW + 2x1300MW the total evaporation may be of 3m3/").

2) The construction of five humid atmospherical refrigerants and the construction of a dam near the site. Its capacity may be 8M of m3 for a surface of 95ha. This dam works as an additive refrigerant between the towers and the river.
Considering these equipments, the heating may be less than 0,3°C, the major part of the year only upper than these value 36 days by year. The maximum being of 1,5°C four days/year.

3) The eutrophisation risk is linked to the concentration of nutrients in the effluent after evaporation and is not very easy to limit. Studies follow on this subject to determine the possible utilisation of immerged macrophytic plants and the validity of other propositions.

4) This problem is general along all the stream. It is important to limit some acute pollution (organic pollution, mineral pollution, pollution by heavy metals). The radionucleids are not of our competence, but are the major problems.

5) Ecotoxicological studies go on specially on chlorine and its derivatives : chloramines and chlorophénols. Also on antitartars.

Consequently, the economical aspects of the necessity to preserve the fluvial ecosystem are not unmeaning. If it is necessary to develop the electronuclear industry, it is not less necessary to preserve the quality of the water considerating all its uses.

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Contractor : University of Clermont-Ferrand 1

Contract n° : 213-77-1 ENV F

Project Leader : BEYTOUT Daniel

Title of project : Adsorption of viruses on natural and artificial supports ; influence on the enumeration of viruses in water ; interest in suppressing viral - pollution.

OBJECTIVES OF RESEARCH

1) To demonstrate that, in sewage and surface water the most important part of viruses is adsorbed on solid phase ; find the most reliable technique of elution and count.
2) To explore new techniques of detection of viruses
3) To study different adsorbing materials and techniques
4) To evaluate spontaneous or induced denaturation of viruses in natural conditions.

I - ELUTION OF VIRUSES ADSORBED ON SLUDGE (2)

1) Material and methods :

Material : a) waste-water before treatment (2.5 lit, each sample)

b) sludge of decantation pool.

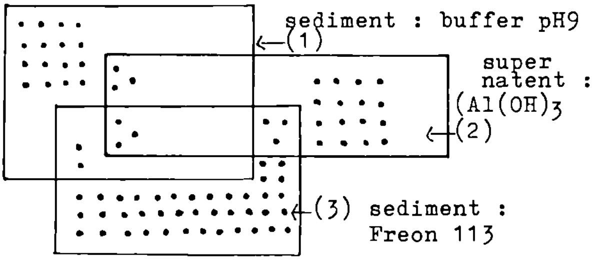
Methods : a) separation of supernatent and sludge by decantation.

- supernatent : adsorption-elution of virus onto $Al(OH)_3$ (Melnick's method)
- half part of sludge : elution in glycin-buffer pH 9 with 10 % serum.
- half part of sludge : agitation with Freon 113 and decantation. Inoculation of equal aliquot of each extract on 4 strains of cells.

b) Sludge is sterilized by ebullition;addition of titrated poliovirus ; extraction by, respectively, glycin buffer-serum, glycin buffer-serum-tween 80, Freon 113, Freon 113+glycin buffer-sérum-tween. Titration ; ratio output/input.

Results : (a)

(1)	24	isolated	strains
(2)	26	"	"
(3)	48	"	"
$1 \cap 2$	6	"	"
$1 \cap 3$	5	"	"
$2 \cap 3$	6	"	"
$1 \cap 2 \cap 3$	3	"	"
total	83	"	" in 48 assays



COMPARISON OF VIRUS EXTRACTION TECHNIQUES EFFICIENCY

b) % of virus recuperation

	pH 9 Buffer	Freon 113	Tween 80 + Buffer	Tween 80(0,5%) +Freon+Buffer
1st ASSAY	3,5 %	10 %	4 %	X
2hd ASSAY	4 %	30 %	4 %	38 %
3rd ASSAY	3 %	40 %	4 %	40 %
4th ASSAY	1 %	2 %	1 %	2 %

Conclusion and comments :

The best method of virus extraction (Freon 113) is lacking in reproductibility ; therefore quantitative study of viruses in sludge and muddy water is not reliable.

2 - NEW TECHNIQUE OF REVELATION : DETECTION OF VIRUS BY IMMUNO-ENZYMOLOGIE METHOD (E.L.I.S.A.)

Material and methods :

- aqueous suspensions of Feces of Rotavirus infected children (1 : 4 dilution)
- rabbit antibody against rotavirus (crude and labelled by alk. phosphatase).
- polystyrene microtrays.

20 l. of water are contaminated by 0.5, 0.05 or 0.005 ml of feces suspension. We apply our technique of adsorption-elution onto cellulose nitrate filter (ref. Beytout D. et al; 1976 Ann. Microb. (Inst. Pasteur) 127, 439-446) and perform ELISA test on 0.10 ml of eluate (sandwich method).

RESULTS : We obtain positive results even in the minimal concentration ; every control is negative ; we experiment four times with same results : we can detect rotavirus in 10^{-7} feces contaminated water.

Then we use this method to look for virus in the hospital room of a rotavirus infected child ; we wash the floor and detect viruses in washing-water.

Comment : It is necessary to have a very specific and strong serum at disposal to execute this test ; we think it may be used to detect non cultivable viruses such as Hepatitis A virus.

3 - STUDY OF DIFFERENT ADSORBING PRODUCTS AND METHODS

We have explored virus adsorbing properties of yeast cells (various strains of Candida, Rhodotorula and Saccharomyces), alive or sterilised or modified by enzymatic digestion. We obtained irregular and unsatisfactory results.

We know the adsorbing properties of cellulose nitrate (loc.cit), but the use of filtration is limited by rapid clogging of the filter. Therefore we disolve the filter in acetone and precipitate it onto iron filings : viruses may be adsorbed by stirring water through this suspension without risk of clogging, and extracted by magnetic attraction.

Recently Sarrette et al (Water Research 1978 vol 11 p 355) and Schwartzbrodt et al (Microbia 1978, 4, 55-68) have described adsorbtion-elution method onto glass-powder by very useful apparatus ; so we stop our own assays to confirm the convenience and usefulness of their procedure. But, even in clear water inoculated by titrated virus, output fluctuates between 60 % and 15 % of input. Sarrette and Vilagines obtain similar variation of efficiency with surface water ; the ratio output-input is lower with waste water.

CONCLUSION : Adsorption-elution on glass-powder is the most simple method for viral analysis of large volume of water ; but efficiency control is still too imperfect for accurate quantitative estimations.

4 - VIRUS DENATURATION

4.1 - Virus survival under natural conditions

Material and method : Sabin strain of poliovirus II in natural water is closed in dialyse bags and immersed in river or lakes ; bags are collected at different times, stored at - 30° C ; all bags are completed to 250 ml, and titration is performed by plack count on the same day with the same cells (microtechnique as we described in 1975, BEYTOUT et al, Ann. Biol. Clin 33pp 379-384). Log. titre - time regression line is calculated : virus denaturation may be expressed by slope of the straight line, or 50 %, or 99 % denaturation time

RESULTS : VIRUS DENATURATION IN NATURAL CONDITIONS

PLACE	DEPTH LIGHTING	WATER TEMPERATURE	SLOPE	50% DENATURATION IN DAYS	99% DENATURATION IN DAYS	NOTES
PISCICULTURE	1 meter shadow	8° C	0.046	6.5	43	Fresh water place very protected from sun
PISCICULTURE	1 meter shadow	9° C	0.042	6.9	46	ID
LAC PAVIN	1 meter sun	15° C	INACTIVATION > 999‰ IN 48 H.			
LAC PAVIN	15 meters sun	4° C	0.024	12.5	83	
LAC PAVIN	55 meters	4° C	0.0205	14.5	97	
LAC PAVIN	90 meters	4° C	(0.005)	(60)	400	r^2= 0 34; non signif. slope; much sludge in water
LAC D'AYDAT	1.50meter shadow	14° C	0.104	2.9	19.5	
LAC D'AYDAT	1.50meter total obscurity	14° C	0.083	3.5	24	dyalyse bags protected in a basket

Comment : survival is long especially if temperature is low and lighting absent. It is probable that adsorption of virus on solid phase promotes their sedimentation in mud, where they are protected of light, and where temperature is lower than on water surface ; consequently this phenomenon prolongates virus survival.

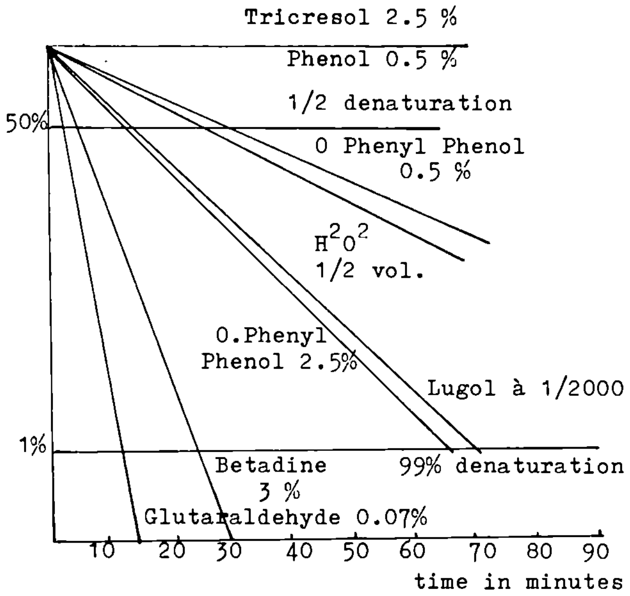
4.2 - Assay of antiseptics and disinfectants (1)

We study in vitro the kinetics of the virulicidal action of different antiseptics and disinfectants.

Material and method : described in paper entered in reference number. The virucidal activity may be expressed by a single parameter provided that the inactivation curve is linear (this is not the case for chlorinated substances). We test each chemical at its usual concentration.

Then we test a commercial disinfectant ("lysoformine" Peters lab.)according to the instructions for use, trying to reproduce the natural environment of virus, i.e. in the presence of proteins, or of organic particles such as in fœcal suspension, or adsorbed on paper.

RESULTS



KINETICS CURVE OF DENATURATION (POLIO II)

(LYSOFORMINE)

Medium	50 % Inactiv.
Water	$2^m\ 14^s$
Water With proteins	$1^m\ 09^s$
Water With fœcal susp.	$1^m\ 20^s$
on paper	$4^m\ 38^s$

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Journées Franco-Québécoise de virologie
Strasbourg 27-28 août 1978 (à paraître dans l'Union Médicale du Canada).

CONTRACTOR : INSTITUT PASTEUR
77 rue Pasteur - 69365 LYON CEDEX 2 - FRANCE -

CONTRACT n° 222 - 77 - 1 ENVF

PROJECT LEADER : Dr. J. VIAL

TITLE OF PROJECT : " Method for : coliforms, faecal coliforms, faecal streptococci count specially surface waters" -

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1. *OBJECTIVE OF THE RESEARCH*

Some C.E.C directive describe rules of capacity for sanitary uses of water. They demand limits of concentration for some bacteriological parameters, specially "coliforms organisms" (total and faecal coliforms) and faecal streptococci.

Far these directives, count of these organisms wants adequate accuracy for avoiding, as for as possible, subjects of contestation in the interpreting of results.

Current methods for surface waters are these one used for drinking water analysis : the most probable numbers (MPN) method, the membrane filtration method, both methods able to get good enough sensibility for drinking water. But MPN precision is andequate to be used for surface water analysis : in the best conditions obtained with difficulty in donkey work, for a 95% probability, confidence limits are : -70% and 200 %. Precision is better with colony counts.

Some censures can be against membrane filtration method, that colony counts in pour plates can avoid. This method is not enough sensible for drinking water, but is really suitable for surface waters. A disadvantage, however, is the difficulty to mark the difference between colonies : the choised media and conditions of growth have to inhibite all the organisms except those to be enumerated. To describe these media and the conditions of growth are the main object of these researchs.

2. *MATERIALS AND METHODS*

Plate count (P.C) and membrane filter (MF) methods were used. Plates were incubated at 37° and 44° for 24 hours (coliforms) or for 24 and 48 hours (streptococci).

From former data and preliminary tests, following media were chosen: for coliforms, Tergitol TTC medium (Tt) from Chapman modified from Buttiaux, and Teepol medium (Te) i.e "Membrane Enriched Teepol Broth" with agar; for faecal streptococci, Slanetz and Bartley medium (SB) and Pfizer selective enterococcus agar (P). A nutrient agar (N.a), non selective medium was often used as blank. Every one is used in P.C and MF methods, except for P, only used in P.C.

Inoculation of the culture medium was got either with purified organisms from local streams (coliforms, faecal streptococci or other organisms) or samples of surface water.

In the first case, after isolation, purification and identification organisms were inoculated in nutrient broth; then, they are separated, washed and diluted with a natural water, sterilized by membrane filtration for getting about 10^8 organisms in 1 ml. From that suspension, eight decimal dilutions were prepared. One ml of each dilution was inoculated into each medium, for other organisms that coliforms and streptococci; for these last organisms, were inoculated one ml of the dilutions giving an adequate number of colonies in plates or on membrane with a non selective medium (usually from 10^{-5} or 10^{-6} to 10^{-9} were used).

In the second case, samples of river water were directly inoculated : 20,2,0.2, sometimes 0.02 ml were used for M.F (volumes usually used in water laboratories); 2,1, 0.5 sometimes 0.2 and 0.1 were used for P.C.

3. *RESULTS*

Coliforms organisms

Thirty eigth of Escherichia coli and 34 strains of the other coliforms; among them 12 "thermoresistants".

The comparative enumeration (with the same dilution) on Tt, Te, Na, with FM and P.C methods, and with 37° and 44° incubations, show :

- At 37° for total coliforms, the values on FM are lower than the values on Na (about 5%); the values on Tt and particularly, on Te are lower than the values on Na.

- At 44°, Escherichia coli and thermoresistant coliforms have about the same growth, and the values are always lower than the corresponding values at 37°, even on Na (on an average : 10%).

To sum up, P.C method give values higher than "FM" method, incubation at 37° higher than incubation at 44°, Tt medium higher than Te medium (although Te is lightly inhibitor with regard to Na medium). All these differences are small (on an average 10%) and usually are not significant with regard to statistic methods).

Examination is easier , with FM method, on Tt than on Te medium, especially for differenciation of "coliforms" (and of the different germs among them). On the contrary, with P.C methods, "halos" of colour with lactose fermentations are more visible on Te, especially whan there are a lot of colonies.

Forty five trains, other that coliforms, were studied to estimate the hazard of errors when there are some of these organisms in a plate and when enumeration is done without seeing halos. At 44°, there was no growth on the three media, for dilutions more diluted than 10^{-4} (from initial suspension described in former chapter). A growth was seen, for 10^{-2} and 10^{-3} dilutions, on only 13% of the strains on Te, 17% on Tt with P.C method (20 and 24% with FM method). The hazards are not great, in particular on Te with P.C method. On the contrary at 37°, they are not negligible: with P.C method 11% and 18% of the strains grow on Te and Tt, for the dilutions from 10^{-4} to 10^{-6}, 13% and 17% for the dilutions 10^{-2} and 10^{-3}.

At last, 63 samples of river waters were studied. As before with P.Cmethods, selection against other strains than coliforms organisms, but also inhibition against these coliforms (which is not so large on Tt than on Te) and influence of temperature are clear : they explain that the values on Tt are 1,5 time larger than the values on Te at 37°, but only 1,1 time larger at 44°.

If the values on Te with MF method, almost are the same as the values on Te with P.C methods, the values on Tt with MF, are much lower than those one, and, of curse, the values on Tt with P.C methods. In the same way, the values on Na with MF method are lower than the values on Na with P.C methods : it would seem that after membrane filtration on river waters an obstacle forbids the way to nutrients through the membranes, and that this obstacle is thrust out by Teepol...

In the experimental conditions of these researchs (cheaper with P.C method than with MF method), P.C methods allows better enumerations of coliform organisms, with regards to statistical rules, than MF method (number of colonies between 30 and 120 with MF method, or 300 with P.C method). For obtaining these good conditions with MF methods, it would be necessary to do twice more dilutions, and the price would be twice expensive.

Faecal streptococci

The problems of selective media is not so hard for streptococci that for coliform organisms. At first, tests were made in river waters. Results show, with regard to :

- media : the grow is faster on P than on S.B.
- methods : counts with MF are lower than with P.C
- temperature : at 37° counts are larger than at 44°.

Some complementary studies were justified with the following questions : Is the incubation at 44° more selective for really groupe D faecal streptococci ?

Does the "colmatage" with river waters explain the lower results with MF methods ?

The temperature, at 44°, is certainly inhibitor to faecal streptococci : Twenty strains, from rivers, biochemicaly identified as S. faecalis or S. faecium, showed a lower grow (6% to 8%) at 44° than at 37°. But is it more selective ? some studies on colonies incubated at 37° or 44° are not conclusive.

Probably, "colmatage" exist with MF : if after deposit the membrane on medium, another laying of medium is poured on that one, counts is as good as with P.C method. But perhaps this fact does not explain everything : if instead of river water, suspension of organisms in filtered water is used, counts are also lower with MF than with P.C: but the difference is very small.

4. *CONCLUSIONS*

The plate count method, which is described in the complete report for application to river water, is more precise, faster, cheaper, than the membrane filtration method and than multiple tubes (MPN) method. Teepol medium, eventually Tergitol TTC medium are fit for the count of coliform organisms thermoresistants à 44°, not so good for total coliforms at 37°. Slanetz and Bartley medium is the best, in our opinion, for enumeration of faecal streptococci. The choice of temperature, 37° or 44°, is still to be discuted. These media can be used with MF methods when surface water is not enough polluted for P.C method(ten or twenty organisms in 100 ml), and both the methods allow to analyse a very large scale of pollution.

Contractor : COMMISSARIAT A L'ENERGIE ATOMIQUE

Contract n° : 224-77-1 ENV F

Project leader : R. MASSOT

Title of project : AN ECOLOGICAL STUDY OF THE OXIDATION PRODUCTS OF AMINO-2 BENZIMIDAZOLE, RESIDUE OF FUNGICIDES, IN SURFACE WATERS.

SCOPE OF THE RESEARCH

This report forms the 2nd part of the study of the products resulting from degradation of amino-2 benzimidazole (A-2B), fungicide residue, under the action of the main agents used for the treatment of drinking water : sodium hypochlorite and ozone.

Following some additional results concerning the action of sodium hypochlorite[*] we are submitting the whole of our work on the action of the ozone.

I. ADDITIONAL STUDY OF CHLORINATION

It is known that A-2B forms an intermediate stage in the natural degradation processes of fungicides such as benomyl and methyl thiophanate. Other products are integrated in this chain - either before A-2B : methyl benzimidazole carbamate (BCM), or after : benzimidazole and, certainly, ortho-phenilene diamine.

1.1. Procedure

1 g of BCM (or benzimidazole) and 150 to 200 ml of 12° chlorometric sodium hypochlorite are brought together in 200 ml of distilled water to which hydrochloric acid has been added.

The organic compounds formed are extracted and analysed by high-resolution mass spectrometry.

1.2. Results

There is mainly chlorination of the benzenic cycle with the formation of the following products.

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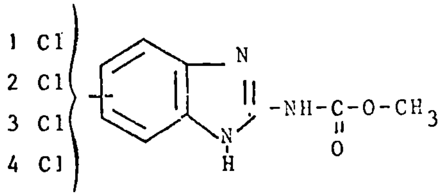
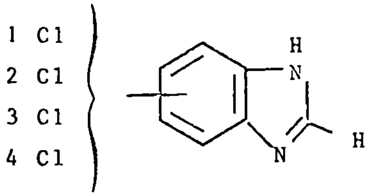
[*] The study of the reactions between A-2B and sodium hypochlorite was the subject of a previous contrat (N° 100-75-1 ENV F) with the C.C.E. End of contrat report (January 1977).

Starting with BCM

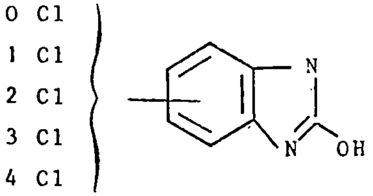


monochloro BCM (very small quantity)
dichloro "
trichloro "
tétrachloro "

Starting with benzimidazole



monochloro benzimidazole
Dichloro "
Trichloro "
Tétrachloro "



Hydroxy-2 benzimidazole
Monochloro " "
Dichloro " "
Trichloro " "
Tétrachloro " "

very small quantity

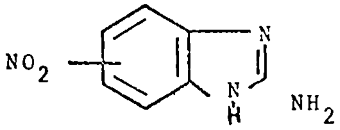
2. ACTION OF OZONE

We operated at pH 8,7 and pH 5,5, authorized legal limits for effluents, with solutions of 1 g of A-2B in 200 ml of ozoned water during 12 hours at the rate of 25 mg of O_3 per hour. After complete evaporation and column chromatography separation we have carried out the analysis by mass spectrometry.

RESULTS

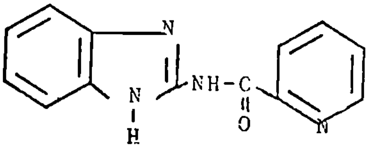
Wa have been able to identify several compounds resulting from the reactions.

M = 178



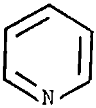
Nitro 5 (6) A-2B

M = 238



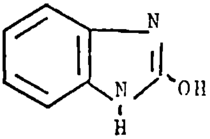
$C_{13}H_{10}N_4O$

M = 79



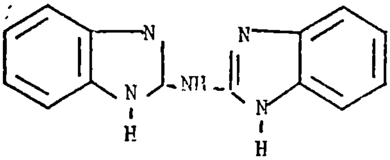
pyridine

M = 134



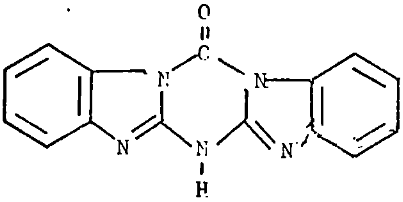
Hydroxy-2 benzimidazole

M = 249



Benzimidazolyl amino-2 bendimidazole

M = 275



C'est une S-Triazine

M = 202 $C_9H_6N_4O_2$) (it was not possible to) specify the structures (

M = 159 $C_8H_5N_3O$)

3. TOXICOLOGY

3.1. Methodology

We operated in accordance with the method described in the above-mentioned report with populations of gold-fish (carassius auratus) and, in some cases, guppies (lebistes reticulatus).

3.2. Results

These tests were made on products resulting from the reaction ozone-A-2B (2 benzimidazole hydroxy) as well as on compounds considered as being intermediate metabolites (2 benzimidazole methyl carbamate) or terminal metabolites (benzimidazole, ortho-phenilene diamine in the environment of fungicides derived from benzimidazole.

The results are given in the following tables :

3.2.1. Gold-fish (carassius auratus)

	Hydroxy-2 benzimidazole	Benzimidazole	O-phenilene diamine	BCM	A-2B
CL_{50} (24) ⁑	200 ppm	200 ppm			450 ppm
CL_{50} (48)	135 "				
CL_{50} (96)		100 "	120 ppm	130 ppm	
CL_{50} (30 j)	35 "	50 "			150 ppm

3.2.2. Guppies (lebistes reticulatus)

	Hydroxy-2 benzimidazole	Benzimidazole	Méthyl-2 benzimidazole carbamate (BCM)	A-2B
CL_{50} (24)	200 ppm	200 ppm	400 ppm	
CL_{50} (48)	160 "	150 "		300 ppm
CL_{50} (96)	130 "	110 "	110 ppm	210 ppm

⁑ CL_{50} (24) = dose of product which kills 50 % of the fish in 24 hours.

It is to be observed that all the products studied are more toxic than the A-2B, the compound on which this study was started. However, this toxicity is considerably lower than that shown by the products resulting from the action of the sodium hypochlorite on the A-2B. We would recall for exemple the values obtained for the dichloro-5,6 A-2B

CL_{50} (24) = 25 ppm
CL_{50} (48) = 9 ppm
CL_{50} (96) = 5 ppm
CL_{50} (30 j) = 3 ppm

CONCLUSION

The object of this study was to investigate the products resulting from the action of agents used for the treatment of drinking water, ozone and sodium hypochlorite, on the intermediate or terminal metabolites in the environment of fungicices derived from benzimidazole and to evaluate their toxicity.

In the first part we had examined the action of sodium hypochlorite on amino-2 benzimidazole. We have presented here the results concerning the action of ozone on this product as well as that of chlorine on the metabolites which are the antecedents or derivates of A-2B.

We have thus been able to reveal and identify numerous compounds, the toxicity of which, although no deadly, is higher than that of the original product.

It is possible that other pesticides, under the same conditions, might give very much more toxic molecules.

We feel that the study of the future of products widely distributed in the environment and their possible transformation under the action of natural or man-used physico-chemical agents is one of the leading directions of ecotoxicology to-day.

Contractor : Fondation Universitaire Luxembourgeoise
Contract n° 161 - 77/1 ENV. B.
Project Leader : A. SCHMITZ - R. WOLLAST
Title of Project : STUDY OF AQUATIC FRESH WATER ECOSYSTEMS

In small rivers the distribution of pollutants depends above all on the dilution effect caused by tributaries and the longitudinal dispersion effect due to water turbulence.

The study of urban runoff in Arlon (18,000 inhabitants) is made according to the MUSKINGUM model. During rain periods highflow in collectors causes considerable pollution charge. Thunderrain (Sept. 6th,1978) is assumed to have taken away from wastewater polishing 760 kg of DCO in 3,800 m3 of water, which goes beyond the regular effluent-norms in treatment plants; that is, 5,630 inhabitant-equivalents per day. Stocking and treatment of water when highflow begins is absolutely necessary.

It is true that attempts in quantification of the sedimentation process have proved rather inefficient, but this study has nevertheless stressed some facts which are important to a better understanding of factors checking the water quality of the Haute Semois. In short, it has been observed that :

- the elimination potential of the organic pollution charge and of the suspended particles by sedimentation process is relatively unimportant
- however, in spite of the low elimination rate, important quantities of solids accumulate in main sedimentation areas during low flow periods
- in high flow periods sedimented sludge returns into suspension and highly increases the pollution charge flowing downstream
- this effect of return to suspension largely outgrows the dilution effect due to the increased runoff of the river.

The study of oxygen concentrations in the Haute Semois has allowed a quantitative definition of cinetic parameters checking re-aeration as well as planktonic and benthic respiration. Thus the necessary parameters are known in order to estimate a priori oxygen variations in conditions similar to those used in the course of this study. The same parameters can be added to the dispersion-dilution equation mentioned above in order to describe the evolution of the organic charge of the river.

The most interesting conclusion of this study is certainly on the one hand, the evidence of the particular importance of benthic respiration in the process of the decomposition of organic matter in the case of a rather shallow river such as the Semois. On the other hand, the considerable action of the sediment surfaces working as bacterial beds in the rotting-process of organic matter of the water volume seems to be another important factor neglected so far.

Thus, on an average, the two-thirds of the respiration are due to sedimented organic material and one third only to organic solids suspended in the water.

Polishing of industrial and urban effluents through a natural marsh has been observed. In spite of an average residence time of 13 to 22 hours, polishing is very efficient. The main factors of this treatment are the decomposition of organic molecules, sedimentation, the assimilation of mineral substances by plants and the delay of high charge levels. When leaving the marsh the water has reached real drinking quality except for the presence of mineral substances due to industrial discharges. The following are the average values :

- BOD : when entering : 20 to 100 mg/l; when leaving : 2 mg/l
- Nitrogen : high elimination rate of all forms; 99% for nitrate
- Metals : elimination of iron (78 to 98%), manganese (80 to 90%), zinc (10 to 65%)
- Bacteriology : elimination of all bacteria at 22° C and 37°C (96,54 and 99,23 %); Nitro-and nitroso-bacteria (93,4 and 88%); total and faecal coliform bacteria (99,52 and 99,97%);faecal streptococcic bacteria (99,56%).

The wastewater polishing of urban effluents has been experienced with *pseudo-marshing* or passage through basins used for plant-cultures. The experimental station of Viville has five series of four basins of about 0,5 m3. A total residence time of about one week during the vegetation period will eliminate 90% of nitrogen; 93% of phosphorus in May and 39% in July; 21% of chlore; 80% of potassium; 19% of calcium; 29% of magnesium; 78% of detergents and 80% of DCO (non filtrated).

Quick and considerable elimination of nutrients is characterized by a productivity reduction of the same culture from the first to the second level, and then from the third to the fourth.

Typha latifolia gives a production per ha on the first, second and third levels of 50,2; 37,7 and 15,6 tons

Phragmites australis produces, on the second,third and fourth levels, 19,8; 2,5 and 2,9 T/ha

Carex acuta produces more than in natural areas, but is less sensible to concentration : on first and third levels : 16,7 and 15,3 T/ha

A full-size project of pseudo-marshing will be realized in Bertrix (5000 inhabitant-equivalents). Plans provide for three levels :

Upper with Typha,Phragmites, ... with gravel
Medium with Scirpus lacustris, then fodder-plants and vegetables, with gravel
Lower : large pisciculture basins
The average residence time will be 21/2 to 71/2 days.

The study of the macrophytes of the Semois continues a previous study of Diatoma and of Protozoa as water quality indicators. Macroscopic algae will also give valuable data. However, not all species can be used for quantitative determination, as their frequency depends on other factors than water quality : reaction to high flow, need for particular substratum ... Certain filamentous algae are used as indicators only because of their frequent occurrence. As to Cladophora glomerata and Lemanca, their occurrence is directly related to the high biological value and oxygenation.

The biotic indices according to Tuffery and Verneaux have permitted to estimate the biotic quality of the Semois, the Beldian drainage area of the Attert and the upper part of the Wiltz, the Serpont and the Marsoult, two tributaries of the Lesse. Coloured maps enable to show the evolution of the water quality.

In the brooks of the Ardennes the very clean waters of springs generally reach index 7 ranging from 9 to 10 with small pollution. However, if pollution increases, the index decreases and goes parallel with saprobial indices.

The ichthyological study of the Semois has been made principally thanks to electric fishing and interpreted in its dynamic aspect. We can conclude that :

- in Central Rulles the introduction of the grayling has permitted to take possession of an available ecological niche
- casual or non casual introduction of any other species has failed
- the chub can live in very eutrophic, even highly polluted waters
- the distribution of species depends on the diversity of the biotopes.

Suggestions have been made as to halieutic dispositions.

The eutrophication of waters is a relatively recent and troublesome phenomenon. After a high piscicultural mortality rate in the Semois in the springs of 1975 and 1976, a Franco-Belgian working-party started to study the natural or accidental phenomena involved.

The present report gives first of all a synthetic view of the results obtained by the different teams having participated in this project during the year 1977. It results from the different studies that among the potential causes of piscicultural mortality in the Semois, the most obvious might be the amount of ammonia nitrogen (form NH_3). The presence of this component in its non ionized toxic form is mainly linked to the variations of pH in the river.

A detailed study of primary productivity in the Semois during 1978, has allowed us to carry out the mathematical model of oxygen dynamics and pH depending on photosynthesis.

The oxygen model based on the registration method of the parameters in two neighbouring stations enables to estimate the different factors responsible for oxygen balance (photosynthesis, aeration, respiration).

Re-aeration is a highly significant factor in spite of slight differences as to saturation.

The general study of the Semois drainage basin in the Ardennes shows

- the eutrophic quality of waters mainly due to increasing tourism
- the presence of mechanic or chemical obstacles to fish migration in certain tributaries, which explains the poverty or the instability of fish populations.

The conclusions refer to the halieutic dispositions concerning the rivers and the numerous ponds of the basin; they also refer to the species which are to be encouraged, to the arrangement of spawning-grounds and rough crossing-spots, as well as to problems of legislation and administration.

The Serpont drainage basin shows such high industrial pollution upstream that all fish life disappears. The tributaries have clean waters,but poor and sometimes acid.Many trout spawning-grounds have become inaccessible as a result of the pollution of the Serpont working as an impassable barrier to generators.Deforestation of river-banks considerably improves biological water quality. It will be interesting to follow the recovery of the Serpont when the wastewater treatment plants of Ila and Oréal work.

The Belgian Attert drainage basin suffers from considerable pollution by the villages but has an active auto-epuration. There is a natural distribution of fish species except upstream Schadeck where a barrier makes it impossible for the grayling to go upstream whereas the trout succeeds in passing and takes possession of the area.The minnow is very abundant. The consequences of the 1976 drought can still be felt and the biomass still remains low. The cleaning out of rivers and pollution also account for this relative poverty. As a whole, the piscicultural situation could be improved at little cost.

Some special methods have been applied.
The quantitative analysis of methane and propane serves to study the evolution of the concentration of a gas injected into water. It allows to estimate the reoxygenation-capacity

of the water. The measures are performed by gas-chromatography. It is necessary to use a polyethylene syringe without "memory"-phenomenon but with a large needle-section in order to avoid the rupture of gas-equilibrium when exhausting the sample.

The estimation of anionic detergents in water has been carried out by an automatic analysing-method which serves to prepare the outlines of a normalization project. The proposed method uses Azur A but the various origins of this reagent cause results of considerable variation, independent from attempts of purification, dessication and extraction. This report gives all details as to the proposed procedure which comes out to be clearly more reliable than those proposed before.

Research in epurating mechanisms involves titration of specific enzymatic activities and this report deals with the details of extract-preparation. As a first step disintegration by ultra-sound (using a Virsonic apparatus) during five minutes at 4° C has proved the best and most necessary procedure. Then, centrifugalization at 20.000 t/min (48.000 g) during 30 minutes gives a supernatant layer of maximum activity. But it appeared that freezing the crude extract after disintegration and before centrifugalization was an important step allowing to reduce the latter to 10.000 t/min (12.350 g) during 10 minutes. Details about the reagents and the material as well as directions for use are given.

The device of a method for automatic measuring of DCO has become necessary because this parameter is intensively studied in water analysis and because its measuring implicates long and expensive manipulations. We first tried to make manual procedures automatic, but the numerous methods published have quickly proved to be of little use.

The manifold which was designed has a coil heated at high temperature and highly concentrated reagents; trivalent chromium resulting from the reaction is continuously titrated by colorimetry. This technique allows to treat 12 samples per hour and needs little supervision. The study of oxidation efficiency and of chloride-interference permits to judge the validity of the method.

Contractor : Katholieke Universiteit Leuven
Contract n° : 258-77-7 ENV B
Project leader : Prof. Dr. H. van de Voorde
Title of project : Ecology of the pathogenic amoeboflagellate Naegleria fowleri in surface water, with special emphasis to thermally polluted waters.
Ecology of pathogenic Acanthamoeba spp.

Objective of the research

Naegleria fowleri and strains of Acanthamoeba spp. are free-living amoebae causing meningoencephalitis in humans, which is a chronic and probably secundary infection when caused by the latter but acute and mostly fatal when caused by the former. Previously we have focused our attention on pathogenic N. fowleri and shown that this amoeba is present in thermal discharges in Belgium. Since the importance of man-made thermal waters we intended to quantitate N. fowleri in the waters involved. On the same occasion we tried to find out how far this agent could be dispersed in the connected water at a detectable rate, since people are using these surface waters for recreational purposes while public survices use it to prepare drinking water.

Nonpathogenic seropositive N. fowleri variants have shown to occur in the same ecological niche as pathogenic N. fowleri. We therefore wanted to establish their exact nature in order to know whether these strains could become virulent or on the contrary are descendants of N. fowleri which became nonpathogenic. A few strains have therefore been investigated in depth in collaboration with a laboratory in the USA.

While we have been able to establish an easy and specific method for pathogenic N. fowleri, the isolation and identification of pathogenic Acanthamoeba spp. still has a long way to cover. This because Acanthamoeba strains show different degrees of virulence and also belong to different species. We gathered typestrains of 19 different species to compare their pathogenicity and to look for quick and specific methods for isolation and identification.

Using these methods thermal discharges and surface waters were compared to find the incidence and origin of pathogenic Acanthamoeba in nature. We have examined the influence of metal ions, which may adversely or advantageously influence the growth of these amoebae. On the same occasion the use of copper sulphate as an amoebicide was investigated.

Material and methods

Samples were concentrated by filtration or centrifugation and incubated on living E. coli at 45 °C for N. fowleri and at 37 °C for Acanthamoeba spp. N. fowleri was identified by a selective medium and its virulence confirmed by intranasal (IN) instillation in mice. Acanthamoeba spp. were identified by axenic growth, cytopathic effect (CPE) in Vero cell culture and IN and/or intracerebral (IC) inoculation in mice.
Antisera against different Naegleria and Acanthamoeba spp. were prepared by subcutaneous injection of amoebic antigen and used in the indirect immunofluorescence technique for specific identification. Typestrains of all available Acanthamoeba spp. were compared for temperature dependence, growth in axenic medium, CPE and for virulence in mice.
The influence of heavy metals was assessed by incorporating metalsalts in agar and growing amoebae on it with washed bacteria. The amoebicidal capacity of copper sulphate was investigated by suspending amoebae in copper sulphate solutions and assessing their survival by subculture at given intervals.

Results

A quantitative study has shown that N. fowleri is present in some thermal discharges in such high numbers as to cause a serious health problem. Their number is diluted out downstream. Pathogenic N. fowleri do not disappear during winter months,although a decrease in number is observed. No relation was found with the number of bacteria, coliforms or E. coli present in the water. This quantitative study also demonstrates that it is better to handle small samples but treated in large numbers for the isolation of pathogenic N. fowleri.

While the percentage of nonpathogenic variants increases with increasing volume, the percent pathogenic strains within the Naegleria isolates is lowered. Also sampling solids yields many variants but a poor percentage of pathogenic strains.

Serological, ultrastructural and lectin binding studies have shown that seropositive N. fowleri variants should be separated from N. fowleri and a new species was therefore erected : N. lovaniensis sp.n. Although pathogenic N. fowleri strains (especially environmental isolates) loose virulence in continuous axenic culture, they never showed the typical characters of the nonpathogenic variants. Moreover, nonpathogenic strains could not be induced to become virulent. It is therefore doubtful that one strain can easily change into the other.

Several typestrains of 19 Acanthamoeba spp. are investigated and it was not possible to predict virulence with 100 % certainty by comparing temperature dependence, axenic growth and CPE in Vero cells. Moreover, pathogenic strains belong to different species and within an Acanthamoeba sp. pathogenic and nonpathogenic strains can occur. A high proportion of strains is virulent IC, but only few kill mice when instilled IN. This same pattern of virulence was found with environmental isolates.

The impact of thermal pollution on the incidence of pathogenic Acanthamoeba is not so drastically as for N. fowleri. Pathogenic Acanthamoeba strains are found in control waters as well as in thermal discharges, although a rise is noticed in the latter where also the most virulent strains are found. It is found that Acanthamoeba is not the most prevalent amoeba in natural waters as stated by other investigators, although it is the predominant genus in chlorinated swimming pools. Also in fish-tanks it is restricted in number, where larger numbers of Naegleria may occur due to the higher temperature.

Copper sulphate was found to be a poor amoebicide, while heavy metals have only minimal influence on the growth of Naegleria and Acanthamoeba.

Conclusions and additional comments

1. The isolation and identification methods for N. fowleri are now at a level to be feasible in every microbiology laboratory. Pathogenic N. fowleri can be present in thermal discharges year-round and are demonstrable in 1 ml samples.

Measures should be taken to destroy them. There is no direct evidence for transition from nonpathogens to pathogens or vice-versa.

2. The isolation and identification methods for pathogenic *Acanthamoeba* spp. are still laborious. Axenic growth and cytopathic effect in cell culture are not 100 % reliable for determination of virulent strains.
   Pathogenic *Acanthamoeba* occur everywhere although a higher concentration and the most virulent strains were demonstrated in thermal discharges.
   Although the epidemiology of *Acanthamoeba* infections is not directly related with swimming, thermal discharges contribute to the proliferation and dispersal of pathogenic strains in the environment.

3. As these organisms cannot be distroyed easily, it is best to prevent circumstances which enhance their growth. The most important factor seems to be water with a high temperature.

responsible author : Dr. J.F. De Jonckheere

References on contract research

1. DE JONCKHEERE J.F. Cytopathic effect in Vero cell culture in relation to virulence in mice within the genus Acanthamoeba. Acanthamoeba Conference, June 14-16, 1978. Columbus, Ohio (USA). Abstract.

2. DE JONCKHEERE JOHAN F. The importance of nonpathogenic Naegleria fowleri strains. IV International Congress of Parasitology, August 19-26, 1978. Warszawa, Poland. Section A, Abstract 33-34.

3. STEVENS A.R., E. GALLUP, J. DE JONCKHEERE and E. WILLAERT. Differences in ultrastructure and lectin sensitivity of pathogenic and nonpathogenic Naegleria fowleri. IV International Congress of Parasitology, August 19-26, 1978. Warszawa, Poland. Section A, Abstract 31-32.

4. DE JONCKHEERE JOHAN F. Quantitative study of Naegleria fowleri in surface water. Protistologica 14, 475-481, 1978.

5. DE JONCKHEERE JOHAN F. Occurrence of highly pathogenic amoebae in thermal discharges. Proceedings of the 2nd Conference on Waste Heat Management and Utilization. December 4-6, 1978. Miami Beach, Florida (USA).

6. STEVENS A.R., J. DE JONCKHEERE and E. WILLAERT. Naegleria lovaniensis sp.n. : isolation and identification of six thermophilic strains of a new species found in association with Naegleria fowleri. Submitted for publication.

- Other publications are in preparation.

Contractor : University of Cambridge
Contract No. 165-76-12 ENV UK
Project Leader : S.M. Haslam
Title of project : River macrophytes of the European
Economic Community

Objective of the research

Earlier research has led to a preliminary understanding of the vegetation of British watercourses, which is now published in "River Plants", Cambridge University Press, 1978. The present investigation is to:

(1) Bring the level of knowledge throughout the other EEC countries up to that in Britain. Other work on macrophytes in these countries has been absent or localised, the most detailed studies being those of Professor Kohler in S. Germany, and Dutch work on some small watercourses.

(2) Pursue more detailed research into the more difficult or more long-term aspects uncovered during the earlier work.

Results and conclusions

In the field seasons of 1977 and 1978, some 6,500 sites were recorded for their vegetation and physical parameters in the non-British countries, and some 450 soil samples were collected for nutrient and heavy-metal studies. Most of this material has not yet been interpreted (and some will be interpreted more efficiently if 1979 and 1980 data are added before analysis).

The preliminary analyses have shown:

1) Rock type and flow regime are the most important controlling factors, as in Britain, but that

2) Landscape and precipitation, when considered throughout the EEC, are not linked as they are in Britain, and a suitable method of estimating their joint effect on water force (and so vegetation) has yet to be devised.

3) Vegetation can be classified in terms of rock type, upstream-downstream variation, and man's activities in each region in which the water force is stable. It is therefore possible to predict vegetation from these factors.

4) The effects of man's activities are similar to those described for Britain in "River Plants", but the exact species which are e.g. the most tolerant to town pollution may vary in different countries.

5) It should be possible to produce macrophyte pollution indices for all the EEC.

6) Nutrient regimes (of interstitial water) are related to rock type, and hence to vegetation. There is a consistent pattern, for trophic status and species assemblage, in nearly all types investigated, both non-British and British. (The most important exceptions are Dutch ditches where, except on sand, management patterns seem more important than nutrient status in determining vegetation).

7) There is some geographical variation, which has not yet been analysed. However, sandstone vegetation is recognisably similar from France to Denmark, limestone from Italy to Germany, etc. Wherever outcrops of a certain rock type occur, the vegetation is similar (as long as the flow regimes are similar and man's activities have not severely damaged the rivers).

In Britain, surveys of c. 1,200 sites per year have been continued. These are now mainly for special purposes such as studying the effects of pollution, monitoring changes in pollution, investigating long-term changes with variations in rainfall, and linking rainfall, landscape and vegetation through an estimation of water force.

"A Ready Reckoner for River Plants" is nearly ready for Press. This small book identifies and classifies British watercourse communities, provides pollution indices, and gives the probable effects of, and predictions for, man's activities.

Information on the effects of pollution is being gathered through field observations and experiments. The chemical(s) in British sewage most damaging to vegetation appear to be organic, and not easily measured or tested. Further investigation of these, and of the side-effects of pesticides and herbicides, would be expensive.

An interesting sideline is the discovery that Elodea canadensis can be altered morphologically by different chemicals, including pollutants and herbicides.

Publications etc.

(excluding papers listed in the previous Final Report)

1) Book published

Haslam, S.M. (1978). River Plants. Cambridge University Press.

2) Main items in preparation

Everitt, M.P. Ecology of River Plants (Macrophyte ecology with special reference to rivers in France and Ireland). Ph.D. Thesis, University College of North Wales, Bangor.

Haslam, S.M. and Wolseley, P.A. A Ready Reckoner for River Plants.

3) Reports

Haslam, S.M. and Everitt, M.P. Reports to C.E.C. (and D.o.E.) in June and December, 1977 and 1978.

Short excerpts have been included in two Nature Conservancy Reports.

S.M.H. was a supervisor for:

Grose, M. and Ship, D. (1978). Water Plants of the Ouse Washes. Royal Society for the Protection of Birds.

4) Oral communications

Everitt, M.P. *Elodea* morphology. Annual Aquatics Meeting, Weed Research Organisation.

Haslam, S.M. A Ready Reckoner for River Plants. Annual Aquatics Meeting, Weed Research Organisation.

Haslam, S.M. Changes in river vegetation in recent years. Annual Aquatics Meeting. Weed Research Organisation.

Haslam, S.M. River Macrophytes and Pollution. 2nd International Congress of Ecology, Jerusalem.

Haslam, S.M. The identification of watercourse habitats. Botanical Society of the British Isles.

Haslam, S.M. Primary production in freshwaters. Cambridge Institute of Education.

Contractor : Freshwater Biological Association/Natural Environment Research Council

Contract No : 278-77-1 ENV U K

Project Leader : Dr. J. Gwynfryn Jones

Title of project : A study of nitrification and denitrification in freshwaters including those enriched by sewage

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Objectives of the research

1. To develop methods for the determination of rates of nitrification, denitrification and other relevant reactions in the nitrogen cycle.
2. To measure these rates in suitable samples.
3. To determine the fate of certain inorganic nitrogen ions at the sampling sites.

Materials and methods

Sampling: Water samples were taken with an integrated 5 m tube sampler and a Friedinger water bottle. Sediment samples were taken with a Jenkin surface mud corer. Dissolved oxygen and temperature were measured in situ and gas release from the profundal zone was measured with a gas trap (area = 1350 cm^2)

Analytical Chemistry: Nitrate was determined using the automated hydrazine-sulphanilamide - N.E.D. method. Ammonia was determined similarly by the salicylate-dichloroisocyanuric acid-nitroprusside technique. Nitrogenous gases were determined by a double column GLC technique which could separate all biological gases. The columns contained Molecular Sieve 5A and Chromosorb 102 operated at 100°. The carrier gas was He (60 ml min^{-1}, 276 kPa) and the thermal conductivity detector oven was set to 200°C.

Denitrification studies : denitrifying bacteria were estimated by an MPN procedure (4 fold dilution, 12 dilutions, 8 replicates, 15°C. 14 days) in Stanier's medium. Nitrate reductase was measured by incubation of enzyme extract from a sonicated sample with nitrate and dithionite/bicarbonate for 1 h. at 35°. The reaction was terminated by aeration and the nitrite determined by the sulphanilamide-N-(1-naphthyl-ethylene hydrochloride) technique. Gas analysis of denitrifying samples was measured in Helium flushed sealed vials.

Nitrification studies : nitrification was measured as dark $^{14}CO_2$ fixation in the presence and absence of the inhibitor N-Serve Controls included formalin treated samples, and samples to which alcohol (the N-Serve carrier) had been added. Incubation was for 12-24 h. at in situ temperatures. The nitrifying bacteria were separated onto 0·22 µm membrane filters and the $^{14}CO_2$ uptake estimated by liquid scintillation spectrometry.

Nitrogen fixation studies : nitrogen fixation was measured by the acetylene reduction technique as applied to un-amended samples and to those which had been amended with carbon and inorganic phosphorus. Tests were conducted aerobically and anaerobically (the latter by flushing with 95% Ar. : 5% CO_2). Acetylene was separated on a Carbosive B column at 215°C. with N_2 as carrier gas and a flame ionization detector. Nitrogen fixing bacteria and algae were determined by an MPN procedure.

Results. These may be divided into 5 main sections.

1. Lake Monitoring. The use of tracer organisms provided useful information on the main route of the inflowing river (containing the sewage effluent) into the lake (Grasmere). A basin towards the western shore was identified as being the most likely to receive most of the incoming materials and to contain the most active microbial population. Subsequent events proved this to be true since deoxygenation, nitrate reduction and ammonia accumulation were most advanced at this site.

2. Denitrification studies. Although there was good correlation between nitrate reductase activities and the release of gaseous nitrogen products from incubated sediments, neither method reflected events in the field (i.e. maximum denitrification at the westerly basin). The gas traps however caught N_2 only in this basin, thus confirming earlier observations that this would be the site of greatest activity. Denitrifying bacteria were more numerous in the sediment (10^7-10^9 ml^{-1}) than in the water column (10^3-10^4 ml^{-1}) and nitrate reduction in the sediment was often maximal below the sediment-water interface N_2 gas release was maximal in late July but then dropped sharply, not recovering until the end of August.

3. Nitrification studies. Nitrification was higher in sandy sediments than in organic oozes. Rates in the water column correlated well with increases in the nitrate concentration in the hypolimnion during July. Inorganic nitrogen species exhibited similar dynamics from year to year. An initial period of ammonification was followed by a period of nitrification when ammonia concentrations declined and nitrate increased. By this time oxygen had become limiting and a period of nitrate

reduction and ammonia accumulation followed.

4. Nitrogen fixation studies. Acetylene reduction rarely occurred without amendment of the samples with either organic carbon or inorganic phosphorus. The only unaltered samples to fix nitrogen came from the rich western basin when conditions were anoxic in the profundal zone. The large input of ammonia from the sewage effluent was considered to be responsible, at least in part, for the absence of nitrogen fixation. Nutrient limitation was suggested as a controlling mechanism for sites more remote from the input.
5. Chemostat studies. These demonstrated that the relationship between nitrate concentrations, organic carbon substrate concentration and type of carbon substrate, was complex and required the sort of detailed investigation which was beyond the scope of this study.

Conclusions and Comments

a. Curtailment of the contract due to the Department of the Environment's change of emphasis to river studies resulted in only basic information being obtained.
b. Denitrification was the major process in Grasmere, being at least an order of magnitude greater than nitrification.
c. Certain problems were encountered when measuring nitrification in sediments.
d. Nitrification played a significant part in the deoxygenation of the hypolimnion.
e. Current MPN methodology for nitrifiers is not satisfactory.
f. Field event confirmed our predictions on the relative importance of various sites of denitrification in the lake.
g. Loss of gaseous nitrogen was equivalent to ca 50% of the nitrate removed from the water column.
h. Nitrogen fixation was not observed in the majority of samples.
i. The sediment water interface was the major site for all the processes examined.

Oral Communications : to DOE and EEC freshwater contact group meetings.

Publications

1. Full details of this work may be obtained from DGR/480/330. A study of nitrification and denitrification in freshwaters, including those enriched by sewage effluent. Final report to DOE.

2. Hall, G.H., Collins, V.G., Jones, J.G. & Horsley, R.W. (1978). The effect of sewage effluent on Grasmere (English Lake District) with particular reference to inorganic nitrogen transformations. Freshwat. Biol. 8, 165.

3. Horsley, R.W. (1978). A technique for the enumeration of heterotrophic nitrate-reducing bacteria. In: Techniques for the study of mixed populations. (Ed. by D.W. Lovelock & R. Davies) Soc. Appl. Bact. Tech. Ser. No. 11. Academic Press: London.

4. Horsley, R.W. (1979). The heterotrophic, nitrate-reducing bacterial flora of Grasmere, English Lake District. J. appl. Bacteriol. 46. In Press.

Contractor: University College Dublin
Contract No.: 217 - 77 - 1 ENV EIR
Project Leader: Dr. D. A. Murray
Title of Project: Paleolimnological studies on Lakes of known differing trophic status to establish eutrophication rates and standards

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Introduction and objectives of the research:

Sediments accumulating in lake basins are composed of numerous materials that are derived from the catchment and the lake itself. These sediments, therefore, incorporating the morphological and biochemical remains of organisms that lived in the lake and materials from the drainage basin, reflect conditions that exist in the lake and its catchment. The accumulated material is a potential fossil record but, like all fossil records "it is exasperatingly incomplete although the amount and diversity of information present is really tremendous" (Frey 1974). This fossil record contains potential information on productivity within the lake through a study of biological pseudofossils - the remains of organisms or biochemical derivatives of organisms e.g. pigments derived from aquatic algae. The chemical constituents, additionally, provide information on disturbances of the drainage basin since during periods of active soil disturbance, elements such as magnesium and potassium are subject to greater erosion and may thus show increased concentrations in the sediment.

Principal aims of the present research:

(i) to relate selected constituents of the surficial sediment from a variety of lakes to the known level of productivity in an attempt to provide an additional criterion for assessing lake productivity.

(ii) to trace the development of these lakes by analysis of 1.0 metre sediment cores in an attempt to determine whether or not, and how rapidly, changes in trophic status had occurred.

(iii) to relate sedimentary phosphorous content to the current enhanced productivity and distribution of fauna in the organically enriched Lough Leane.

Materials and Methods

In this study surficial sediment and sediment cores were obtained from a total of forty-eight lakes (forty-six in Ireland and two in Italy). Surficial sediment was recovered from one additional lake in Ireland and from two additional Italian lakes including nine basins of Lake Lugano.

Collections of surficial sediment were obtained by Ekmann grab. The upper 2.0 cm of sediment was treated as surficial and this represents from two to ten years accumulation. Such sediments were deep frozen until analysed. Samples from the Italian lakes, likewise frozen, were air freighted to Dublin in polystyrene containers.

Sediment cores were obtained, as far as possible, at the deepest point of each lake, with a piston corer (Murray 1976). Cores, obtained in plexiglass tubes, were transported intact with a minimum of disturbance to the laboratory. Sediment was extruded vertically upwards in 1.0 cm sections for the upper 30 cms. Thereafter the sampling interval was 5 or 10 cm to the 100 cm level. In each case a sample constitutes a 1.0 cm deep cross-section of the core. Samples were stored, deep frozen, in labelled petrie dishes until analysed as follows - Organic content was determined as loss on ignition to 550°C. Organic carbon was determined using chromic acid oxidation. Dry sediments were brought into solution by treatment with hydrofluoric, perchloric and nitric acids and analysed for total Ca, Fe, K, Mn, Mg and Na by atomic absorbtion spectrophotometry. Total P was determined on aliquots of the same extract using colorimetric methods with ammonium vanadate and a series of suitable reference standards. Exchangeable or soluble P was extracted from 1.0 ml sub-samples of wet sediment with acetic acid/sodium hydroxide ("Morgans") solution and determined colorimetrically as for total P. Sedimentary pigments were extracted with 90% Acetone at 4°C. Results are expressed as sedimentary pigment degradation units (SPDU) and are calculated on the basis of optical density at the selected wavelength for carotenoid (480nm) Chlorophyll (665) and, when detectable, bacteriochlorophyll (750nm) derivation. SPDU's are calculated per gm dry weight, organic weight or per gm organic carbon. Special studies on pigment diversity were carried out on surficial sediments from thirty-seven samples (thirty lakes), following the 2D chromatography method of Sanger and Gorham (1972). Oscillaxanthine, specific to the blue-green alga <u>Oscillatoria</u>, was extracted from L. Ennell (No. 17) sediments using the method of Griffith et. al (1975).

Sedimentation rates for two lakes were determined by the ^{137}Cs method and some pollen analysis was also carried out.

Results

The results comprise some 23,000 analyses and may be summarised as follows:-

(a) Surficial sediment parameters in relation to the present day trophic assessment:

The most significant parameters for use as additional assessment factors are sedimentary pigment derivatives since these reflect the level of primary productivity. Chlorophyll derivatives and carotenoids preserve well in sediments, the latter being preferentially preserved in lakes which develop an anaerobic hypolimnion, consequently the ratio of chlorophyll to carotenoid derivatives has been proposed as an index of productivity.

Productive lakes should have a low pigment ratio index (PR) while lakes of low productivity should have a high PR index. The results obtained in this study are shown (Table 1). The assessment of trophic status, given as high (H), medium (M) or low (L), is that quoted by independent workers. In the case of the lakes denoted by an asterisk no previous assessment has been made. The results obtained, in general, support the use of the PR as a useful index. Apart from the anomalous result of L. Egish all lakes assessed as having high productivity have a PR index of less than 0.4 and the more enriched systems a value of 0.3 or less. The highly enriched L. Ennell has the lowest index obtained, 0.17. On the contrary lakes of poor productivity in general have a higher PR index.

IRISH LAKES	PR	PD	Status				
Allen	.65	35	L	Leane	.28	41	M/H
Arrow	.44	47	H	Lene	.65		M
Beltra	.60	34	L	Looscannagh	.63	37	L
Bofin	.58	33	L	Mask	.75		M
Brin	.62		L	Muckno	.28		H
Caragh	.50	30	L	Mullagh			H
Carra	.34		H	Nacung	.53	28	L
Conn	.39	43	H	Nafooey	.41		M/L
Corrib	.67	36	M	O'Flynn	.42	37	H/M
Cullin	.40	42	H	Oughter	.43	40	H
Derg upp.	.33		M/H	Owel	.35		M/H
Derg low.	.38		M/H	Ramor	.38		H
Derravarragh	.31		H	Rea	.37	40	H
Doo Mayo	.53		L	Ree	.48	40	H
Doo Kerry	.21	46	H	Sheelin	.23	41	H
Easky	.78	28	L	Sillan	.43		H
Egish	.58?		H	Templehouse	.30	45	H
Ennell	.17	46	H	Upp. Killarney	.80		L
Feeagh	.72	27	L	ITALIAN LAKES			
Finn	.97	31	L	Comabbio	.25	39	H
Gara	.50	40	M	Monate	.56	39	L
Garadice	.28	44	H	Varese	.33	40	H
Gartan	.38		L	Lugano Lakes			
Gill	.40	32	H ?	Agno	.29	47	
Gowna	.35		H	Capolago	.30	41	
Gowna	.40		H	Figino	.31	46	
Graney	.60	36	M	Gandria	.29	41	
Guitane	.62		L	Lugano	.40	38	
Inagh	.47		M	Melide	.31	42	
Inchquinn	.26	43	H	Morcote	.31	39	
Key	.53		H/M	P. Tresa	.35	43	

Table 1. Summary of pigment ratio (PR) pigment diversity (PD) and present day status designated as high (H), medium (M) or low (L) productivity.

The pigment diversity results are also given. (PD) in Table 1. The PD is primarily a measure of pigment diagenesis in which the initially high pigment diversity of algae is increased during decomposition. Sanger and Gorham (1972) recorded maximum PDs of 10, 23, 47 and 50 in woodland litter, swamps, holomictic and meromictic lakes respectively. Their results indicate that the higher PD values are recorded for more productive lakes and this is attributed to a greater autochthonous input. It is suggested that this index may prove of use in lake productivity assessments. The range of values obtained in this study lies between 27 recorded for the unproductive L. Feeagh and 47 recorded for Lough Arrow. A value of 46 is recorded for the Fignio basin of Lake Lugano. The ten Irish lakes assessed with low productivity (Table 1) have PD indices ranging between 27 and 39 with a mean value of 32, the productive lakes have indices in the range 40 to 47 with a mean of 42. One lake, L. Gill, assessed as naturally eutrophic by Flanagan and Toner (1975) has a relatively low PD. However, these authors had stressed the need for clarification of their assessment of L. Gill. On the basis of the trends observed in the Irish lakes it would appear that the majority of lakes or lake basins sampled in Northern Italy could be considered moderately or highly productive.

(b) Recent development of lakes

Profiles of chemical and biochemical parameters prepared for all the lakes cored yield some information on their development and disturbances in their catchments. In a number of the lakes sampled marked increases in organic content were noted, probably related to the period of extensive tree felling from 1600 AD to 1900 AD. The profiles for sedimentary pigments and PR provide some insight to recent trends in productivity. In the majority of cases increased productivity is noted but some results indicate former more productive conditions. This result is explicable on the basis of the Irish population trends. Prior to the famine the population of Ireland was about 8.0 million, whereas to-day the figure is approximately 4.0 million. The greater population density in earlier times could have enhanced or disturbed lake productivity. In more recent times the use of fertilizers, detergents and sewage disposal accounts for most of the productivity increases noted. An estimate of the rate of change may be obtained in the PR profile. Greatly enhanced productivity in recent times is reflected in the rapidly decreasing PR values obtained in the upper 10 cm – 20 cm of core sediment of many of the lakes surveyed.

(c) Sedimentary phosphorous in L. Leane

Initial findings of limnological studies on this lake in the period 1971-1973 demonstrated that a problem of accelerating eutrophication exists. Partially treated sewage is discharged to the lake at Ross Bay and some other point sources. The effects however are localized and considerably alleviated by the high turnover rate of the lake. The amounts of exchangeable P in the sediments is low - average 20 µg/ml apart from the areas where nutrient input occurs where values exceed 100 µg/ml. There is no evidence of large scale release of P from the recent sediments in the deeper areas of the lake. Sediment cores, analysed in detail for chemical, biochemical and biological

parameters give a record of recent changes. The sedimentation rate, determined by ^{137}Cs analysis at CCR Euratom Ispra, is currently 1.0 Cm/yr. It is evident that an overall net accumulation of P has occurred for the past 40 years.
In Ross Bay a sediment "nutrient" gradient in terms of exchangeable P is observed decreasing with distance from the sewage outflow. A system of biotic indices, ranging from 1 (grossly polluted) to 5, (clean) has been devised to demonstrate quality ratings based on the lakes fauna. A correlation of biotic and abiotic parameters using Kendalls and Spearman's rank correlation gives a significant negative correlation with distance from the point ofentry of the sewage. A positive correlation is noted between sedimentary exchangeable P and organic content.

Conclusions

(i) Surficial sediments analysed for pigment derivatives provide reliable additional information to the determination of lake trophic status and productivity.

(ii) Sediment cores contain a record of lake development. The technique has limited application in shallow lakes where the benthic sediments may be disturbed by wind and wave action. In deep lakes ($>$ 15m), however, the results appear more reliable and greatly enhanced productivity trends may be observed.

(iii) In L. Leane, currently phosphorous is accumulating in the sediments. In the littoral regions biotic quality ratings provide a reliable index of water quality.

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Oral communication - addition to 1-3 above. Paleolimnological studies on Irish and Italian lakes - delivered to the Biological Sciences Seminars, Indiana University, Bloomington, U.S.A., October 1978.

TOPIC 16 : WATER QUALITY

Epidemiology

Contractor:	Federal Health Office (Bundesgesundheitsamt) Postfach D-1000 Berlin 33 Federal Republic of Germany
Contract No.:	196 - 77 - 1 ENV D
Project leader:	Dr. M. Sonneborn
Title of project:	Epidemiological Studies of the Influence of Drinking Water Ingredients upon Morbidity

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1. The influence of water quality on the morbidity and mortality of various diseases has been studied for more than 20 years. From these diseases, those of the cardiovascular system have been playing a special role. The underlying hypothesis of these studies is hard water = low mortality by cardiovascular diseases; soft water = high mortality from these diseases. There have been studies establishing such association between water ingredients and cardiovascular diseases but also other studies which do not confirm this association or even represent converse results. Frequently, there have been methodological errors in the studies published thus far. The most important ones have been an inadequate choice of samples, a choice of unsuitable characters, a lack of attention to time-related and geographical associations and non-consideration of the varation range of water parameters. In addition to studies which either accepted or rejected the hypothesis of a water-related factor there has been a considerable number of publications referring other regionally different variables to cardiovascular mortality. These are again and again climatic characteristics, social factors as well as geographical latitude and longitude, type of nutrition, or fluoridation of drinking water, to mention a few.

Information on the effects of change in the composition of drinking water may essentially contribute to a solution of the problem of associations between water quality and the incidence of diseases. Although the available studies on this subject confirm the hypothesis mentioned in the beginning, they do not give regard to the fact that irrespective of alterations of water quality during the observation period, there may have also been alterations of other regional and time-relatet influences.

In addition to mortality, also a number of morbidity parameters is associated with water quality. The most important ones are blood pressure, serum cholesterol, serum triglycerides, and other serum lipids, ECG signs, and vascular changes recognizable by X-ray examinations. The results that have been described in literature thus far do not present a uniform picture. It may be that one of the characters typical for a defined region in respect of mortality may be water hardness or a combination of several water ingredients. If considering only the dynamics of the mortality from ischemic heart disease in the Federal Republic of Germany which have shown a continuous rise over the years considered despite almost unchanged water quality, there is considerable doubt that even part of the mortality is due to a waterrelated factor. In the United States of America, cardiovascular mortality is decreasing while there has been also almost no change in water quality.

2. In seven villages and towns of Hesse as well as in the cities of Nordenham and Brake, Lower Saxony, and in Berlin, both drinking water ingredients and criteria of the morbidity of the population were studied. More than 15 000 persons were involved in the study. The morbidity data refer to cardiovascular diseases and the associated risk factors.

   Additionally, the composition of the drinking water has been analyzed for another 80 water supply areas of the Federal Republic of Germany. From these,areas of different water quality are to be selected for additional investigations of the problem of health relevance of drinking water.

3. So far, the following ingredients of drinking water have been measured:

   Na, K, Ca, Mg, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb,
   Zn, Cl, F, I, TOC.

   The methods of analysis used were atomic absorption spectrometry, photometry (for iodide) and electrometry by means of ionsensitive electrodes (chloride, fluoride).

   Medical screening comprised some 60 parameters and a questionnaire. Evaluation is to include the results of ECG and X-ray examinations, measurements of lung function, measurements of blood pressure, and serum analyses (including cholesterol, tri-glycerides, uric acid, Na, K, Ca, phosphate, chloride). Furthermore, the social and economic conditions of life of the test persons were recorded.

4. For the 7 localities of Hesse studied, the indicators of morbidity were correlated with the water parameters. The signs of all statistically confirmed ($p < 0.05$) correlation coefficients (Spearman's rank correlation) are mentioned, the remaining non-significant correlations have not been considered. As expected, contradictory correlations in some cases. For reasons of conspicuousness, the numerical values of the correlation coefficients have thus been omitted. Information on possible associations between water ingredients and morbidity will not be available until evaluation has been extended to include a considerably higher number of localities.
   In a comprehensive data bank, more than 19.000 data on drinking water in accordance with standard analytic procedures under the Drinking Water Ordinance are available (see enclosure).

Beyond this, detailed analytical data (approx. 40 parameters) from our own studies have been evaluated for more than 600 water supply areas. These data have been prepared for mathematical evaluation.

5. Also mortality data on ischemic heart diseases and all causes of death for 1968-1974 from the Statistical Offices of the Federal Länder were complied and prepared for uniform evaluation. Work is under way for a further subdivision of the comparatively large regions into rural or urban areas to enable a possible grouping by water supply areas.

   For comparison of regional mortality figures, the standardized mortality rate (SMR) and standardized proportional mortality rate (SPMR) were chosen.

   The SMR indicates the proportion of deaths from a defined cause that would occur among the population of an area if the age distribution of such population were identical with that among a standard population. The SPMR indicates the proportion of deaths from a defined cause among deaths from all causes within an area if in such area the age distribution of deaths from all causes would be identical with that of the standard population.
   Both mortality indices were calculated for the Länder of the Federal Republic and the years 1968-1974. Generally, they exhibited the same tendency for ischemic heart disease (International Classification of Diseases (ICD) 410-414), in part they differed, however, quite considerably in numbers. In addition to the cities of West Berlin and Bremen, the three southern Länder of Rhineland-Palatinate, Baden-Württemberg, and Bavaria exhibited obviously the lowest SPMR while the highest SPMR were found to be present in the northernmost Land of Schleswig-Holstein, the city of Hamburg, and the Saarland. Nevertheless, this coars regional classification does not suffice to establish any north-south differences, nor would it be meaningful to assign corresponding water parameters. This will require sub-grouping into smaller regional units.

6. Since a number of authors have established an association between mortality and climate which may, like water, exhibit regional differences, we also studied the seasonal variations of mortality from ischemic heart disease. Mean monthly temperatures for the Federal Republic of Germany in 1972-1976 were assigned to the numbers of deaths per month from ischemic heart disease and the correlation coefficient was calculated. The resulting correlation coefficient of $r = -0.540$ could be statistically confirmed for $p < 0.000005$. For the ICD groups 470-474, the coefficient of correlation with the mean monthly temperature of $r = -0.776$ could also be statistically confirmed ($p < 0.000005$). It is quite imaginable that the mortality from ischemic heart disease was influenced by temperature largely through the occurrence of influenza illnesses since when the influence was eliminated, the partial correlation coefficient between mortality from ischemic heart disease and mean monthly temperature of $r = -0.297$ at $p < 0.05$ was only about one half of the correlation coefficient that considered the influence by influenza illnesses.

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Biotechn. Umschau 2, 383-386/1978

Contractor : CERCHAR, INSERM, IRCHA, IRCHA-LHVP, Paris

Contracts n° 245, 249, 263, 270

Project leaders : P. LAZAR, R. CABRIDENC, R. FERRAND, B. FESTY

Title of project : Coordinated study concerning the relationship between water quality and health parameters

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OBJECTIVE OF THE RESEARCH

The epidemiological studies conducted in France had two main objectives :

1. To find areas contrasted for the quality of drinking water distributed, in order to compare vital statistics and establish a possible relationship.

2. To look for some primary effects of water (and more generally environment components) on foetal environment (microanalysis of amniotic fluid)

METHODS

1. Selection of study areas for water sampling

The areas primarily selected were Paris and Alsace. Later, some regions of the Center of France were added.

The a priori reason for choosing Paris is the fact that in this city four main water resources are used, two coming from the rivers Saine and Marne (superficial waters) and two susceptible to be considered as deep waters (Avre and Varne). Apart from this multiple water alimentation, Paris may be thought to have a relative homogeneity for the kind of life, which should reduced the role of confounders. As it will be seen however, it was not possible to eliminate the confounding influence of the socio-economic factors completely.

The a priori reasons for choosing Alsace is first the fact that this region has two main contrasted water resources : one which feeds the eastern plain and which is constituted by a single subterrenian stratum, and the other which feeds the western part and which is constituted by mountain springs. The former is hard water while the latter is soft.
In addition various industrial and mining activities introduce important sources of mineral and organic pollution which affect the quality of deep water along south-north oriented gradients. Furthermore, Alsace is a region in which mortality rates are quite high.

The first observations collected in Paris having shown contrasts smaller than expected, we decided to look for some additional data coming from a region with an a priori low level of pollution : several "departments" from the Center of France (south of the Seine plain) were selected for sampling superficial and deep waters.

2. Sampling of amniotic fluid

Amniotic fluids at birth were collected at two stages of the work. First, they were sampled without any selection and analysed in order to have some information on the distribution in such fluids of the micropollutants usually found in drinking water. In a second stage, various samples selected according to the occupation of the mother and the status of the child at birth, were used for a comparative analysis.

RESULTS

1. Water quality in Paris

As expected, many highly significant differences appeared between the four waters feeding Paris. Some differences can also be established between the reservoirs, in which water is first accumulated and the faucets used for local sampling.
It is not possible to summarize all those differences in a very few words, since they are not quite consistent. Most pollutants are at a higher level in superficial waters (as for instance, the micropollutants extracted with chloroform at pH 7), but some of them are more highly represented in deep waters (nitrates for instance). This clearly means that if any health difference should appear, it would not be quite easy to designate the specific component responsible for it. But obviously this could be a second step to perform.

Paris can be divided into areas fed with water coming almost exclusively - or in great majority - from the four basic resources and into other areas fed with various mixtures of the four categories. About two thirds of the 80 basic districts ("quartiers") belong to the first kind ("pure" resource). The eastern part of Paris is fed with Marne water (superficial),the south east with Seine (superficial), the western part with Avre (deep) and a corridor along the River with Vanne (deep) (Figure 1).
Table 1 gives some of the main water characteristics of these "pure" areas.

2. Mortality data in Paris

A basic study of the evolution of the population in Paris between 1968 and 1975 revealed some significant variations in terms of age and socio-economic structure. However these modifications do not seem important enough to modify deeply the differences in mortality between the different districts of the city.

Generally speaking, mortality rates decrease from the eastern to the western part of the city. This is true for the main causes of death and when age is taken into account.
Table 1 gives the main mortality and socio-economic data for the "pure" areas (the area along the river has been divided into three parts in order to take the east-west gradients into account).

3. Correlation between water characteristics and mortality data

As there is an east-west gradient for mortality and as water feeding also varies from eastern to western parts of the city, a crude correlation would show a significant relationship. However such a correlation, if not meaningless, would not be demonstrative of any causal relationship between the quality of water and mortality since many other factors, among which the basic socio-economic ones, do also vary from East to West. That is the reason for which a special attention was devoted to the fact that, within the eastern part, an area fed with deep water (area n° 4) was located between two areas fed with superficial waters (areas n° 5 and 6). However no trends of different mortality could be observed according to this water distribution structure (see table 1).

4. Comparison of the three regions investigated in France

Table 2 gives the main water and mortality characteristics of these regions. No clearcut correlation appears between the two sets of data.

5. Amniotic fluid examinations

Table 3 shows that it was possible to find some micropollutants in the first sample of 61 amniotic fluids (the percentages of contaminated fluids varied from 8% to 49% according to the class of pollutants).
The second step of the survey is still in progress.

CONCLUSIONS

1. Comparison of various areas

No correlation could be clearly demonstrated between the quality of water resources and available mortality data either within Paris or in the three French regions compared.
Within Paris some correlation might be shown, but the coincidence of the water and the socio-economic east-west gradients makes it difficult to infere causal relationship from the observed co-variation between water quality and mortality trends.
The basic data established for water quality will be quite helpful for the follow-up studies, trying to overcome the difficulties pointed out by the present work. Two ways should be explored : i) trying to find health parameters susceptible to be influenced faster than life duration and ii) eliminating the socio-economic component as much as possible. These will be the two main objectives of the survey conducted in Paris nurseries to study child growth among 6 month-3 year old children.

2. Fetal environment

As the basic technical study showed that the study of amniotic fluid micropollution is feasible and justified, it is worth developing these investigations at a larger scale, in parallel with other measurements of primary effects of environment on fetal development, among which accumulation of heavy metals in fetal hair. A special effort will be devoted to determine the contribution from possible pollution of drinking water consumed by the mothers.

Table 1 -

			1 Avre	2	3 Vanne	4	5 Marne	6 Seine
WATER	Oxydability	(mg/l)	0,70	0,42			1,02	1,01
	Nitrates	...	6,2	6,3			3,6	3,9
	Silica	..	13,7	10,2			6,7	6,1
	Sodium	..	12,0	6,1			9,3	16,2
	Micropollutants (average value - pH 7)	..	0,098	0,094			0,168	0,157
SOCIO-ECONOMIC	% housing with confort		61,7	52,5	30,7	25,0	41,5	48,6
	Age (mean)		38,3	40,1	41,1	39,9	36,3	35,1
	Non manual workers (M)		56 %	50 %	33 %	33 %	34 %	45 %
MORTALITY RATES per 100.000 h (45-64 years)	Mortality all causes	M	899	1185	1488	1492	1352	1449
		F	491	583	657	718	652	635
	Ischeamic heart diseases	M	111	148	151	132	135	161
		F	25	27	20	32	29	32
	Cerebrovascular diseases	M	51	90	89	88	88	107
		F	31	41	46	53	50	48
	Tumor	M	333	399	464	520	474	473
		F	245	266	249	283	266	271

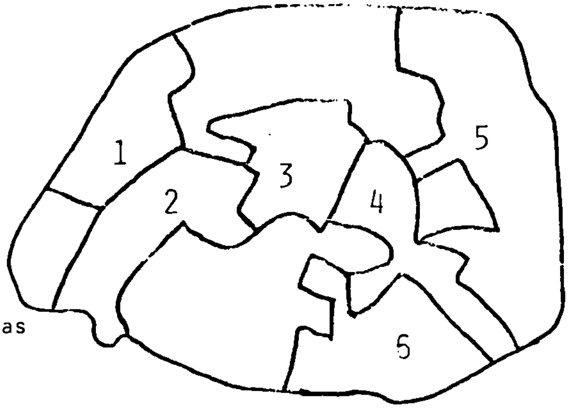


Fig. 1 : Paris areas

Table 2 - Average value of the amount of organic micropollutants (mg/l) and age-standardized death rates/1000h

		PARIS	ALSACE	CENTER
Micropollutants (pH7)	Deep water	0.084	0.056	0.087
	Sup. water	0.113	0.202	0.120
Age-standardized death rates/ 1000 h	M	10.5	14.2	10.0
	F	9.2	12.7	9.9

Table 3 - Micropollutants in amniotic fluid samples

	Presence non detected	Presence detected
Amines Aromatiques (Aromatic Amines)	43	18 (29%)
Azocycles (Cyclic Azocompound)	31	30 (49%)
Chloro-phenols (Chlorophenols)	54	7 (11%)
Hydrocarbures chlorés (Chlorinated hydrocarbons)	56	5 (8%)
N - Nitrosamines (N - Nitrosamines)	44	17 (28%)
Organo-metalliques (Organometallic)	44	17 (28%)
Phtalates (Phthalates)	35	26 (43%)
Polycycles hydrocarbures (Polycyclic hydrocarbons)	42	19 (31%)

Contractor:	University of Pavia, Italy (Centro Ricerche Nutrizione umana e dietetica)
Contract n°	201-77-1 ENV I
Project Leader:	Ermanno Lanzola
Title of project:	Mineral characteristics of water and Coronary Hearth Disease

OBJECTIVE OF THE RESEARCH

Interest in research concerning a correlation between coronary hearth disease (CHD) and the mineral characteristics of local water supplies dates back about twenty years to the first studies by Kobayashi (1) in Japan and by Schroeder (2) (3) in the United States and has long been supported by the observation that the incidence of CHD has a wide geographic variation. Since 1968, numerous research and ample reviews (4-10) have been published on this subject. However, even today, the responsibility of any of the numerous elements found in water has not been clearly proven except for a well verified correlation between the hardness of water and the incidence of CHD. Numerous hypotheses have been formulated and recently the importance of the Zn/Cu ratio (11-12), of Mg (13) and of Cd (14) have been considered; this last element was considered particularly due to its importance in the etiology of hypertension but, now,its correlation with CHD is controversial. The intent of the present work is to contribute to the epidemiological studies in this field, taking account that the province of Pavia, (situated in the Lombardia region, Northern Italy), has the following particular characteristics: one third of the province is situated in a mountanous area and the other two thirds in the Po plain; many of the various water supplies have different origins and consequently different characteristics. As for the mortality rates for CHD they are widely varied in the different Communities.

MATERIALS AND METHODS

The geographical region included in the survey was subdivided into areas presenting homogeneous mortality rates for CHD in order to establish meaningful guidelines for the water samples to be taken from individual water sources throughout the province of Pavia.

Information concerning CHD mortality rates was collected from the local city halls and the analysis of water was restricted to those adjacent areas exhibiting significant differences in these rates. The mortality rates were standardized according to age and sex using the population of Pavia as a reference point; a six year period (1970-1975) has been considered in order to have a larger number of cases and to avoid the annual fluctuations possible when dealing with small numbers. The following parameters and elements were determined: Hardness, Ca, Mg, Zn, Cu, Cr, Cd and Pb. The characteristics of the municipalities involved in this survey are quoted in Table 1.

TABLE 1 Characteristics of the municipalities

MUNICIPALITIES	AREA	POPULATION N°	FAMILIES N°	*MORTALITY (10^{-5}pop.)
Bagnaria$^{\blacktriangle}$	16,59	647	199	576
Val di Nizza$^{\triangle}$	29,59	921	323	144
Varzi$^{\triangle}$	58,77	4394	1399	146
Casatisma$^{\blacktriangle}$	5,46	891	273	592
Casteggio$^{\triangle}$	17,78	7813	2334	132
Montescano$^{\blacktriangle}$	2,40	366	124	561
Montù Beccaria$^{\triangle}$	15,60	2282	786	150
Canneto Pavese$^{\triangle}$	5,81	1683	608	180
Filighera$^{\blacktriangle}$	8,15	802	271	517
Vistarino$^{\triangle}$	9,25	1009	276	164

▲) category A (mortality for CHD $>500.10^{-5}$)

△) category B (mortality for CHD $<200.10^{-5}$)

* Standardized rate for age and sex

Water Sampling: In each community samples were taken from three different points of the same aqueduct at two different times. The water samples were taken directly from the tapes after running the water at a constants flow for five minutes. At the time of sampling, 1 ml HNO_3 65% was added for each liter of water.

Analytical methods: Pb, Cu, Zn and Cd were concentrated by extraction with solvent using the Diethylammoniumdiethyldithiocarbamate/Xilene system. Ca, Mg and Cr were determined directly on the water solution after diluition or concentration by evaporation. The water hardness was determined using EDTA. Pb, Cu, Zn and Cd were measured by atomic absorption using a Spectrophotometer Perkin Elmer Mod. 460, in which is mounted a background corrector with a deuterium lamp.

RESULTS

The obtained results are summarized in table 2. To compile the data the communities were divided into two categories; A, with a high mortality ($500/10^5$ pop) and B, with a low mortality

(200/10^5pop). The comparison between the values of the relative elements in the two areas show significant difference for copper, with higher values in area B and between the Zn/Cu ratios, with higher values in area A. The linear correlation coefficients calculated between the values of the elements and the mortality rates do not show a significant correlation; only in the case of copper does the coefficient arrive at a value lower than 20%. In addition, a non parametric correlation (from Kendall) was carried out between the mean values for the elements found in the water samples and the mortality rates in the different communities; the negative correlations with Calcium (T=-0.60; S=-27) and with water hardness (T=-0.55; S=-25) proved to be significant. As for the values of Cadmium, Chromium and Lead no inferential analysis was carried out because in most of the water samples the levels were below detectable limits. Therefore, only the mean, maximum and minimum values have been reported (Table 3). In addition, research on the quantities of the same elements in various groups of foods ready for consumption (Spaghetti, soup, rice, meat, fish, tuna fish, sausage, chicken, legumes, potatoes, salad, cheese, fruit, bread and wine) was carried out. As it can be seen from fig.1 the average ratio between water and food, as for the intake of various elements, ranges from 6/1000 for Zn up to 25/1000 for Cr.

TABLE 2 Mineral characteristics of water in communities with different mortality rates.

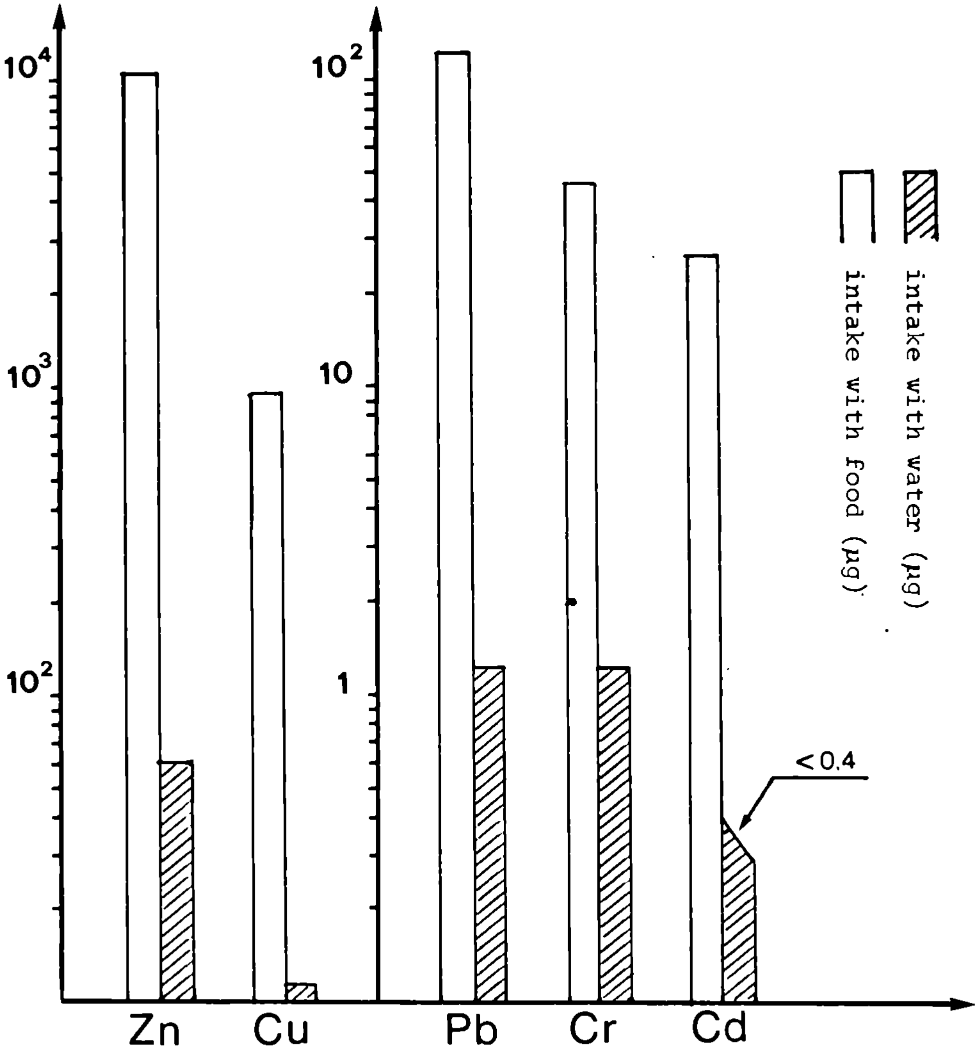
		Category A	Category B	Signif of* comparisons	Correlations coefficient
Ca mg/l	$\bar{X}$	83,66	93,50	0,20 P 0,30	r= -0,069
	ds	39,25	35,53		ns
Mg mg/l	$\bar{X}$	29,85	29,15	ns	r= +0,105
	ds	15,71	14,89		ns
Hardness mg/l	$\bar{X}$	372,54	399,166	ns	r= -0,029
	ds	177,95	155,90		ns
Zn µg/l	$\bar{X}$	313,87	307,30	ns	r= +0,027
	ds	689,75	563,61		ns
Cu µg/l	$\bar{X}$	7,50	11,27	<0,05	r= -0,418
	ds	5,19	7,87		P<0,20
Zn/Cu	$\bar{X}$	61,82	54,30	≃0,05	r= + 0,036
	ds	67,31	60,00		ns

* Analysis on the data transformed in log.

TABLE 3 Water Cd, Cr and Pb content in the Communities of categories A and B.

	Category A			Category B		
	mean* µg/l	min µg/l	max µg/l	mean* µg/l	min µg/l	max µg/l
Cadmium	–	<1,0	<1,0	1,5(2)	<1,0	1,5
Chromium	6,25(8)	<1,0	9,0	5,5(13)	<1,0	12,0
Lead	3,75(12)	<1,0	9,0	8,0(12)	<1,0	28,0

* The mean was calculated only in the case of detectable levels. The number of samples is indicated in parenthesis.



CONCLUSIONS AND COMMENTS

A tendency towards a negative correlation between mortality for CHD and Copper content of water is supported by the significant difference between the values for Copper and for the Zn/Cu ratio of the two areas. Zinc has not shown an association with CHD. This finding agrees with the hypothesis of Klevay who states that in the Zn/Cu ratio the important role belongs to Copper; this finding could also be due to the high variability of the results. The negative association of CHD with water hardness and Calcium can be shown only by using a non parametric correlation, between the expressed values, according to a graded scale.

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Contractor	: National Institute for Water Supply Leidschendam, Nederland
Contract No	: 273-77-1 ENV N
Projectleader	: Drs. B.J.A. Haring
Title of the project	: Epidemiological study on the relationship between water quality and health parameters in The Netherlands. Inorganic water constituents and health parameters

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Objective of the research

In The Netherlands central softening of drinking water was postponed because of the statistical relation which was found between the hardness of drinking water and death rate from ischemic heart disease (Biersteker et al. 1975; Gezondheidsraad 1975). It was recommended to study first in greater detail the possible cause of this statistical relationship.

At present a national program for the study of the relationship between the content of mineral water constituents and morbidity and mortality in The Netherlands is being coordinated by a working group, chaired by Prof. R.L. Zielhuis of the Coronel Laboratory, University of Amsterdam. Participating organisations are: National Institute for Water Supply (RID), Leidschendam; National Institute of Public Health, Bilthoven; Testing and Research Institute of The Netherlands Water Undertakings (KIWA), Rijswijk; Agricultural University, Wageningen; Interuniversity Reactor Institute, Delft and the Ministry of Public Health and Environmental Hygiene, Leidschendam.

This report presents the results of a survey concerning the intake of minerals and metals from drinking water.
Special attention will ge given to those factors which affect increased exposure of consumers to metals released from water distribution systems in households (e.g. cadmium, lead, copper and zinc). Examples of such factors are:
- agressivity of the water vs. the water distribution pipe;
- length of the water distribution pipe;
- water usage pattern of the population e.g. flushing of the tap before the abstraction of water for consumption purposes, volumes of water consumed etc.

The relative importance of these factors in relation to the exposure of the population to metals released from household plumbing systems can be studied by comparing the distribution of metal exposure in different water supply areas. The determination of the intake of minerals and metals by drinking water runs parallel with a survey of the population exposure to lead by drinking water distributed via lead pipes. The watersampling was conducted by KIWA and waterworks laboratories, while the analysis were carried out at RID.

The study was preceded by two preparatory studies:
- a pilot study in Amsterdam to try out "proportional" sampling of drinking water, while furthermore the statistically desirable sample size was determined;

- an inquiry formulated in cooperation with the CBS (Central Bureau of Statistics), which aimed at a better knowledge of the water usage patterns of consumers especially with respect to the quantity of drinking water consumed in relation to the source and hardness of the water.

Materials and methods

Earlier investigations (Zoeteman and Haring, 1976) demonstrated that large differences in the concentration of metals released from household piping materials are found, dependent on the period of standstill of the water in the pipe; therefore, a proportional type of sampling was recommended. A sampling device, which can be connected to the kitchen tap and can sample 5 % of the water used for consumption was designed especially for this purpose (figure 1).
From the volume of water in the sampling bottle and the number of persons living in the appartment an average volume of water consumed per person can be calculated and compared with data obtained from an inquiry relating to consumption patterns.

The performance of the proportional sampling device was tested in houses in the townships of Hoensbroek and Brunssum equipped with copper pipes (Haring et al. 1977; Vos et al. 1977) and in Amsterdam in houses with lead plumbing systems.
The analysis of Pb, Cd and Cu in the proportional samples were carried out with anodic stripping voltammetry on a stationary hanging mercury drop electrode using a P.A.R. model 316 automated cell sequencer (Haring, 1975). All other metals were determined with atomic absorption spectroscopy using Perkin Elmer equipment (models 603 and 306 with HGA-500 and HGA 76B).

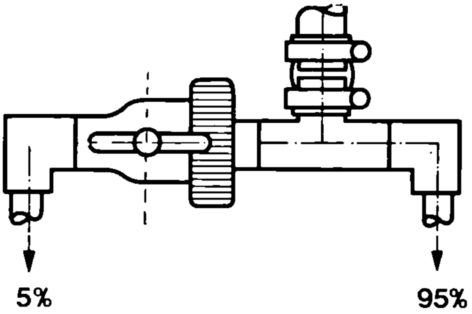

Fig. 1

proportional water sampler
tap open, when water is used for consumption
tap closed, when water is used for other purposes

Results

a. Inquiry on water consumption patterns in The Netherlands

The inquiry was held in June 1976 among 2.000 household which were selected in a representative way. The response of 1.472 households included 4.620 persons located in 89 communities. The selection of households aimed at a representative stratified sample of the population, not only with respect to the distribution of sex and age, but also in relation to water quality (hardness) and raw water source (groundwater, bankfiltered surface water and stored surface water in dunes or reservoirs).

The most important results of the inquiry are:

- In The Netherlands the number of houses with lead piping is estimated at 932.000 ± 91.000 which is about 22 %.
- The average volume of drinking water which is actually consumed increases until the age of 40;there after the level remains constant at 1,3 litres per person per day (figure 2).
- 1 % of the population consumes more than 3,3 litres of drinking water per day.
- About 10 % of the population never drink water as such.
- Drinking water consumption was found to be significantly higher in areas served with ground water compared to areas with surface water or bankfiltered surface water as drinking water source.
- Individual water usage patterns with respect to the flushing of the tap before water consumption appear to be determined by the kind of water use. For coffee or tea making the tap was flushed in 25 % of the households while 44 % of the people flushes the tap before drinking a glass of water.
- Finally it can be mentioned that hot water from boiler or geyser is regularly used for making coffee or tea in 25 % of the households.

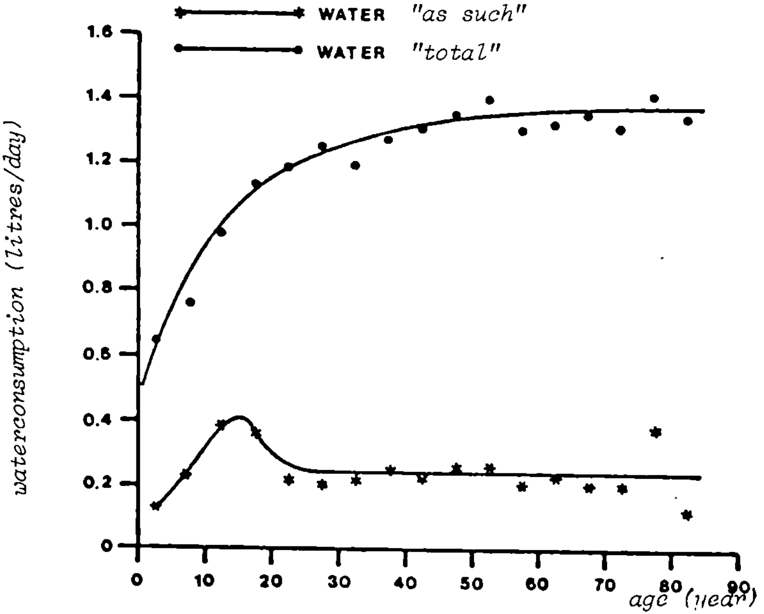

Fig. 2
drinking water consumption and age

b. Pilot study on the exposure to lead, copper and zinc from household water distribution pipes in Amesterdam

In cooperation with the Amsterdam waterworks both early morning samples and proportional samples were collected at 200 addresses. Some results of the pilot study are:

- Lead concentrations in "proportional" samples were found to be 3 times higher in houses with lead plumbing than in houses equipped with copper piping.
- A significant correlation was found between the metal concentrations in "proportional" and "early morning" samples.
- Relations were demonstrated between metal exposure on the one side and length of the household waterdistribution pipe and consumption habits with respect to the flushing of the tap on the other side. Only the first relation was found to be significant (95 % confidence).
- The desired number of samples for proportional sampling was defined as the number of samples necessary to make a significant distinction between e.g. a 5 % and a 25 % exceedance of a certain standard (the percentages are related to the fraction of samples that exceed the standard). According to these criteria the desired number of samples was calculated to be 100.

c. Inventory of daily exposure to lead and other elements by drinking water

The survey of lead exposure via drinking water in 25 communities was primarily initiated to examine the relative importance of factors that enhance lead exposure and also to evaluate the Dutch situation against the background of the standard for lead in drinking water which was recently adapted by the E.C. The results of this survey will also be used in the studies as to the possible cause of the relation which was found between hardness of drinking water and death rate from cardiovascular diseases.

In each community 50 addresses (with lead plumbing) were "proportionally" sampled during one week. The results of 18 communities are available at the moment (December 1978). It was found that the range of lead concentrations (in the proportional samples) within one community is usually much smaller than the differences between average lead concentrations in different communities. It could be concluded that the agressivity of the water (pH) is one of the most important factors in relation to lead exposure. Apart from Pb, Cu and Zn the following elements were determined in the samples: Ca, Mg, Na, K, Si, Li, Sr, Ba, B, Al, Cr, V, Mn, Mo, Co, Ni, Be, As and Sb.

d. Correlations between water quality and death rates

Finally, the statistical relationships between death rate from cardiovascular diseases (C.V.D.) and some inorganic waterconstituents such as Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, pH, Cl^{-}, HCO_3^{-}, NO_3^{-} were studied by calculation of Spearman rank correlation coefficients. The results of this study confirm earlier findings by Biersteker and Zielhuis (1975).
As soon as data on various death causes during 1977 and 1978 become available it will be possible to investigate possible relationships with the data on heavy metal exposure by drinking water which are presented in the report under discussion.

Conclusions and additional comments

The survey of daily exposure to minerals and metals by drinking water has produced valuable information for future epidemiological studies.
There is some evidence that the statistical relationship between hardness of drinking water and death rate from C.V.D. is indeed due to lack of Ca or Mg in the water (Neri, 1978). In this respect, another important field to investigate is the effect of water hardness on the exchange process of minerals and metals in drinking water and food, during food preparation. Besides conclusion of the ongoing epidemiological study one of the main objects for the future is to study the possible extraction of essential elements (Ca, Mg, Zn) from food during cooking with soft water in comparison with hard water.

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Contract No.	: 273-77-1 ENV N
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Title of the project	: Epidemiological study on the relationship between water quality and health parameters in The Netherlands Organic water constituents and health

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Objective of the research

Only in the last decade, significant advances have been made in the area of the quantitative analysis of organic compounds in drinking water. The increased possibilities of the analytical techniques to measure organic constituents have led to the conclusion that the occurrence of these compounds in drinking water may create health problems (1,2).

In the first part of this report the results of a survey concerning the presence of organic constituents in drinking water in The Netherlands have been presented. Special attention is given to those compounds which may casue irreversible changes of the body tissue and of which threshold limit values cannot yet be established on a scientific base, viz. the mutagens and carcinogens (3).
The growing consensus, that the majority of human cancers is caused by environmental agents with special references to chemicals also leads to an increased attention for carcinogens and mutagens in drinking water.

In the second part of this report an epidemiological study has been presented whereby drinking water derived from groundwater and surface water, in one way and the organic constituents found in drinking water in nineteen cities on the other way, were correlated with death rates of five cancer sites.

Materials and methods

Twenty cities were selected for the survey of organic constituents in drinking water and nineteen cities for the correlation studies on the five cancer sites. The selection of the cities was based on the type of raw water source (groundwater or surface water), the type of storage facilities (reservoir, dune or bankinfiltration) and the oxidative treatment (ozone or chlorine).
A composite sample for a certain community or groups of communities was collected in case more than one pumping station was supplying the area. Stainless stell vessels of 200 litres content were used for this purpose. Analysis of the water samples for the presence of organochlorine pesticides and of cholinesterase inhibiters was carried out at the National Institute for Public Health, Bilthoven, by means of gas chromatography (GC) Polynuclear aromatic hydrocarbons (PAH) were determined using a modified thin-layer chromatography (4). Volatile halogenated compounds like chloroform and trichloroethene were analysed by headspace analyses. In addition the concentration method of Grob (5) (closed-loop gas stripping) was also used.

To obtain higher boiling and slightly polar compounds, the concentration method developed by Junk (6) was used. In this method adsorption of organic compounds on macroreticular resins (XAD) is applied and elution of this resin is carried out with diethylether. The organic compounds in this extracts were analysed with gaschromatography-mass spectrometry.

The epidemiological investigation was based on a population of 4.6 x 10^6 inhabitants of The Netherlands, which is about 33 % of the population of the country. Cancer mortality rates for both sexes of all ages were obtained from the Central Bureau of Statistics (CBS) and taken over a period of twelve years (1964-1976). The cancer sites considered were esophagus, stomach, colon, rectum and liver. The standardized mortality rates were correlated to the concentration range of organic groups, viz. trihalomethanes, total alkylbenzenes, total chlorobenzenes, total phthalates and specific organic compounds viz. chloroform, bromodichloromethane and trichloroethene present in the drinking water of nineteen cities prepared from various raw water sources.

Results

a. Presence of organic constituents in drinking water in The Netherlands

A total number of 280 organic compounds has been detected in the twenty types of drinking water investigated. Nearly 100 compounds of these 280 could not be identified.

The most important results of the survey were:

- In drinking water derived from groundwater supplies some halogenated hydrocarbons like tetrachloromethane, trichloroethene and tetrachloroethene were rather frequently detected. The presence of these compounds in drinking water is probably the result of groundwater contamination.
- Nearly twice as much organic compounds were found in surface water supplies than in groundwater supplies.
- The largest number of organic compounds was found in tapwater derived from Rhine water after storage in open reservoirs.
- If during water treatment a higer amount of chlorine was used, it resulted in an increase of the concentration of trihalomethanes.
- Mutagenic and carcinogenic organic compounds could be detected in drinking water at relatively low levels.

b. Correlations between water quality and cancer mortality rates

The statistical relationship between five cancer mortality rates and two raw water sources from which drinking water is prepared (surface water and groundwater) as well as some organic constituents such as trihalomethanes, total alkylbenzenes, total chlorobenzenes, total phthalates, chloroform, bromodichloromethane and trichloroethene was studied by linear regression analysis. All the results should be regarded as preliminary as no confounding factors were included in this study. The results of this study confirm earlier findings of Diehl and Tromp (7) and show higher mortality rates for all five considered cancer sites if drinking water was prepared from surface water (appendix 1 : table 1).

Other preliminary results are : (appendix 1 : table 2-6)

- The concentration of trihalomethanes (THM) in drinking water and cancer mortality rates of esophagus and stomach show a significant positive correlation for.males.
- The concentration of chlorobenzenes in drinking water and cancer mortality rates of rectum show a significant positive correlation for females.
- The concentration of alkylbenzenes in drinking water and cancer mortality rates of stomach and liver in both sexes and esophagus for males show a significant positive correlation.
- The concentration of phthalates in drinking water and cancer mortality rates for the esophaugs for females and stomach and colon for males show a significant positive correlation.
- Between the concentration of trichloroethene in drinking water and mortality rates of all the considered cancer sites no significant correlation was found in both sexes.
- Alkylbenzenes and phthalates in drinking water show more positive correlations with the five considered cancer mortality rates than THM's.

Conclusions and additional comments

The survey of organic constituents in drinking water revealed that a number of organic compounds could not be identified. In order to be able to assess whether these compounds constitute a hazard, existing analytical techniques for identification and quantification should be improved.
From this survey and from earlier observations it can be concluded that carcinogenic and mutagenic compounds are present in drinking water although only in relatively low concentrations. The epidemiological investigation revealed that significant statistical relationships exist between raw water sources from which drinking water is derived and some of the considered cancer sites. This positive statistical relationship is also present for some organic constituents and some cancer sites.
The mortality rates for esophagus and stomach showed to be consistently and strongest in association with the exposure to all the considered groups of organic compounds.
Bladder and lung cancer as well as confounding factors were not included in this investigation. It is evident that these cancer sites and some confounding factors should be included in further epidemiological studies.
The preliminary epidemiological data indicate that there may exist a relationship between the considered cancer sites and some organic constituents as well

as the drinking water sources. The question whether a particular relation between the observed cancer sites, the concentration of some organic constituents and the raw water source exists has to be first clarified by completing the present epidemiological study in the Netherlands.

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Appendix 1

Correlation coefficients between standardized mortality rates (Mt) of five cancer sites in males and females versus two types of drinking water (raw water source, ground- and surface water) as well as organic constituents present in drinking water.

Table 1

Correlation coefficient between mortality rates in both sexes versus surface water/groundwater as drinking water source and α-values for two tailed test

Sex all ages	Site or type of malignancy									
	Esophagus		Stomach		Colon		Rectum		Liver	
	R	α	R	α	R	α	R	α	R	α
Male	-0.36	<0.2	-0.28	ns*	-0.27	ns*	-0.11	ns*	-0.56	<0.05
Female	-0.44	<0.1	-0.34	<0.2	-0.28	ns*	-0.36	<0.2	-0.18	ns*

Table 2

Correlation coefficient between mortality rates in males and females versus levels of trihalomethanes in drinking water and α-values for two tailed test

Sex all ages	Site or type of malignancy									
	Esophagus		Stomach		Colon		Rectum		Liver	
	R	α	R	α	R	α	R	α	R	α
Male	0.43	<0.1	0.33	<0.2	-0.06	ns*	0.05	ns*	0.01	ns*
Female	0.25	ns*	0.11	ns*	0.01	ns*	0.08	ns*	0.20	ns*

Table 3

Correlation coefficients between mortality rates in males and females versus levels of chlorobenzenes in drinking water and α-values for two tailed test

Sex all ages	Site or type of malignancy									
	Esophagus		Stomach		Colon		Rectum		Liver	
	R	α	R	α	R	α	R	α	R	α
Male	-0.03	ns*	0.0	ns*	0.03	ns*	0.19	ns*	0.28	ns*
Female	-0.01	ns*	0.10	ns*	0.19	ns*	0.38	<0.2	0.25	ns*

Table 4

Correlation coefficients between mortality rates in males and females versus levels of alkylbenzenes in drinking water and α-values for two tailed test

Sex all ages	Site or type of malignancy									
	Esophagus		Stomach		Colon		Rectum		Liver	
	R	α	R	α	R	α	R	α	R	α
Male	0.47	<0.05	0.35	<0.2	0.14	ns*	0.21	ns*	0.33	<0.2
Female	0.26	ns*	0.36	<0.2	0.15	ns*	0.18	ns*	0.34	<0.2

Table 5

Correlation coefficient between mortality rates in males and females versus levels of phthalates in drinking water and α-values for two tailed test

Sex all ages	Site or type of malignancy									
	Esophagus		Stomach		Colon		Rectum		Liver	
	R	α	R	α	R	α	R	α	R	α
Male	0.16	ns*	0.39	<0.1	0.33	<0.2	0.01	ns*	0.29	ns*
Female	0.35	<0.2	0.19	ns*	-0.01	ns*	0.0	ns*	0.16	ns*

Table 6

Correlation coefficient between mortality rates in males and females versus levels of trichloroethene in drinking water and α-values for two tailed test

Sex all ages	Site or type of malignancy									
	Esophagus		Stomach		Colon		Rectum		Liver	
	R	α	R	α	R	α	R	α	R	α
Male	0.07	ns*	-0.18	ns*	0.18	ns*	-0.11	ns*	0.01	ns*
Female	-0.16	ns*	-0.07	ns*	-0.04	ns*	-0.0	ns*	0.0	ns*

ns* - not significant (α = 0.2)

Contractor: Water Research Centre, UK

Contract No: 246-77-1 ENV UK Project A

Project Leaders: V.K. Collinge and B.T. Commins

Title of Project: Water quality and cardiovascular disease : change situations in the United Kingdom

---

1. OBJECTIVE

The objective of this project was to identify situations in the United Kingdom where water hardness has changed by at least 25% and to find out whether the changes in drinking water hardness have been accompanied by significant changes in cardiovascular disease mortality rates.

2. METHODS

In an investigation of this kind it is essential to make the observations on fairly large communities. Crawford, Gardner and Morris (CGM) (1) in their important earlier UK study looked at changes in water hardness and mortality rates in the county boroughs of England and Wales. Before the local government changes of 1974 the then county boroughs were the major centres of urban population outside London. The present study has, so far, been based on this same category of 89 towns.

A definition of a 'change' in water hardness has to specify the size of the change and the time period during which it is to have taken place. For size, this contract suggested 25%. CGM had earlier used an absolute change in total hardness of 50 mg $CaCO_3$/l. In the UK about one third of supplies are below 100 mg $CaCO_3$/l and one fifth below 50 mg/l. One tenth are however over 300 mg/l. The two possible criteria just mentioned operate differently at the extremities of this range. We therefore recommended a compromise, to define a 'change' if the water hardness has changed by (25 + 0.125H) mg $CaCO_3$/l where H is the initial or final hardness whichever is the lower.

Information from several sources but mainly that collected specially for WRC studies (not covered by EEC contract) was used to identify changes which took place precisely in the time intervals : 1951 - 1961, 1961 - 1971 and 1951 - 1971, using each definition of 'change' in turn.

Whereas the initial contract had suggested that the mortality experience of each 'change' town should be compared with that in a pair of matched control towns over three five-year periods, the constraints of available data and the difficulty of matching the controls in a scientific and effective way, were reasons for our reverting to an approach and method of analysis similar to Ref. (1). This is essentially a comparison between three groups of towns:

Group A	towns which have decreased their water hardness
Group B	towns which have increased their water hardness
Group C	no change, control group.

For each town, the change in cardiovascular (or other cause specific) death-rate, for a specific age-sex class, is measured by the log ratio,

$$y = \log_e (\text{death-rate at } t_2/\text{death-rate at } t_1),$$

where t_1 and t_2 denote the times between which the mortality rate changes are being defined. The statistical analysis hinges on the comparison of the group means, $\bar{y}_A$, $\bar{y}_B$ and $\bar{y}_C$. Different age-sex classes give rise to separate y variates and the results for these may be assessed either separately or jointly. The method of joint assessment used by CGM has however been corrected to allow for inter-class correlations.

Cardiovascular death-rates for each town were derived for the periods 1948 - 54, 58 - 64, 69 - 73 centred on the National Censuses of 1951, 1961, 1971. Cardiovascular was defined by ICD codes 330 - 334, 410 - 468 (6th/7th revisions) and 393 - 458 (8th revision). We have used rates for four age-sex classes: males 45 - 64 years, 65 - 74 years; females 45 - 64 years, 65 - 74 years.

The analyses comprised first a re-working of the CGM data set and then an assessment of the changes which took place in 1961 - 1971 and then those in 1951 - 1971.

3. RESULTS

3.1. Re-assessment of the paper by Crawford, Gardner and Morris

CGM had claimed a high significance ($p < 0.02$) for the association between cardiovascular death-rates and water hardness changes, but had ignored that the death-rate changes in their four age - sex classes were not statistically independent. The re-assessment thus started with this question of statistical method. However, the re-examination of the data revealed that for Canterbury, one of the five towns which had increased its water hardness,there appeared to have been an exceptional combination of death-rate changes. At first a clerical or arithmetical error was suspected but detective work eliminated this and narrowed the trail to an institutional population. With the help of the Kent Area Health Authority the source of the anomaly was identified.

The correction of the method, but mainly the revision of the Canterbury data, modifies the outcome. The critical probability at which the 'water factor' achieves significance now becomes about 0.1, which is much less conclusive than before. In spite of the tentative correction of these defects in the CGM paper, we continue to have strong reservations about the way in which CGM assigned the towns to groups.

3.2. Results from new data

Using the more stringent criteria for defining changes we have been unable to find enough towns to support a statistical analysis of changes between 1951 and 1961.

For the 1961 - 71 changes, the results may be summarised as follows:

(a) The picture is similar, whichever criterion is chosen.

(b) The number of towns in the 'decreasing' group was generally small (2 to 5). As far as cardiovascular mortality changes were concerned, it was not possible to distinguish statistically between this group and either the controls or the 'increasing' hardness group.

(c) The cardiovascular mortality changes in the 'increasing' group were significantly different from those of the controls. This was due mainly to the results for male mortality, which support the 'water story'. Figure 1 shows the comparisons for males and females aged 45 - 64, based on the recommended definition of hardness change. For males aged 45 - 64 the 'increase' group shows an average mortality 9% below the control group. The 95% confidence interval for this effect is from 4% to 13% of the control group mean. However, the effect on females tended to be smaller and in the opposite direction.

The set of changes between 1951 and 1971 in general showed no significant association between cardiovascular mortality and water hardness. Whilst over the longer time period more towns changed hardness, the scatter of points representing mortality changes in the control towns also increased, making any possible association more difficult to detect.

3.3. Power of method

The wide scatter of death-rate changes raises the question whether the design of this study and its statistical method are capable of detecting a water effect if such an effect really does exist. This question has been examined theoretically. For a ten year study (1961 -71 for example) with five towns in each of groups A and B, the method would stand a very good chance of exposing a water factor which systematically produced a change of 10% in cardiovascular death-rates among the 45 - 64 age groups of males and females. If however the effect were only of size 5% the chance of finding it would be only around 50%. The calculations would give similar results with 2 and 9 towns in groups A and B.

4. CONCLUSIONS

This project has not produced conclusive evidence either for or against the possible effect of water hardness on cardiovascular disease. It has undermined the previous strong conclusions of Crawford, Gardner and Morris(1). The analysis of new data (1961 - 71) reveals a difference between the towns which increased hardness and the control group, for males, which is unlikely to have arisen fortuitously. This is offset by the inconsistent results for females and for the small number of towns whose hardness decreased.

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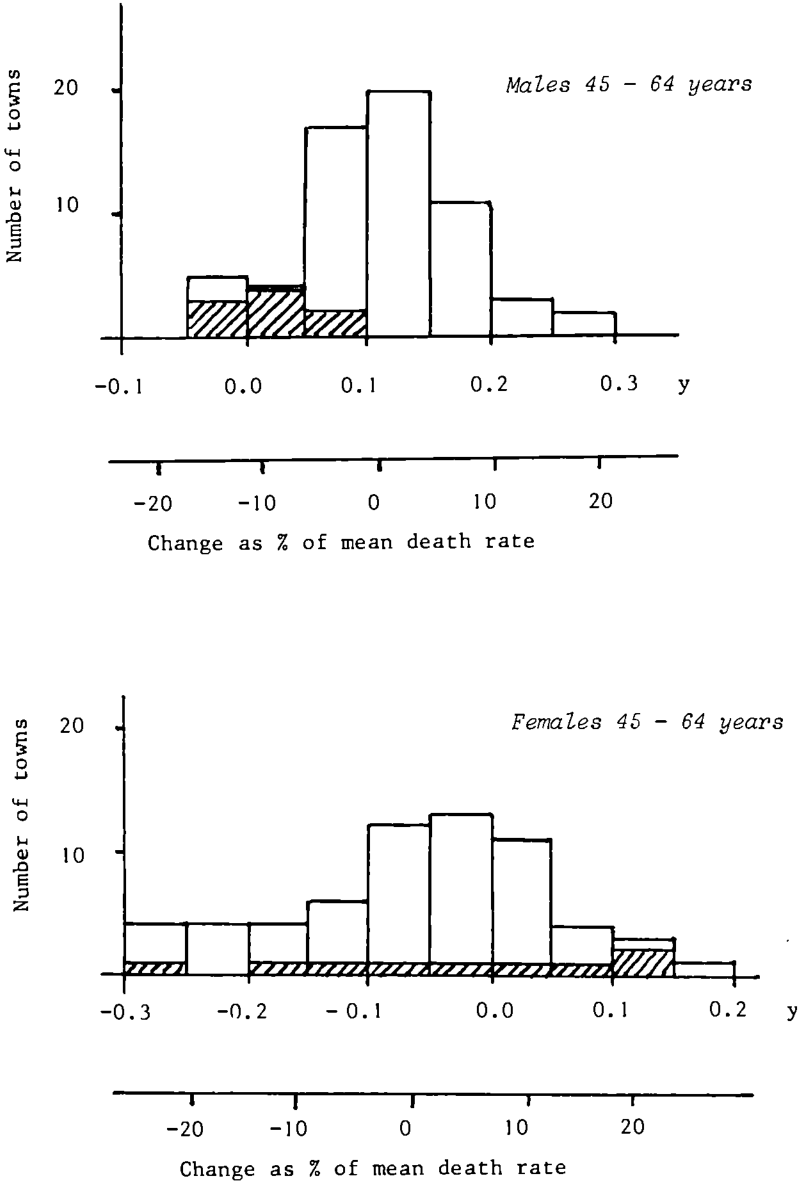
Fig. 1 Death-rate changes 1961 - 71 for
increased hardness group (9 towns) ▨
and control group (62 towns) ☐



Contractor :	Water Research Centre, UK.
Contract no:	246-77-1 ENV UK. Project B
Project leaders:	V.K.Collinge and R.F. Packham
Project title:	Population exposure to metals from piping materials for water distribution in the United Kingdom

1. OBJECTIVE

To investigate and evaluate methods of estimating population exposure to metals released from piping materials used for water distribution in the United Kingdom.

2. METHODS OF ASSESSING POPULATION EXPOSURE

There are two main factors which influence population exposure to trace metals in drinking water, i.e. the quantity of tapwater consumed per individual and the concentrations of the trace metals. The quantity of water consumed can be determined by surveying drinking water habits. Trace metal concentrations can be determined on discrete samples or those collected by a proportional sampler; alternatively they may be predicted from a knowledge of the factors influencing trace metal concentrations in water and information on household water usage patterns.

Progress has been made on the programme set out in the contract but certain aspects are incomplete. The greatest attention has been given to lead because of it's importance in the UK. A large number of samples have been collected however which are to be analysed for 30 trace elements using an Inductively Coupled Plasma Quantometer. A delay in commissioning this instrument has been due to the staff problems and the necessity for comprehensive checks on possible interactions between the different elements being measured.

2.1. Drinking water habits

Drinking water habits of middle aged men involved in a Regional Heart Study have been surveyed and further information obtained from a comprehensive nationwide drinking water habits survey (funded separately by the Water Research Centre.)

2.2. Sampling methods

First draw, fully flushed and random daytime samples have been collected from people's homes, and in experimental situations. A proportional sampler developed by RID in the Netherlands which collects 5% of the water drawn for drinking purposes has also been tested and used.

2.3. Factors influencing concentrations of metals in tapwater

The most important factors which have been studied are the effect of stagnation time and flow rate. *In situ* lead service pipes were studied in Scotland (very soft water) and in the South East of England (very hard water).

2.4. Water usage patterns

From information on household water usage patterns and from a knowledge of the factors which influence levels of metals in water it is considered possible to predict metal concentrations in tap water drawn for drinking purposes. Information was obtained for different household sizes using diaries and data loggers attached to flow meters.

2.5. Population exposure and computer simulation models

The ultimate intention is to combine information on drinking water habits with concentrations of trace elements. A computer simulation is being developed for uniting the various parameters for theoretically predicting exposures.

3. RESULTS

3.1. Drinking water habits

Data on drinking water habits of 518 middle aged men has been collected and analysed. The results from the questionnaires used indicate a wide range of volumes of water consumed, varying between 0.5 and 5.2 litres per head per day;43% of the men allowed some water to run to waste before drinking and 7% drank first draw water in the morning. A nationwide drinking water habits survey in which diaries and questionnaires were used is providing more comprehensive information.

3.2. Sampling and analytical results

About 1600 discrete tap water samples have so far been collected in the course of a Regional Heart Study. This represents 40 each of first draw, fully flushed and random daytime samples in each of 13 study towns. The samples have been primarily collected for trace element determination. In the experiments carried out to assess the factors influencing concentration of trace metals in tap water so far 500 samples have been collected for lead analysis using atomic absorption spectrometry. Concentrations of lead up to 1800 μg/l have been recorded.

Results obtained so far have confirmed that the proportional sampler developed by RID provides a reliable method of sampling tap water.

3.3. Factors influencing concentrations of metals in tap water

3.3.1. Influence of flow rate on lead concentrations

Two experiments were carried out on a lead pipe supplied with hard water one in which the pipe had not been used for a period of about 3 months and the other where the water had been continuously flushed before the experiment. In both cases there was clear evidence of a decrease in lead concentration with increasing flow-rate up to about 7 litres/min flow; in the first experiment there was a highly significant increase in lead concentration above this flow but this effect was only just apparent in the continuous flush tests. This is shown in Fig.1a. The same effect, although not so marked was observed in a soft water area.

3.3.2. Effect of stagnation in lead pipes

A number of experiments showed that lead concentrations increased sharply during the first few hours of stagnation and levelled off to a fairly constant level after 15 - 30 hours. An example is shown in Fig.1b.

When the stagnation time was kept constant (5 minutes) and the flow rate was varied it was found that for both hard and soft water situations there was a relatively small but nevertheless statistically significant increase in lead concentration with increasing flow rate.

3.4. Water Usage patterns

Data has been collected for various household types. This has been used for the computer simulation to assess the stagnation times of water in the pipe prior to use for drinking purposes.

A computer simulation model is being developed to predict exposure to lead under a variety of conditions and to indicate the factors which most affect the intake of this element. Preliminary tests have yielded encouraging results but further work is needed. It is proposed to develop this approach for other trace metals when the analytical data is available.

4. CONCLUSIONS

1. Lead concentrations in tap water in both soft and hard water situations were found to be highly dependent on the period of stagnation during the first few hours before water is used. They are also dependent on the flow rate at which the water is drawn.

2. Encouraging results have been obtained with a computer simulation model for predicting lead exposure by combining information on domestic water usage patterns and factors affecting levels in water.

3. It is apparent that there are a number of approaches for assessing population exposure and at this stage these have been subjected to only preliminary investigation. The work suggests that it is unlikely that there will be one universal method for assessing population exposure for all situations.

Figure 1a. Relationship between steady state lead concentration and flow-rate under flushing conditions (hard water).

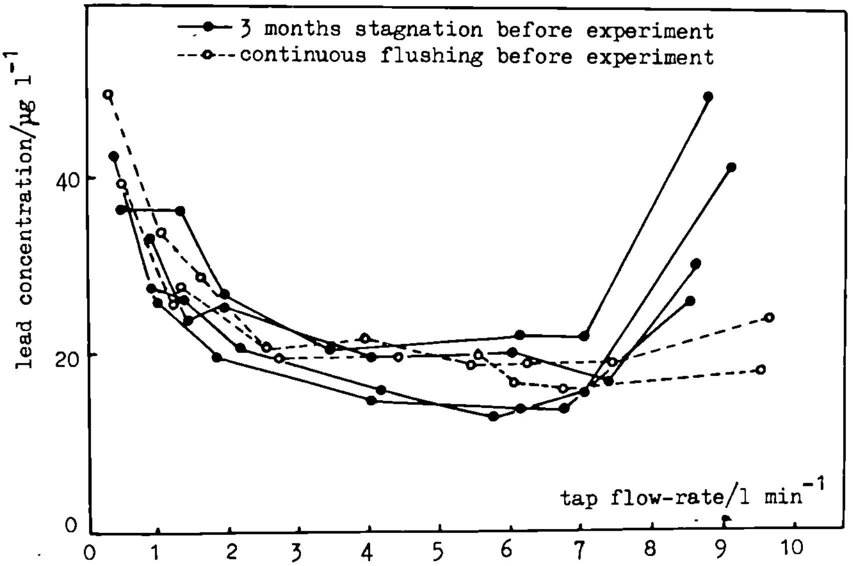
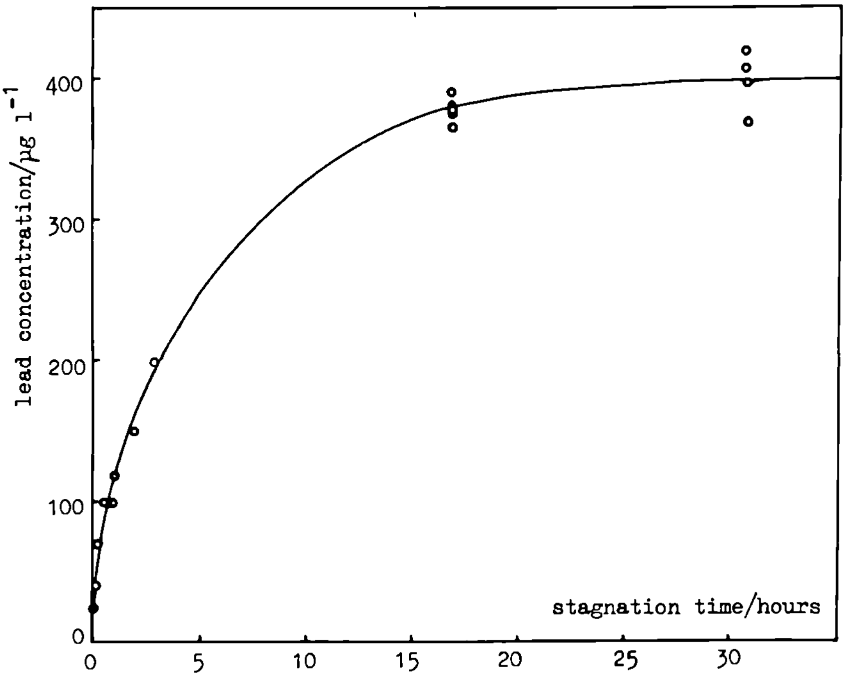


Figure 1b. Example of the shape of the stagnation curve.



TOPIC 17 : WASTE HEAT
&
TOPIC 18 : MARINE POLLUTION

Contractor: Kernforschungsanlage Jülich GmbH, D-617 Jülich,
Federal Republic Germany

Contract n^{o}: 173-77-1 ENV D

Project Leader: Prof.Dr.H.W.Nürnberg, Institute of Applied Physical Chemistry, Nuclear Research Center (KFA),Jülich

Title of project: Comparative Studies on Heavy Metal Pollution and Balance in Selected Regions of European Seas

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Objectives of the research:

Among the pollutants and potentially ecotoxic substances in the marine environment toxic metals constitute a significant class. The potential hazards are related to the fact that toxic metals are biologically nondegradable and exert tendencies to accumulate in marine organisms (plankton, mussels, crustaceans, fish). Via the marine food chains they reach man where a certain fraction is deposited in vital organs according to the degree of sea food consumption and exerts progressively increasing toxic effects of chronic nature. Moreover, there might be the still largely unexplored hazard of perturbations in marine ecosystems due to ecotoxic effects on marine life. Particularly vulnerable are the relatively shallow waters of coastal zones and estuaries which are subjected predominantly to the continuous intake from polluted rivers and all kinds of coastal waste water outlets. Yet these are the marine areas from which stems a significant amount of sea food (mussels, crustaceans and also many kinds of fish) and where in the future marine protein food production is to be developed on a large scale and by a modern technology of aquaculture and fish farming. With respect of toxicity are of predominant general significance the metals cadmium (Cd), lead (Pb), mercury (Hg), but also cooper (Cu), nickel (Ni), arsenic (As) and others are of interest. Reliable data on the levels, fate, behaviour, species and their distribution exist in European coastal waters at present still to a completely unsufficient extent. This is mainly related to the methodological difficulties which had to be overcome because the toxic metals exist and act in the marine food chains at the trace level and even at the ultra trace level in the dissolved state in sea water, the medium via which the transfer from the sediment and particulate matter deposits to the organisms at different trophic levels of the marine food webs occurs (1-4).

Thus, the objectives of our research were focussed on the following topics (1-6):

1. Comparative base line studies on the levels of Cd, Pb, Hg and Cu, dissolved and bound to particulate matter in coastal waters of the North Sea (from Oostende to Den Helder and Emden to Isle of Sylt and in German Bight) and of the Ligurian and Tyrrhenian coast (Ventimiglia to Cape Anzio). Additionally long term observations including the investigation of salt marsh plants are carried out in the Scheldt and Rhine estuaries in collaboration with the Delta Institute, Yerseke.

2. Comparative studies on the toxic metal content (Hg, Pb, Cd and more recently also Cu, Ni and As) in sea food (plankton; edible parts, intestinal organs and bones of benthic and pelagic fishes; crustaceans and mussels) from Atlantic, North Sea and Western Mediterranean Sea.

3. Elucidation of the predominant dissolved toxic metal species in sea water, as they are the relevant chemical forms for the entrance of toxic metals into the marine food chain and for the interactions of these metals with sediments and particulate matter. These studies were aimed to results of general validity on the identy, stability and the kinetics and mechanism of formation of labile inorganic complexes and the rather stable organic chelates of toxic metals under the specific influences of the salinity components.

4. Methodological developments leading to reliable procedures, free from artefacts producing contamination, for sampling, sample pretreatment and digestion and accurate, precise and highly sensitive methods for trace analytical determinations and physicochemical investigations at the actual trace levels at which toxic metals occur usually in the marine environment.

Methods:

The applied *determination methods* for metals in sea water and particulate matter are preferentially specially improved modes of differential pulse stripping voltammetry (DPSV) at a new version of the mercury film electrode (1-8,11) (MFE) for the simultaneous determination of Cd, Pb, Cu, Zn, Bi and Sb. For Hg a new DPSV-procedure in the subtractive mode at a twin gold electrode has been very successful (9,1o). These electrochemical procedures combine extreme sensitivity (determination limits typically 1 ng/l!) with satisfactory precision (RSD$\leq$ 8 % at the 1o ng/l level and above) and an inherently good accuracy. For Hg in sea water as alternative an automated procedure based on cold vapour atomic absorption spectrometry (AAS) has been introduced (12,13). While, expect for Hg, in sea water and other natural waters the voltammetric determination of toxic trace metals is due to its superior performance definitely the method of choice (1-6), for the trace metal content in biological material modes of flameless AAS (electrothermal for Cd, Pb, Cu, Ni, Zn (14); cold vapour for Hg (12,13) and hydride mode for As, Sb, Se (31)) remains an important and versatile alternative. Both methods, voltammetry and AAS, are due to their potentialities, their relatively moderate cost requirements and their compactness (installation in mobile laboratories on vans and ships) for environmental trace metal chemistry and surveillance the two most important methodological approaches (2,3,4,6). Particular significance for monitoring the ingestion of toxic metals from the atmosphere with rain and snow will gain a new procedure with automatic rain sampling and subsequent voltammetric trace metal analysis (15). Of course, biological materials, particulate matter or sediments require reliable, i.e. contamination free and losses avoiding, *digestion* procedures, which had to be developed and were tested by radiotracers for their chemical yield (16-19). For fishes and other organisms contamination free dissecting procedures with quartz knives have been systematically improved and developed (29,3o). The necessary sample pretreatment for sea water samples is simpler and involves o.45 /u-filtration, subsequent pH-adjustment and according to a new procedure UV-irradiation to decompose metal binding components of dissolved organic matter (DOM) (11). Besides these sample pretreatment procedures *sampling* itself is one of the most critical and vulnerable stages concerning risks of analytical contamination and resulting accuracy deficiencies of the determined data. These problems are successfully overcome by new unorthodox water sampling approaches (surface water from rubber boats or small wooden cutters with all plastic devices, deeper waters with a new device based on Boyle-Mariotte law) (11,2o). The systematic and consequent development of rigorous *cleaning* procedures for all used labware (21), the development of *working standards* for fish and marine organisms (22) and the full adaptation of all manipulations to the requirements of demanding trace analysis were vital basic prerequisites for reliable results in the performed case studies (22).

Results and Conclusions:

Base line values of the *levels* of Cu, Cd, Pb in *coastal waters* (dissolved and at particulate matter) have been established in a comparative study (11,25) from over 4oo sampling stations at the Ligurian and Tyrrhenian coast (23,24) and along the North Sea Coast (Southern Bight, including Scheldt and Rhine estuaries, and German coast from Emden to Isle of Sylt, including Wadden Sea, Weser and Jade estuaries, and parts of German Bight (11,25). The data reflect a rather static situation in the studied Mediterranean area while at the North Sea coast hydrographic parameters (tides and tidal currents) exert strong influences. The content of particulate matter was usually at the North Sea significantly higher. Total Cd- and Pb-levels (dissolved plus particulate) are about a factor 5 higher (average: Cd o.o5; Pb 1.oo; Cu 1.24 /ug/l) than in the studied Mediterranean coastal zone (average: Cd o.o14; Pb o.133; Cu o.6 /ug/kg). The pattern of the total toxic metal profiles could be correlated to a certain extent to known local pollution sources (shipping routes, ports, waste water outlets). Studies on Hg-levels have been performed for the German Bight and are to be extended to other areas (26).

Now *long term studies* to reveal seasonal influences and generally to obtain more detailed long term based informations are at selected sampling points under way, e.g. in the Scheldt and Rhine estuary. The investigation of the toxic metal input into the latte estuary is supported by a nearly completed comprehensive study on the Rhine up to the Lake of Constance (27) . The estuarine studies are extended to salt marsh plants and marine organisms, to sediments and to the input from the atmosphere (15) applying the new automatic rain samplers.

A comprehensive comparative study on over 85o specimens of over 5o species of *pelagic* and *benthic fishes*, *crustaceans* and *molluscs* from the Western Mediterranean, Atlantic off the Strait of Gibraltar and the North Sea has revealed the Hg-levels in the edible part (fillet for fish) of this sea food (28-31). The Hg-level is for many Mediterranean, particularly pelagic, fish species definitely higher than for similar species from Atlantic and North Sea. The whole range of determined Hg-values goes from $<$ 2o to $>$ 2ooo µg/kg FW. Large prey fishes as tuna and sword fish have usually the highest Hg-burdens (33). Contents of Cd and Pb remain toxicologically unsignificant in the edible part of fish, but are accumulated in the liver and other organs (3o,31). Rather substantial amounts of Cd and Pb can occur according to the local pollution situation in filter feeders, as mussels, other molluscs and crustaceans. In the Mediterranean these studies based on our marine station at Fiascherino/La Spezia have been partly performed in collaboration with Prof.Renzoni (Univ. of Siena) (32-35) and the CNEN-Laboratory (28,29,32), Fiascherino, also as contributions to the UNEP/FAO-program (MED II).

The particular property of the voltammetric approach to be in principle sensitive to dissolved chemical species of trace metals has enabled very elucidating *speciation studies* yielding results of general validity for the sea (1,2,4,5,6,).

For the first time the carbonato complexes of Pb and Cd have been identified and characterized with respect to their stability in sea water (36,37) and improved data on Pb-chlorocomplexes have been determined. Based on these experimental results and literature data the distribution of the total dissolved content of Pb and Cd in the sea over their major *labile complex species* MeX_j could be quantitatively evaluated, taking as well into account for the first time influences of ion pairing effects between the salinity components (38).

Voltammetric studies with the defined organic chelators NTA and EDTA, by the way also already becoming anthropogenic pollutants in coastal waters, have revealed for the trace metals Cd, Pb and Zn the general specific influences of the salinity components on the stability of *organic chelates* (39-41) and the kinetics and mechanism of their formation (42-44) in the sea. All measurements could be performed due to the excellent sensitivity of voltammetry at the actual trace metal levels. Due to the competition of the Ca- and Mg-excess for the organic chelators trace metal chelates are virtually only formed by ligand exchange with previously formed alkaline earth chelates. From concentrations of NTA required for the chelation of Cd, Pb and Zn in sea water the demanded concentration of potential natural chelators from dissolved organic matter (DOM) can be predicted and conclusions on their ability to contribute to the speciation of toxic trace metals in the sea can be drawn (45). In this context it is to be expected that in the open sea the contribution of *dissolved humic materials* to the chelation of Pb, Cd and Zn remains negligible (45,46). This conclusion has been recently experimentally confirmed by electrophoresis with radioactive tracers of the mentioned trace metals (46-49) and by voltammetric studies (5o) for humate materials of terrestrial and of marine origin. The studies also indicated that nevertheless humate films at particulate matter or sediments might due to the significantly enhanced interfacial concentration act under these circumstances as chelators for the trace metals (45,46,5o).

In general it can be concluded that during the 1st phase of the 2nd Environmental Research Program for certain European coastal regions a number of accurate data and findings could be assembled forming a reliable basis for the toxic metal balances and the observation of the development of the toxic metal inventory of the studied marine ecosystems in the future.

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Contractor:	Federal Institute of Hydrology (Bundesanstalt für Gewässerkunde), Koblenz, Federal Republic of Germany
Contract No.:	207-77-7 ENV D
Project leader:	Dr. H. Bergmann
Titel of project:	Investigations on the hydrochemical state and the transport of trace metals in estuaries

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1. Objective of the research

The objective of the research program is to get more detailed information on the spatial distribution of trace metals and its time-dependent changes in an estuary. It finally aims at the mechanisms and approximate calculation of the metal transport within estuaries, and at the influx of trace metals from land into the sea.

2. Materials and methods

At the beginning of the program an extensive literature review with respect to trace metals in estuaries and coastal waters was carried out. The collected literature (ca. 1000 publications) were sorted into two areas:
a) Trace metal concentrations in water, particulate matter, sediments, plants, and animals; b) Processes within estuaries involving trace metals. An evaluation of the publications was attempted with the aim of describing the present state of knowledge.

Field investigations on the transport of heavy metals were carried out in the Ems estuary (Dutch/German border). At one location at the head of the estuary (oligohaline range), samples were taken hourly during a tidal cycle (13 h) in different water depths. At the same time and depth of sampling current velocities were measured. Water samples were analysed for Fe, Mn, Cu and particulate matter. From these concentrations ($c_{t,z}$) and the corresponding velocities ($v_{t,z}$) the transport ($Tr_{t,z}$) at the time t and the depth z was calculated for each component:

$$Tr_{t,z} = c_{t,z} \cdot v_{t,z} \qquad (\text{in g} \cdot \text{sec}^{-1})$$

For these calculations partial cross sections of 1 m width were assumed, as there was no information on the overall distribution of concentrations and velocities across the whole cross section. The water layer above the ground (approx. 0.5 m) was not included in the measurements and calculations. By integration of the time-dependent functions of the transport over the whole tidal cycle, partial loads (in kg) through partial cross sections in different depths were obtained. From these results net loads (difference between loads during ebb and flood) could be calculated.

Trace metal analysis was done by atomic absorption spectrometry (AAS) following recommended procedures. In addition, particulate matter was determined in separate samples taken simultaneously with the metal samples.

3. Results

3.1 Literature reviews

In compiling the literature review [1, 2] on heavy metals in coastal waters (water, particulate matter, sediments, plants, animals), the selection and listing of data was frequently handicapped by lack of information on sampling, sample preparation and analysis. Another, graver difficulty was the comparability of analytical data caused by the wide differentiation of research objectives and by specific differences within the matrices, which render a comparison of data problematic. Therefore, one result of the review is the finding that most analytical data on heavy metals in coastal waters found in the literature probably cannot be used for a genuine comparison. Consequently, when reporting research results it would seem to be appropriate to take into account the criterion of "comparability of data". No doubt, the tables of concentration values indicate tendencies of heavy metal loads, but at present they cannot give definite minimum and maximum concentrations in coastal waters.

One application of the comparison of data is the biological monitoring, here specifically related to trace metal concentrations in littoral plants and animals. Standardization of bioindicators has not yet been possible because of insufficient knowledge of suitable organisms. In addition, the correlations between trace metal contents of the organisms and their environment, and the kinetics of accumulation in the organisms are only vaguely known. However, without knowing this no reliable conclusions concerning the heavy metal load of coastal waters can be drawn. The most reliable data is offered at present by some internationally-coordinated investigative and monitoring programmes, where from sampling to evaluation great emphasis is placed on the comparability of data.

A second literature review of chemical, physical, biological and hydrological processes in estuaries involving heavy metals was compiled (360 publications [3]). The evaluation itself was started in a similar manner as described above. As it turned out to be far more complex, it could not be finished to date. As partial result, however, could be found that concentrations of dissolved and particulate heavy metals in rivers are world-wide about the same, and that, therefore, a close relationship exists between the (average) river discharges and the metal load of the rivers. By taking into account and improving our knowledge of the aforementioned processes taking place within estuaries this fact may possibly be used to develop a method for assessing heavy metal influxes into the sea through estuaries.

3.2 Transport of heavy metals [4]

Presently available information on the transport of dissolved and undissolved heavy metals in estuaries is rather scarce. An attempt was, therefore, made at finding a way to determine transport and loads. The investigation was limited, for the time being, to a small part of the cross-sectional discharge area in the Ems estuary at Terborg. The benthic layer (approx. 0.5 m) was neglected.

For the four selected components copper, iron, manganese and particulate matter, the loads in the individual water layers and over the tidal cycle could be computed from concentration and velocity measurements. The results of the first cruise were as follows:

(a) In the area investigated, the transport of dissolved heavy metals is far lower than that of undissolved metals (0.1 % - 25 %, depending on the metal concerned).

(b) In the course of the tidal cycle observed, a net load of the four components concerned moved seaward in the topmost water layer and landward in the lowest water layer.

(c) Overall computation for the examined water depth and tidal phase show a net load of heavy metals moving landward, and of suspended sediments seaward.

These results were not representative, because they were strongly influenced by, inter alia, the meteorological conditions. Even so, however, the investigation methods used were found to be well suited for the in situ study of transport processes in the estuary.

The investigation was repeated in the same way two more times. Result a) (see above) was confirmed, whereas those in b) and c) showed a less consistent picture. A more detailed analysis of the data will be necessary to find out the reasons for the differences within the three series of measurements. Maybe then can be judged whether the method employed is capable of providing useful information on the transport of heavy metals in estuaries, or if weather conditions and other factors (river discharge, extreme tides etc.) influence the metal transport in an unpredictable way.

3.3 Hydrochemical state of heavy metals

For several years dissolved (< 0.45 µm) and particulate metals were determined at different sampling sites (salinities) in the Ems estuary. The purpose was to find out how the metals are distributed between the two phases, and how this distribution may vary in relation to salinity, pH and other parameters. The evaluation of present data is still in progress and will be finished shortly.

A little problem of special interest was the determination of dissolved Cr^{3+} and Cr^{6+}. A method for the separate analysis of these two ionic species at the ppb level could be worked out [5]. A field investigation of the occurrence of Cr^{3+} and Cr^{6+} in natural waters has been finished. A first survey indicates that in coastal waters the percentage of dissolved Cr^{6+}, compared to total dissolved chromium, is less than 20 % [6].

4. Conclusions and additional comments

The short time of investigation does not allow any definite conclusions. Some remarks, however, may be made:

a) Field investigations in estuaries of the mass balance of metals and their influx into the sea is strongly influenced by weather conditions. Further investigations have to show whether this influence renders any assessment of the metal influx into the sea unpredictable.

b) Another way to assess metal discharges into the open sea seems to be that of combining metal concentrations and water discharges of rivers with an improved knowledge of estuarine processes involving heavy metals.

c) First results indicate that the metal transport in estuaries occurs mainly in the particulate phase. However, it is questionable whether particulate or dissolved metals are of greater importance to litoral organisms in terms of bioaccumulation and toxicity.

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5 H. Bergmann and K. Hardt, Analysis of dissolved Cr^{3+} and Cr^{6+} in water by APDC-MIBK extraction and atomic absorption spectrometry, (1979) (submitted)

6 H. Bergmann and K. Hardt, Occurrence of dissolved Cr^{3+} and Cr^{6+} in natural waters (unpublished results).

Contractor : Laboratoire de Physique et Chimie marines à Villefranche sur Mer; Université Pierre et Marie Curie, Paris, France.

Contract n° 152-77-1 ENV F

Project Leader : C. COPIN-MONTEGUT

Tittle of project :"Répartition naturelle des éléments métalliques lourds dans l'eau de mer et le plancton".

Objective of the research :

As a result of current interest in heavy metal pollution, information is accruing on the present levels of metals in the marine environment and on the effect of these elements on marine organisms.

Although general concern has been expressed on the widespread pollution of the Mediterranean with certain metals, yet few systematic data exist at present on the trace metal distribution. In the first part of our study, an investigation has been carried out on cadmium and copper distribution in a limited area of the Northwestern Mediterranean. Levels in sea water and in plankton have been researched. Far less information exists on the elemental content of phytoplankton and zooplankton species, although these species are important in term of total biomass, their position in food web and their ability to concentrate and transport large amounts of metals in various ways.

In the second part of our study, experiments have been carried out in controlled conditions in order to determine the effects of cadmium and copper on some phytoplankton species. Preliminary results concerning toxicity thresholds and concentration factors have been obtained.

1 - Distribution of cadmium and copper in sea water and plankton samples of the Northwestern Mediterranean.

Materials and Methods :

Samples of sea water and plankton were collected throughout the year and along two selected transects in order to assess the possible seasonal and spatial gradients in metal distribution.

Twelve cruises were completed over a period of one year. One transect extended along the French coast between Monaco and Antibes and included 10 coastal stations approximately equally spaced. The other transect was perpendicular to the coastline and included 3 stations located at 7,10 and 20 miles offshore. In the restricted geographical area which was concerned contamination from land-based sources was to be expected.

The samples of sea water and plankton were analyzed both for cadmium and copper.

Surface water samples were collected with a 5 l Niskin bottle and stored in borosilicate bottles with tefloned topper. For cadmium and copper analyses, no treatment to stabilize the samples was performed after collection. Upon return to the laboratory, filtration and trace metal treatment were completed. Samples were filtered on Sartorius membrane filter (0.45 µm pore size) and a coprecipitation procedure using zinc ammonium pyrrolidine dithiocarbamate was performed immediately to preconcentrate cadmium and copper. The zinc-chelate matrix containing the coprecipitated metals was collected on a 0.45 µm membrane filter. The analyses were carried out by flameless atomic absorption spectrophotometry on an aliquot fraction of the membrane filter which was introduced into the graphite cup. The analytical sensitivity was 0.3 ng Cd/l and 8 ng Cu/l and the analytical precision was 20% for cadmium and 15% for copper.

Plankton samples were collected by making a vertical haul covering a depth range of 0-100 m, by means of towing together two 0.50 m diameter plankton nets: the first net (WPII type , 200 µm mesh) was used for zooplankton and the second one (53 µm mesh) for micro- and phytoplankton. After collection, a filtration procedure was conducted to separate the plankton in 3 different-sized subsamples : - fraction 1 (organisms of size >1,000 µm) containing macroplankton and salps - fraction 2 (organisms of size between 200 and 1,000 µm) corresponding to zooplankton in the case of the zooplankton net and" mixed plankton " in the case of the phytoplankton net - fraction 3 (organisms of size ranging from 53 µm and 200 µm) containing larval zooplankton and phytoplankton. Metal analyses were performed on fraction 2 of each net. Digestion was carried out on dry plankton material with nitric acid. Determinations were made by flame atomic absorption spectrophotometry on the digested solutions. The analytical sensitivity was 100 µg Cd/l and 350 µg Cu/l and the analytical precision was 20% for cadmium and 15% for copper.

Together with the metal determinations of sea water and plankton, hydrological parameters (such as temperature, salinity, dissolved oxygen...) were measured and the biological activity was estimated by the measure of the biomass of the plankton samples.

Results and discussion :

Results are shown in tables 1 and 2 with regards to dissolved cadmium and copper in sea water samples.

The average values are 0.037 µg/l for cadmium and 0.24 µg/l for copper. The degree of scatter of the results, indicated by the standard deviation of the mean, is high for cadmium (var. coeff. 81%) and less important for copper (54%).

Spatial variations are taken into consideration : coastal stations present mean values of 0.046 µg Cd/l and 0.25 µg Cu/l, whereas open-sea stations are characterized by mean values of 0.034 µg Cd/l and 0.20µg Cu/l. Nevertheless, no significant nearshore-offshore gradient could be assessed.

Seasonal variations : a significant increase in cadmium and copper concentrations is noticed during summer. Considering all results together,the mean value for cadmium is 0.054 µg/l during summer, whereas it is 0.030 µg/l during the other seasons. In the case of copper, the mean value is 0.39 µg/l during summer and 0.17 µg/l during the other seasons.

The increase in cadmium and copper concentrations is more significant when we consider coastal and open-sea stations separately :

Coastal waters are characterized by mean values of 0.056 µg/l for cadmium and 0.41 µg/l for copper during summer, instead of 0.031 µg Cd/l and 0.18 µg Cu/l during the other seasons. Open-sea stations present mean values of 0.049 µg/l for cadmium and 0.34 µg/l for copper during summer whereas mean values are 0.028 µg Cd/l and 0.14 µg Cu/l during the other seasons. Under these conditions, the variability of the results decreases significantly for copper.

Hydrological and biological phenomena could explain the increasing concentrations of cadmium and copper during summer:

Spring and summer are characterized by an increasing hydrographic stability (no vertical mixing of the water column) in the upper layer of the photic zone, resulting in thermal stratification.

Biological activity is high during spring and summer (especially in the beginning of thermal stratification) and dissolved organic compounds released by plankton might interfer and complex trace metals. Some authors have shown that copper, zinc,and nickel, complexed with dissolved organic compounds, have presented higher rates during summer.

An attempt to correlate trace metal distribution in sea water samples with hydrological parameters and biomass indicator will be made in further studies. Nevertheless,hydrographical and biological conditions seem to play an important and determinant role.

Results concerning the concentrations of cadmium and copper in plankton are shown in tables 3 and 4. Results are expressed in µg metal/ g dry weight.

Our results indicate that copper concentrations in plankton are highly variable whereas cadmium concentrations are more stable.

Copper appears to be taken up more efficiently by smaller organisms: the total median is 57.3 µg Cu/g for mixed plankton and 28.4 µg Cu/g for zooplankton.

Cadmium concentrations do not differ significantly according to size organisms. The median is 1.9 µg Cd/g for zooplankton and 2.0 µg Cd/g for mixed plankton.

Copper concentrations in mixed plankton and zooplankton do not show any significant correlation between them, whereas cadmium concentrations in mixed plankton and zooplankton are positively coupled.

Copper concentrations in zooplankton are not related to biomass throughout the year, whereas copper concentrations in mixed plankton exhibit negative correlation with biomass during the period of high variability of the biomass. This negative relationship might be due to high growth rates which could result in dilution of the amount of copper per unit mass of plankton.

No seasonal trend was observed in cadmium concentration, whatever be the net used. Moreover, no spatial trend between nearshore and open sea waters was noticed in cadmium and copper concentrations whatever be the net used.

2 - Experimental studies on the toxicity of metals to phytoplankton

Materials and Methods :

Tests in controlled conditions were carried out on phytoplankton strains in culture. Toxicity thresholds were determined by estimating the influence of these metals on cell growth. The uptake of metals by a phytoplankton species Cricosphaera elongata was studied.

Phytoplankton cultures were grown in local offshore water, filtered through a 0.2 µm membrane filter, enriched with nitrate, phosphate, silicate and iron. The enrichment of sea-water was great enough to allow a high growth rate without introducing potential complexing agents. Algae were cultured at 18°C under continuous light. Growth curves were obtained from cell counts. Toxicity experiments were carried out on culture volume of 10 ml and uptake experiments on volume of 2 liters.

Toxicity threshold : the toxicity threshold was considered as the metal concentration at which no growth occurred. Experiments were carried out at various concentrations: between 0.05 and 4 mg/l for cadmium and between 0.006 and 6.25 mg/l for copper.Metal was added to the culture medium 24 hours before inoculation and the subsequent growth rates were determined. These rates were compared with control rates in the absence of metal. Experiments were stopped when the control cultures reached the end of the exponential phase.

Uptake experiments : the species Cricosphaera elongata which is especially sensitive to copper, was chosen as a "test-species".The sublethal concentrations used in these experiments were 10 to 20 times lower than the toxicity threshold : cultures were contaminated with 10 and 20 µg Cd/l and 2.5 to 25 µg Cu/l. Contaminant was introduced in the culture medium at the end of the lag phase. Experiments were stopped at the end of the exponential phase. Culture media were filtered on a 8.0 µm membrane filter and cells were collected, then dried and digested. Analyses were carried out by flame atomic absorption spectro photometry.

Results :

The toxicity thresholds as regards copper correspond to 0.03 mg/l for Asterionella japonica, 0.39-0.78 mg/l for Coccolithosphora sp., 6.25 mg/l for Dunaliella salina and 0.025-0.050 mg/l for Cricosphaera elongata. As regards cadmium, the toxicity threshold corresponds to 0.4-0.5 mg/l for Cricosphaera elongata. There is much difference in the sensitivity of the various phytoplankton species to metals. Inhibitory effects on cell division rates are noticed at much lower concentrations than the toxicity threshold, especially for Cricosphaera elongata .

In the uptake experiments : the concentration factors range from 4,200 to 3,100 for culture media spiked with 10 and 20 µg/l respectively. In the case of copper, the concentration factors are nearly stable for concentrations in the culture media inferior to 12.5 µg/l (C.F. ranging from 2,100 to 2,900) whereas they increase significantly for 15 and 25 µg/l.

These preliminary experiments have allowed us to develop a methodology adapted to the toxicity studies of our program. They should lead to a more systematical research on other phytoplankton species. The study of acute and subacute effects of metals on these species will be continued.

Dissolved trace metal concentrations in sea water.
Spatio-temporal variations.

	Table 1 : Cd (µg/l)		Table 2 : Cu (µg/l)	
Stations	n	Mean	n	Mean
coastal :	106	0.046±0.031(2)	110	0.25±0.13
summer(1)	29	0.056±0.040	30	0.41±0.13
other seasons	77	0.031±0.025	80	0.18±0.06
offshore :	30	0.034±0.023	30	0.20±0.10
summer	9	0.049±0.031	9	0.34±0.06
other seasons	21	0.028±0.014	21	0.14±0.04

n = number of separate samples
(1) july,august,september
(2) standard deviation of the mean 1 σ

Trace metal concentrations in plankton
(expressed in g metal/g dry weight)

	Table 3 : Cd (µg/g)			Table 4 : Cu (µg/g)		
	n	Mean	Median	n	Mean	Median
Zooplankton	42	2.0±0.9	1.9	151	66.3±80.2	28.4
Mixed plankton	42	2.4±1.1	2.0	136	96.2±108.5	57.3

Reference :

HARDSTEDT-ROMEO M., LAUMOND F. : Eléments métalliques traces dans les organismes planctoniques de la Méditerranée Nord-Occidentale. XXVI$^{\text{ème}}$ Congrès-Assemblée plénière de la C.I.E.S.M.. 24 Nov- 2 Dec 1978, Antalya (Turquie)

Contractor : Collège de France, Laboratoire Biologie Marine, Concarneau

Contract n° 205 - 77 - 1 ENV F

Project leader : Dr. Yves ROUGER

Title of project : Studies on the incidence of agricultural pollution on the biology of estuarine organisms

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RESEARCH OBJECTIVE

In order to determine some criteria for pollutants and potentially toxic chemicals from agriculture, we initiated experiments with diluted waste from pig farms and with the pesticide methyl parathion.

Our aim was to determine behavioral criteria as a sensitive and quick measure of agricultural pollution.

MATERIAL AND METHODS

We worked with six through eight month old sea bass, one year old sea brem and five month old Mulet. The age of the shrimp was unknown.

We tested the animals in different situations ; chemical discrimination, Y maze, open field situation, natural environment.

For behavioral purposes the only method employed was visual observation.

RESULTS

1 - High bacterial levels in the Aven river estuary due to pig waste spray on the soils.

During spring 1977 we studied the pollution of the Aven river, because of the high levels of bacteria, the commercialisation of mussels and oysters has been prohibited. We started ressearch to localise the origin of the pollution.

After periods of rain, we found a high level of bacteria in the river with a larger concentration in the branch of the estuary where the pig farms were located.

At this point, the level of bacteria was as high as at the exit of the PONT AVEN sewage station.

There was a significant correlation between the bacterial level in some branches of the river and the existance of the pig farms (fig.1).

The origin of the high levels of bacteria was the run off on each side of the river valley. Large amounts of pig waste have been used as fertiliser, In comparison to the amount of bacteria and the concentration of waste, we can measure the pig waste dilution in the river to 10 ppm.

2 - Effect of pig waste on the behavior of fish and shrimp

We studied the effects of low concentrations of pig waste on fish (sea bass ans Mulet) and shrimp (Palaemon serratus) and the dose the dose response curve.

It appeared in a multiple situation choise that the sea bass was not able to distinguish between stimulation from pig waste and no stimulation. But there was a significant effect on the total activity of the animal and the threshold level situated at 0.07 ppm.

The shrimp and the mulet were able to discriminate between stimulation and no sitmulation after 0.04 ppm and 0.07 ppm respectively.

In another experiment we bred sea bass in a pig waste solution of 0.07 ppm and the animals were tested in a Y maze situation or in open field tests.

There was significantly less activity in the animals raised in polluted water compared with those raised in clean water.

In the open field situation, there was a significant difference between the two groups of animals, there was fin raising movement and less flight reactions in animals raised in 0.07 ppm pig waste solution.

The social behavior of sea bass raised in 0.07 ppm showed a significant difference compared to the animals raised in clean waters. There was less fin raising movement in pig waste dilution (15.0) versus 24.96 in clean water. There was less flight response in pig waste dilution (4.2) versus 8.72 in clean water. These results were similar in spite of the differentes techiques used : pig waste at low cencentration (0.07 ppm) affects the behavioral responses of sea bass and produces a slow down of activity (locomotory activity, fin raising movement, flight reaction) which indicated that all the defense mecanisms of the organism was affected (fig. 3 and 3).

3 - Effect of Methyl Parathion on the sea bass, sea brem and shrimp behavior.

After determining the lethal dose of methyl parathion for these different species (12 ppm in sea bass, 5 ppm in shrimp) we studied the effect of the pesticide at very low exposures to the animal.

Sea bass bred for 48 hours in a 5 ppm methyl parathion solution showed a significant difference of behavior after 8 day .

There was a lower activity in the animal raised in methyl parathion solution (161.2 versus 316.6) but there was a significant increase in fin raising and flight responses. This was a very different response in comparison to results from the pig waste experiments.

The shrimp was found that there was a decrease of activity after a concentration of 0.02 and 0.08 ppm of methyl parathion.

The shrimp was not able to discriminate between the stimulus and non stimulus in a choice situation which was a different response in comparison to results from pig waste sitmulation.

In summary the comparison of the response of the three species and the two substances showed that there was a selective response within the speices and within the substances.

Mulet unlike the sea bass showed and escape reaction when it was presented to pig waste.

Sea basse ans sea brem showed a decrease of fin raising and flight response when presented to pig waste but increased when presented to methyl parathion (fig. 4).

Publications

- Bacterial pollution of the Aven river estuary, Y. Rouger, B. Keravel, to be published in MARINE POLLUTION BULLETIN.
- Behavioral response of shrimp (Palaemon serratus) sea bass (P. labrax), and Mulet (C. laborsus) to diluted pig waste. A comparison betwenn bio-assay methods based on behavioral studies and bacteriological methods ; Y. ROUGER, L. LE COZ, L. OLLIVIER to be published in MARINE POLLUTION BULLETIN.
- Effect of pig waste at low concentration on the chemical orientation of sea bass, Y. ROUGER, S. GABRIEL, to be published in MARINE POLLUTION BULLETIN.
- Effect of pig waste at low concentration during breeding on the emotional behavior of sea bass. Y. ROUGER et C. ANDERSON, to be published in MARINE POLLUTION BULLETIN.
- Effect of methyl parathion on the behavior of sea bass, sea brem and shrimp. Y. ROUGER, L. LE COZ, to be published in MARINE POLLUTION BULLETIN

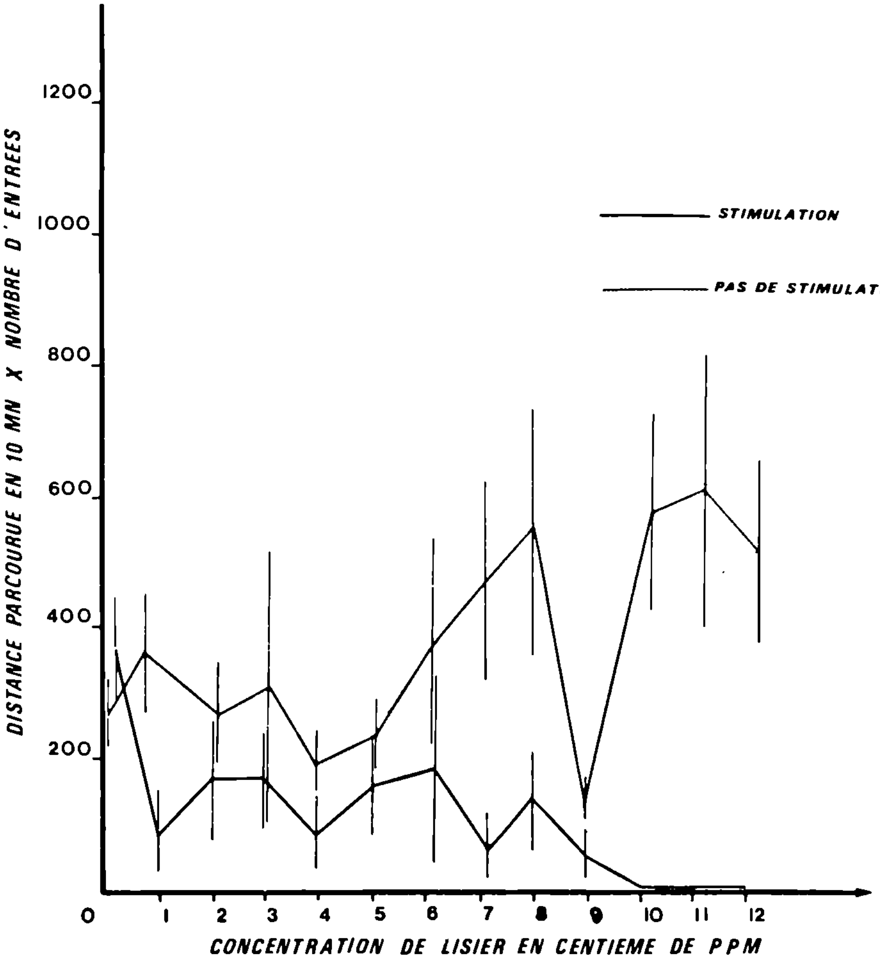

Figure 1

BACTERIAL CONCENTRATION IN AVEN RIVER ESTUARY

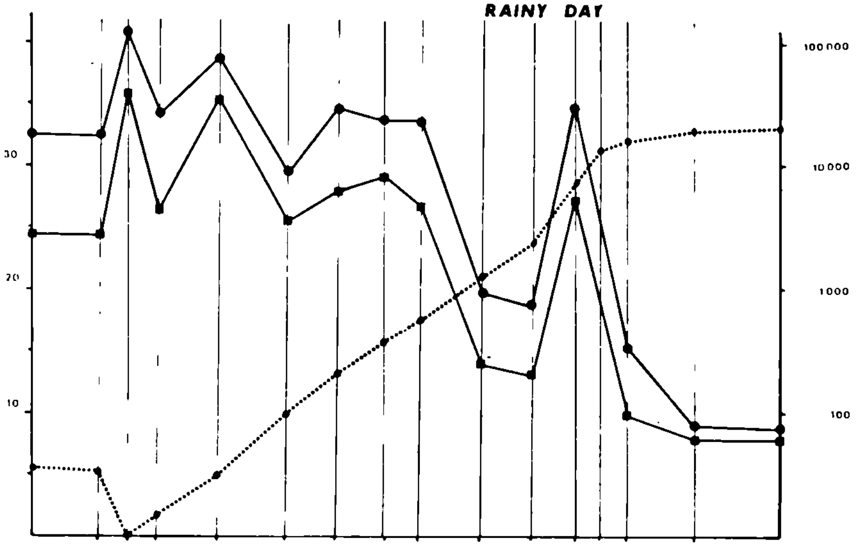

Figure 2

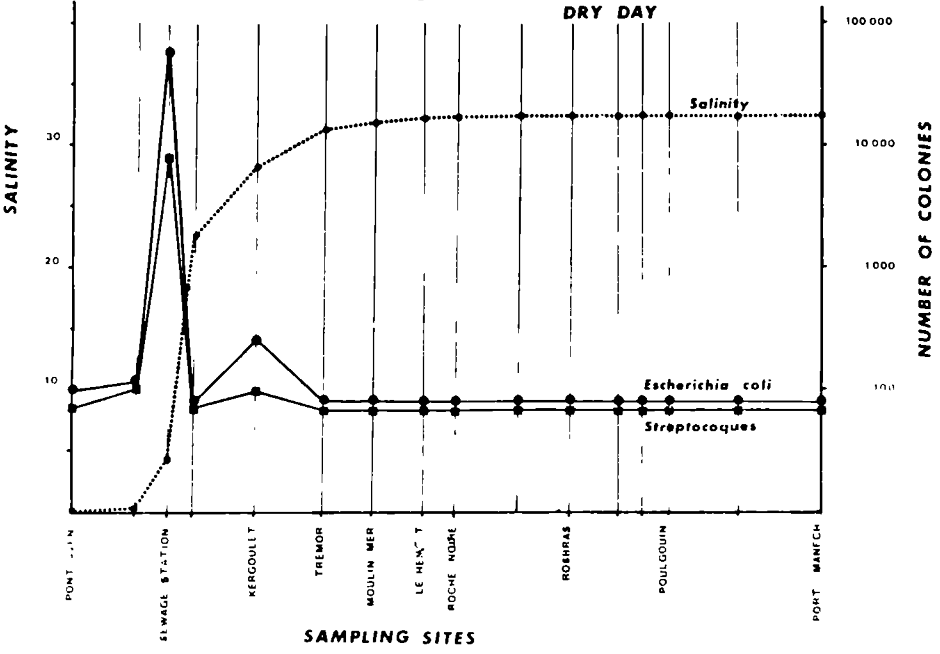

Figure 3

EFFECT OF METHYLPARATHION ON BLACK SEA BREAM

0,005 PPM

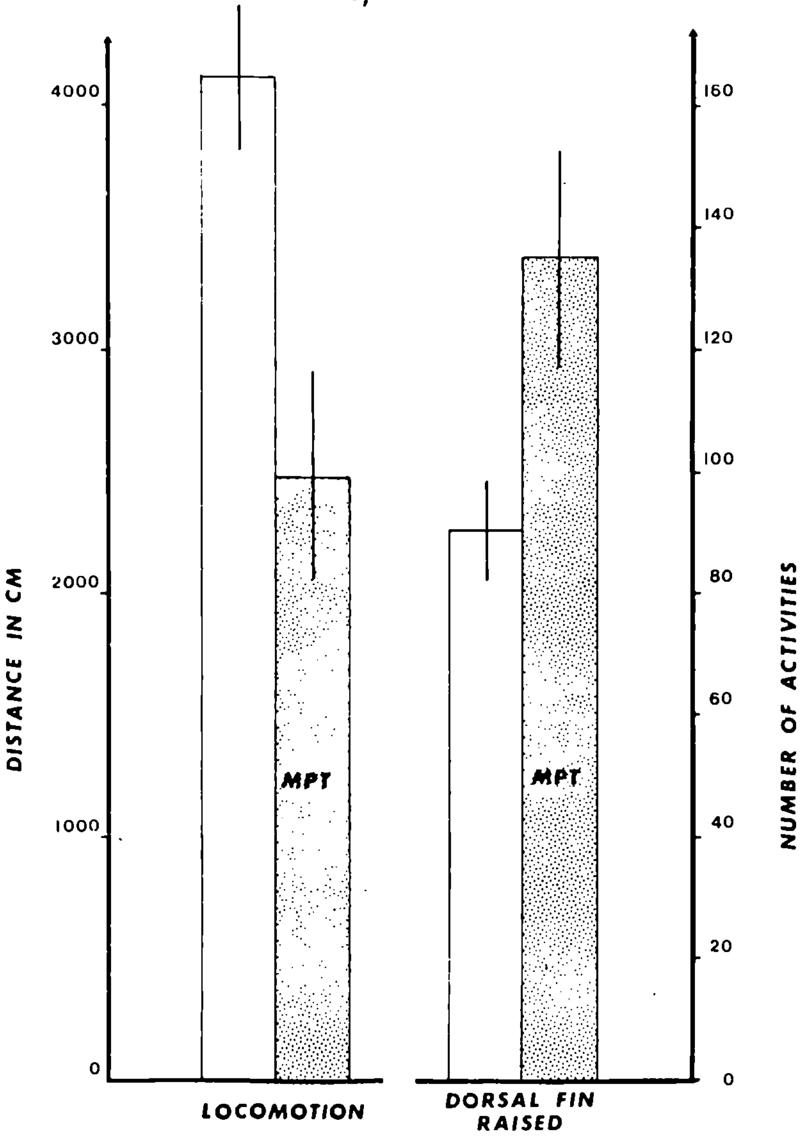

Figure 4

Contractor : Consiglio Nazionale delle Ricerche, Rome, Italy

Contract n° 283-77-7 ENV I

Project Leader : B. Battaglia (co-investigators: L. Chieco-Bianchi and V.U. Fossato)

Title of project : Chemical Pollution of Lagoon Waters and Carcinogenic Hazard

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Objective of the research

The work represents an extension of a previous program carried out with the financial aid of the E.E.C. (contract n° 060-74-1 ENV I).

The current objectives are: a) the evaluation of levels of certain chemical substances that have recognized carcinogenic properties (polynuclear aromatic hydrocarbons, chlorinated hydrocarbons, elements such as Cd, Cr, As) in the Laguna Veneta and the adjacent Adriatic Sea by monitoring their presence in the tissues of the blue mussel (Mytilus sp.), one of the most widely distributed filter-feeders present in this area. This research was carried out at the Istituto di Biologia del Mare, C.N.R., Venice; b) a study, using a mouse bioassay system, of the carcinogenic effect of tissues of mussels collected from areas having high and low contamination, including commercial mussel parks. The animal studies were conducted at the Istituto di Anatomia Patologica, University of Padua.

Since mussels are particularly efficient in accumulating persistent pollutants from water and the mussel culture represents an important sector of the fishery in the Laguna Veneta, these organisms were used as indicators for monitoring the pollution in this area as well as indicators of the carcinogenic risk associated with chemical pollution of lagoon waters.

Materials and Methods

During the period covered by the research program, mussels were periodically collected from natural populations at nine stations located inside the Laguna Veneta and at one station located in the Adriatic Sea, about eight miles offshore.

The primary extract to be used in the animal studies was prepared by refluxing one Kg of drained soft parts of mussels in 5% alkaline ethanol for eight hours. The resulting solution was diluted with distilled water and repeatedly extracted with 200 ml portions of petroleum ether. All extracts were combined, shaken successively with equal volumes of 20% NaCl and 10% HCl solutions and washed with water until neutral. The organic layer was dried over anhydrous sodium sulphate, decanted, then the solvent was evaporated under reduced pressure in a rotary evaporator. One Kg of mussel tissues give on the average 1.6 g of crude extract.

For the determination of 3,4-benzopyrene (BaP) and perylene (Pe), about 0.2 g of crude extract were purified on a column of alumina, the hydrocarbons then separated by thin-layer chromatography on activated silica gel plates followed by quantitative determination by spectrofluorometry of the BaP and Pe fractions. Details on the analytical procedure are given by Fossato et al. (1979).

Since analyses of chlorinated hydrocarbons and metals were carried out at a later date, soft parts of mussels were freeze-dried and stored in glass bottles.

Extraction of the chlorinated hydrocarbons was accomplished by refluxing an aliquot of the freeze-dried sample in a Soxhlet apparatus for eight hours with n-hexane. Coextracted substances were removed using the general method of partitioning between n-hexane and acetonitrile, then PCBs were separated from DDT and its metabolites (DDD and DDE) by chromatography on silica gel column using n-hexane, which elutes the PCBs, followed by benzene, which contains DDT and its metabolites. Quantitative analysis was done using a 5750 Hewlett-Packard gas chromatograph equipped with a Ni 63 electron capture detector. Details on the analytical procedure are given by Fossato and Craboledda (1979).

For the determination of cadmium, chromium and arsenic, about 3 g of lyophilised material were digested with 90% nitric acid. The resulting suspension was filtered through a 0.45 µm membrane, made up to 100 ml with demineralised water and analysed by a Perkin-Elmer Model 372 atomic absorption spectrophotometer. Cadmium and chromium were determined directly in an air-acetylene flame, while arsenic was separated by reduction to gaseous arsine with sodium borohydride and transported by the carrier gas to the quartz cell heated in the air-acetylene burner.

In vivo carcinogenesis assay. Inbred NFS/N mice of both sexes were used for the experiments. Groups were set up as follows.

Group 1. Six weekly intramuscolar injections starting with mice six to eight weeks of age.

1a. 19 mice receiving 3 mg injections of mussel extract of 4/77 (mussels collected from heavily polluted area), dissolved in 0.10 ml olive oil.

1b. 20 mice receiving 3 mg injections of mussel extract of 3/77 (mussels collected from lightly polluted area), dissolved in 0.10 ml olive oil (negative controls).

1c. 37 mice receiving 0.1 ml injections of olive oil (vehicle controls).

1d. 36 mice receiving 20 µg injections of 3,4-benzopyrene, dissolved in 0.10 ml olive oil (positive controls, high dosage).

1e. 27 mice receiving 2 µg injections of 3,4-benzopyrene, dissolved in 0.10 ml olive oil (positive controls, low dosage).

Group 2. Single subcutaneous injection in newborn mice, 24 to

48 hours old.
2a. 18 mice injected with 3 mg mussel extract of 4/77, dissolved in 0.05 ml olive oil.
2b. 12 mice injected with 3 mg mussel extract of 3/77, dissolved in 0.05 ml olive oil.
2c. 19 mice injected with 0.05 ml olive oil.
2d. 15 mice injected with 2 µg 3,4-benzopyrene.
N.B. Numbers of mice refer to actual number of mice weaned at 5 weeks of age.
Group 3. Mice, starting age six to eight weeks, given 14 weekly intragastric doses of 0.30 ml lyophilised mussel samples suspended in tap water (25 mg/ml), for a total of about 105 mg.
3a. 21 mice receiving lyophilised sample of 5/77 (mussels collected from heavily polluted area).
3b. 17 mice receiving lyophilised sample of 6/77 (mussels collected from lightly polluted area).

Results

A considerable body of analytical data has been accumulated during the course of this study, permitting the synthesis of pattern of the levels and the geographical distribution of some chemical carcinogens in mussels of the Laguna Veneta.

Ninety samples of mussels were analysed for their BaP and Pe content. A large range of concentrations was observed: 0.5-70.8 µg/kg of wet tissue for BaP and 0.1-15.6 µg/kg for Pe. The highest recoveries of these compounds have been from tissues of mussels taken from waters adjacent to the industrial area of Porto Marghera and near the cities of Venice and Chioggia. The lowest levels were found in mussels from the Adriatic Sea and in the central basin of the Laguna, farthest from pollution sources. This distribution indicates the presence of local inputs and confirm the likely anthropogenic source of the major portion of the BaP and Pe found in mussels of the area investigated. The range of concentrations of BaP and Pe reported for mussels from the Laguna Veneta does not differ markedly from those measured in bivalves from similar areas throughout the world.

Residues of chlorinated pesticides, such as aldrin, dieldrin, hexachloro cyclohexane (BHC), dichloro-diphenyl trichloroethane (DDT), which for decades have been heavily used in agriculture, and polychlorinated biphenyls (PCBs), which are contained in formulations used for industrial and technical purposes, were determined on sixty samples of mussels. Aldrin and dieldrin were present only in small quantities (0-0.4 µg/kg wet weight for aldrin, 0.2-1.8 µg/kg for dieldrin). The α and γ isomers of BHC were detected in all the samples (ΣBHC 0.6-4.1 µg/kg). DDT and its metabolites represent the major fraction of chlorinated pesticides (ΣDDT 8.5-27.2 µg/kg), but PCBs are the most important residues at all stations regardless of season (ΣPCB 30-155 µg/kg). The distribution of chlorinated pesticides is quite uniform, while for PCBs significant differences were observed for samples collected inside and outside the Laguna, indicating the presence of local inputs. Current levels of chlorinated hydrocarbons in mussels of the Laguna Veneta are generally lower than in the majority of those reported for other industrial and urban areas of the Mediterranean Sea.

Forty samples of mussels have been analysed for their Cd, Cr and As content. Cadmium concentrations were 0.1 to 2.0 mg/kg wet weight; the higher values were found in samples taken from waters adjacent to the industrial port area, however, the number of analyses is not enough to judge whether a concentration gradient truly exist. A lower range of concentrations was observed for chromium and arsenic (0.2-0.7 mg/kg for Cr, 0.2-1.2 mg/kg for As), and moreover these elements did not show significant differences in mussels collected in different basins of the lagoon indicating an almost uniform distribution in this area. Although metals showed considerable fluctuations during the year, the data are insufficient to demonstrate a seasonal pattern. Research on this aspect is in progress.

Mice of different groups have been observed weekly for tumor development. Up to date only groups 1a, 1b, and 1d have reached the end of the observation period (18 months), and the surviving mice have been sacrificed. No relevant gross tumor development was noted at necropsy in the experimental group 1a as compared with negative control group 1b; the histological examination of these groups has not yet been completed. Mice of the remaining groups will be sacrificed between April and July 1979.

Conclusions

The results of analyses of mussels from the Laguna Veneta show the ability of this bivalve to accumulate large amounts of persistent chemicals and confirm the possibility of utilising this organism as a useful indicator of chemical pollution of marine waters.

Previous experiments, carried out under the contract no. 060-74-1 ENV I, suggested that crude extracts of mollusc tissues administered by single subcutaneous injection to newborn mice have a weak oncogenic effect. Since the above experiments are in progress (the present contract ends in June 1979), it is not yet possible to draw definite conclusions on the carcinogenic potential of mussels grown in polluted areas.

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Contractor :	Rijksinstituut voor Natuurbeheer, Arnhem
Contract n° :	198-77-1 ENV N
Project leaders :	J.L. van Haaften and P.J.H. Reijnders
Title of project :	Investigations on the causes of fluctuations in the population of harbour seals in the Dutch Wadden Sea

During 1974 to 1978 population dynamics of the harbour seal population in the Dutch Wadden Sea have been studied. Frequent aerial surveys provided data upon the size of the population. During boat trips information about the age composition was obtained by measuring track widths. These data have been used in a simulation model to calculate the birth rate and the initial juvenile mortality.

It was found (1.)that the initial juvenile mortality in the Dutch population is higher than that in Schleswig Holstein ; (2.) that pup production in the Dutch population is low compared to the population in Schleswig Holstein if the ratio adult females to males in the Dutch population is supposed to be equal to that in Schleswig Holstein, or that mortality amongst adult females in the Dutch population is higher than in Schleswig Holstein ; (3.) that the number of subadults in the Dutch population is relatively high compared to a stable population (Schleswig Holstein) and that apparently immigration from elsewhere occurs ; (4.) that the reproductive rate was higher or the juvenile mortality lower during 1974 to 1978 than in the preceding decennium.

N.B. This is the summary of the article " Recruitment in the harbour seal (Phoca Vitulina) population in the Dutch Wadden Sea" by P.J.H. Reijnders published in the Netherlands Journal of Sea Research 12(2): 164-179 (1978).

Contractor : Organization of Industrial Research TNO

Contract n° : 227-77-1 ENV N

Project leader : Jan Kuiper

Title of project: Continued investigations into pelagic microcosms subject to environmental stress by pollutants

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Objective of the research

In nature large and little-understood variations frequently occur in ecologically important parameters, such as population density and species composition. It is therefore difficult to detect long-term effects of environmental stress in the field. Much of our knowledge of the influence of individual pollutants on the marine ecosystem comes from actual dumping practice and from tanker and other disasters.

Although laboratory experiments are indispensable and yield useful information, extrapolation of their results to field conditions is at present difficult, if not impossible. In order to assess the value of experiments in the laboratory, there is a need for experiments with more complex systems that can be regarded as approximating field conditions, more closely (Ringelberg 1973).
To bridge the gap between the laboratory and the aquatic environment, several investigators use large plastic bags suspended in natural waters (Strickland and Terhune 1961, Menzel and Case 1977). Our laboratory started this type of research with Dutch coastal water plankton communities in 1974. When it had been shown that the method of enclosing a plankton community in a plastic bag can be used for toxicological research (Kuiper 1977a), further investigation was aimed at developing the method and at determining the impact of pollutants in low concentrations on the development of the enclosed system.

Materials and Methods

In the period 1976-1978 ten experiments were conducted, which were partly sponsored by the EC Environmental Programme (Contract no. 227-77-1 ENV N). Mercury and cadmium were chosen as model pollutants in six experiments.
In two experiments we added these heavy metals simultaneously, in order to study possible synergistic or antagonistic effects.

In four experiments, selected chlorinated organics were added as model pollutants (phenol, p-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 3,4-dichloroaniline).
These aromatics were chosen because laboratory experiments had shown that they differ widely in biodegradability.

At the start of each experiment 6 to 8 bags were simultaneously filled with 1400 l of natural seawater each. The model pollutants were added in a single dose, since single additions most closely approximate the "normal" field situation if the source of a pollutant is an outfall, a river or a dumping event (Menzel and Case, 1977).
During the experiment the development of the decomposers, phytoplankton and zooplankton was monitored as well as various abiotic parameters affecting the organisms on the various trophic levels in the bags.

Results

The main results of the experiments have been described by Kuiper (1978). During the experiments in which mercury was added to the bags, mercury concentrations in the water decreased rapidly (by 3-32 % per day).
This decrease can be attributed in part to adsorption of the added mercury to suspended particles which settle to the bottom of the bags, so that mercury accumulates in the sediment. After a lag phase of about a month the added mercury was methylated in the sediment. Addition of 5 µg $Hg.l^{-1}$ changed the species composition of the phytoplankton and inhibited the growth of the phytoplankton as long as concentrations in the water remained higher than 1.5-2.5 µg $Hg.l^{-1}$. Similar results were found in an earlier experiment (Kuiper, 1977b) and by other investigators using large enclosures (Thomas et al., 1977). Our results indicate that the no-effect level of mercury on a phytoplankton community depends not only on species composition but also on the concentration of cells and on the ratio of living to dead suspended particles. Addition of 5 and 50 µg $Hg.l^{-1}$ changed the growth pattern of the decomposers and inhibited or killed the zooplankton.
In contrast with that of mercury, the concentration of cadmium in the water does not decrease appreciably in the course of an experiment. One of the most important results of the experiments with cadmium was the finding that addition of 1 and 5 µg $Cd.l^{-1}$ inhibited the growth of the ctenophore *Pleurobrachia pileus*, which had a marked influence on the development of the plankton community.

It is clear that P. pileus is affected by cadmium concentrations of at least a factor of 10 less than reported in literature (Rosenberg and Costlow, 1976). The data of the experiments in which $HgCl_2$ en $CdCl_2$ were added simultaneously, show that the effects of the two metals are additive.

The experiments with the organic compounds showed that the biodegradation of these chemicals in a semi-field situation can differ considerably from that found in laboratory tests, one reason being the differences in nutrient status of the seawater compared with laboratory cultures. The decrease of the concentrations of the chemicals in the water was slower when temperatures were lower, but the biological properties of the system (e.g. species composition and biomass of the bacteria) are also very important. The results show that extrapolation of results from laboratory biodegradation tests to field conditions is still very difficult.
The formation of intermediates (in one of the experiments) resulting from the degradation of p-chlorophenol, dichlorophenol and probably also dichloroaniline, influenced the development of the phytoplankton drastically, whereas the original compounds did not show such marked effects. This finding illustrates the usefulness of the present experimental method, because no such intermediates were found in the laboratory tests.

Conclusions and comments

The results of the experiments clearly illustrate the possibilities of the plastic bag method for toxicological and biodegradation research.
The most important advantage of the method is that it provides the possibility of performing experiments with different organims on a single trophic level, as well as with different trophic levels in a single system under semi-natural conditions. This possibility is of fundamental importance in attempts to estimate the effects of pollutants on natural aquatic ecosystems.

The interactions in the systems in a bag are so complex that any hypotheses about the mechanisms by which chemicals interact with the system will often need to be tested in laboratory experiments. Another advantage of the method therefore is a certain feedback to the laboratory (validation of test procedures, discovery of sensitive test organisms, mechanism of action of pollutants, etc.).

The fact that many species live on one trophic level increases the value of the experimental results, but also constitutes a weak point in the ex-

experimental set-up. The experimenter has little control over the species composition of the enclosed community, so the sensitivity to chemicals may vary widely from one community to another.

Another weak point is that at the start of an experiment nutrient concentrations must be high enough to ensure significant development of the phytoplankton. Addition of nutrients is possible (Takahashi et al., 1975), but this has the disadvantage of making the system less natural and making effects on mineralization processes more difficult to detect. It is better to choose the moment of filling the bags on the basis of measurements of of relevant parameters in the field before filling (e.g. chlorophyll, nutrients).

An important problem is how to determine the optimum duration of the experiment. The duration must be related to the generation time of the organisms in the system. Copepods having a generation time of one or two months, 3-6 weeks would seem to be a minimum for detecting any significant effects on their development.
Although the results show that much longer experiments are possible, a duration of 3-6 weeks appears to be an optimum, because after that time the species composition of the enclosed community begins to deviate more and more from that of natural phytoplankton communities.

From our experiments we can draw the general conclusion that, in toxicological and biodegradation research, the plastic bag method affords a useful tool for translating the results of relatively simple laboratory tests to more complex systems.

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Contractor : University of Liège, Laboratory of Oceanology

Contract n°: 178-77-1 ENV B

Project leader : Prof. A. Distèche and J.M. Bouquegneau

Title of project : Quantitative study of heavy metals transport in marine ecosystems

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Objective of the research

The knowledge of dangers of discharging pollutants in the marine environment requires the study of the accumulation and of the effects of those pollutants in aquatic organisms as well as the study of the influence of environmental factors on them.

The aim of this work is to try and determine in laboratory experiments the main path of entry of pollutants in aquatic organisms and to study the effects of abiotic factors on their uptake kinetics.

The results of our researches can be divided in three parts :

1° the study of the importance of the uptake of heavy metals from water and food by aquatic organisms.

2° the determination of the percentage of ingested pollutants assimilated by the animals.

3° the study of both direct accumulation and elimination of mercury.

Material and methods

Animals have been intoxicated in 80 l. aquaria containing aerated and unfiltered water to which the pollutant was added. Water was changed and polluted every day.

In order to study the elimination of the pollutant, contaminated animals were placed in aquaria containing clean and continuously filtered water.

Tissue samples were analysed for mercury by flameless atomic absorption spectrometry (Coleman Mercury Analyser System M.A.S. 50) after mineralization in H_2SO_4 95% and H_2O_2 30% and for other metals by atomic absorption spectrophotometry (Perkin-Elmer, Model 103) after mineralization in 65% HNO_3.

Results

1° Uptake pathways of mercury in a two levels aquatic food chain *Tubifex tubifex* and *Lebistes reticulata.*

Experiments have been performed in aquaria containing guppies fed either with unpolluted tubifex, or with tubifex contaminated in the same aquarium. The three groups of animals were living in the same polluted water but were separated from each other. Water was polluted by 10 or 50 ppb of mercury (mercuric chloride or methylmercuric chloride) for 12 days. Fishes were fed every day till they refused feeding. The evolution of the mercury concentration in the three groups of animals was measured. Important pollutant concentrations in both species have been observed but no difference in the mercury uptake kinetics of both groups of guppies.

It was concluded of those results that a direct uptake from water was the main route of uptake of mercury into the guppies. This was confirmed by the measurements as described below, of the assimilation of that pollutant from food.

2° Determination of percentages of ingested pollutants assimilated by aquatic animals.

The percentages of ingested heavy metals assimilated (food metal - excreted metal) x 100/food metal) have been measured at the level of three twolevels food chains : *Tubifex tubifex-Lebistes reticulata, Patella vulgata-Serranus cabrilla, Dunaliella bioculata-Artemia salina.* The preys *Dunaliella bioculata* and *Tubifex tubifex* were intoxicated in the laboratory with heavy metal chloride salts added to the water while *Patella vulgata* had been naturally contaminated in the industrially polluted area of the Bristol Channel.

The results are summarized in Table 1 and show the very low percentages of ingested metals assimilated by artemias and fishes except in the case of methylmercury where 30 to 50 percent revealed to be assimilated. This is quite interesting since it is known that most of heavy metals present in the tissues is bound to proteins and that the percentage of ingested proteins assimilated by fishes is known to be about 80%.

Since direct uptake of heavy metals from water showed to be more significant that uptake from food, we studied in detail the kinetics of accumulation of mercury and other metals by some aquatic organisms.

Dunaliella bioculata - *Artemia salina* food chain.	
$CuCl_2$	non detectable
$ZnCl_2$	non detectable.
$CdCl_2$	3.5 %
$HgCl_2$	5.7 %
CH_3HgCl	28.8 %
Tubifex tubifex - *Lebistes reticulata* food chain.	
$ZnCl_2$	10.6 %
$CdCl_2$	.0.1 %
$HgCl_2$	0.1 % - 1.4 %
CH_3HgCl	37.1 % -53.2 %
Patella vulgata - *Serranus cabrilla* food chain.	
Cd	0.8 %

Table 1 : Percentage of ingested heavy metals assimilated by *Artemia salina*, *Lebistes reticulata* and *Serranus cabrilla*.

3° The uptake and release of mercuric chloride by some aquatic organisms.

Recent studies about some sea water fishes reveal mercury uptake kinetics which can be described by the equation

$$Ct = Css\ (1 - e^{-Kt})$$

where Ct is the concentration of mercury at time t, Css is the asymptotic or steady state concentration and K = 0.693/tb1/2, tb1/2 being the theoretical biological half-time.

It is generally assumed that the tb1/2 calculated from the accumulation curve is more or less equal to the half-time of elimination of the pollutant observed when intoxicated fish are treated in clean water. However, our observations about the uptake and release of mercury by two species of fish (*Serranus cabrilla* and *Lebistes reticulata*) lead us to reconsider the generality of this rule. Indeed, both fish showed mercury uptake kinetics which present a very short calculated tb1/2 on the basis of the observed accumulation versus time curve but a much larger one when the release of the toxic material was measured in non-contaminated water. We explain this by the hypothesis of the induction by mercury itself of a decrease of the rate of entry of the pollutant, for example by the increase of the mucus production by the animal. Further tests are carried out in our laboratory to check.

On another hand, studies on the uptake of mercury by another fish *Crenilabrus ocellatus* revealed mercury accumulation kinetics of a sigmoïd form.

So it appears that several kinetics of mercury uptake can be observed in aquatic animals depending on the species. Moreover, those kinetics can be modulated by environmental parameters such as salinity or the presence of dissolved and suspended organic matter in water. We have investigated the effects of the presence of organic matter and of salinity respectively on the kinetics of uptake of mercury by *Lebistes reticulata* and by the crab *Eriocheir sinensis*.

Considering the presence of organic matter in polluted water, our results show that it can strongly reduce (up to five times) the initial rate of entry as well as the steady state concentration of the pollutant.

Salinity too has been shown to have a drastic influence on both toxicity and accumulation rate of mercury uptake by *Eriocheir sinensis*. Indeed, the same concentration of mercury is much more toxic for the fresh water adapted crabs than for the sea water adapted ones. The toxicity of lethal doses of mercury on the fresh water adapted chinese crab was shown to be a rupture of the osmotic balance of the animal. Considering the accumulation of the pollutant, the initial rate of uptake revealed to be faster in animals adapted to lower salinities. Those differences have been related to the different physiological states of the crabs depending on the salinities they had been adapted to.

Conclusions

The presence of pollutants such as heavy metals in the marine environment leads to an increase of toxic materials concentrations in living organisms. Those concentrations are depending not only on the concentrations of pollutants in water but also on the species and its position in the food chains, the route of entry of the pollutant in the organism and the influence of main environmental parameters such as temperature or salinity.

In a first step, the influence of those parameters has to be studied separately.

When considering most of the heavy metals, our results show that the place of the species in the food chain is not of great significance according to the very low percentages of ingested pollutants being assimilated.

When considering mercury, this metal, present in water, is rapidly accumulated in aquatic organisms by way of the gills. Several different kinds of uptake kinetics can be described depending on the studied species. Most of them have an exponential shape while some are sigmoïd.

The rate of accumulation of mercury is decreased by the presence of organic matter in the medium. An increase of the salinity decreases the initial rate of uptake too but seems to have only slight effect on the concentration of pollutant at steady state. The results suggest that heavy metals are apt to be more toxic for estuarine species which have to withstand osmotic stresses.

Temperature is another fundamental environmental factor whose effect on metal uptake should be studied., and, finally, as marine ecosystems parameters are continuously adapting, further research is needed in order to estimate their relative sensitivity when heavy metal accumulation mechanisms are involved.

Publication

The uptake of mercuric chloride from sea water by *Serranus cabrilla*.
D. RADOUX and J.M. BOUQUEGNEAU. To be published in the Bulletin of Environmental Contamination and Toxicology, vol.22, n° 6, 1979.

Contractor: University College of Swansea
Contract no^{o} 164-77-1 ENV UK
Project Leader: Professor J. A. Beardmore
Title of project: Detection and assay of genetic damage caused by environmental chemicals in marine environments

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Period covered - 1st phase of the 2nd environmental research programme 1.1.77 - 31.12.78.

Introduction. That estuarine waters contain significant, and in some cases high, concentration of substances capable of causing genetic change is now well established. The mussel (*Mytilus*) is a filter feeder and tends to concentrate such substances in its tissues. During the first environmental research programme the contractor demonstrated that electrophoretic analysis of populations of *Mytilus* exposed to different concentrations of heavy metals revealed significantly higher frequencies of rare variants at a number of loci in populations exposed to higher levels of heavy metals. (Beardmore 1979). During the first phase of the second programme attention has been directed towards the following:-

1. Extension of geographic range of the populations assayed.
2. Tests of validity of method.
3. Measurement of metal concentrations in animals.
4. Correlation of assay with *in vitro* methods.

Results

1. A large number of populations (now in excess of 100 sites) have been sampled round the coasts of Britain. The results of this survey show that *Mytilus galloprovincialis* is present in many areas sympatrically with *M. edulis* (Ahmad & Beardmore 1976). In some areas there appears to be little interbreedin but in other areas there is evidence of significant genetic exchange between the two taxa (Skibinski & Beardmore in press). This, while interesting in itself, considerably complicates the problem of assay of rare variants as some genes which are common in one are rare in the other species. Despite this difficulty it has proved possible to identify a number of populations which can with reasonable confidence be regarded as pure *M. edulis*. Assay of genes has involved 11 polymorphic loci (Table 1).

Table 1. Electrophoretic assay systems used.

No.	Abbreviation	Enzyme coded
1	AAT	Aminoaspartate transferase
2	S AAT	Aminoaspartate transferase
3	AP-1	Aminopeptidase
4	EST-D	Esterase-D
5	IDH	Isocitrate dehydrogenase
6	LAP-1	Leucine aminopeptidase
7	LAP-2	Leucine aminopeptidase
8	MDH	Malate dehydrogenase
9	6-PGDH	6-Phosphogluconic acid dehydrogenase
10	PGM-2	Phosphoglucomutase
11	PHI	Phosphohexose isomerase

The assay methods for all systems except nos. 1 and 2 are in Ahmad *et al* 1977. Methods for 1 and 2 were developed by Dr. T.C. Cross.

Table 2. Populations chosen for more intensive study and estimates of frequencies of rare variants based on 11 loci.

Populations		Frequency of rare variants $^0/oo$	N
Burry Port	(S. Wales)	22.63	2558
Llanbedrog	(N. Wales)	20.33	1452
Llangranog	(W. Wales)	28.32	5420
Mumbles	(S. Wales)	32.79	24552
Ravenglass	(Lancashire)	23.32	2200
Seascale	(Lancashire)	29.75	2640
Solva	(W. Wales)	17.26	7150
Windscale	(Lancashire)	23.64	3520

The estimates of the frequency of rare variants, some of which may be the result of new or recent mutation (or quasi mutational processes) are given in table 2. The definition of rare variant used here differs from that used in earlier reports and is specified by genes not normally present in a sample of populations at individual frequencies of >0.01 and in no cases >0.02. Using this criteria the populations having the highest frequency show values about double those in the population with the lowest frequencies.

2. A start has been made on testing the validity of the method by comparing the frequencies at two groups of loci (3+4+6+7+10+11) vs (1+2+5+9+8). The rank orders of the total frequencies at the two groups of loci for only the eight populations listed above is as follows:-

	Group 1 rank	Group 2 rank
Mumbles	1	1
Llangranog	2	7
Seascale	3	2
Solva	4	4
Burry Port	5	5
Ravenglass	6	3
Llanbedrog	7	6
Windscale	8	8

The Kendall rank correlation coefficient is 0.5 with an attached probability somewhat >0.05. Llangranog is an obviously poor fit in the table and omitting it the correlation coefficient becomes 0.71, P = <<0.05. There is evidence cited below that Llangranog is a peculiar population and while the test of validity needs to be extended to a larger sample of populations it seems that there is some internal consistency.

3. Determinations of heavy metal content have been made on animals from some of the populations as shown in table 3.

Table 3. Levels of heavy metal in Mytilus (ppm dry weight)
(ND = not determined)

Population	Cd	Cu	Ni	Pb	Zn
Burry Port	2.80	11.0	1.70	4.70	101.00
Llangranog	1.91	9.41	11.79	37.60	89.89
Mumbles	1.50	7.50	8.75	7.50	187.50
Solva	0.74	13.0	2.7	ND	10.78

This table shows clearly that while in general ecological terms Llangranog, well away from centres of population and industry, might be considered a 'clean' site, the waters in that area are relatively heavily loaded with heavy metals. The figure for Pb is particularly striking. The association between heavy metal concentration and frequency of rare variants demonstrated in earlier reports is borne out by these data.

4. The development of methods like the Ames test and those developed by Parry (1976) permit a direct test of potential mutagenic activity of substances present in tissues, water and sediments. Dr. J.M.Parry has tested some of the populations we have been studying and has shown that mussels in certain areas accumulate mutagenic agents through their filter feeding habit. Extracts of mussel tissue made either by acidic extraction (which should include the heavy metals) and by alcoholic extracts (which

should include many organic substances). Thus extract of Mumbles and Llangranog mussels is highly mutagenic whereas extract of mussels from clean waters in Anglesey is not (Parry and Al-Mossawi 1979). W. Barnes has examined the mutagenic activity of acidic extracts of Enteromorpha using the Ames strains TA98 and TA100, and some of his data are shown in Table 4.

Table 4. Effect of extracts containing heavy metals (data of W.Barnes)

Site sampled	Ratio of Treated/control mutation frequency
Limeslade	6.92
Caswell	6.46
Oxwich	3.77
Port Eynon	5.09
Rhossili	2.09

There is a clear effect which is in all cases statistically significant. Furthermore, the effect diminishes with the distance down the Bristol Channel away from the higher concentration of contaminants found in Swansea Bay.

Conclusion

The direct genetical assay of populations exposed to substances which can act as mutagens has shown association though, as yet, no causal mechanisms have been established. However, there are typically many such substances present simultaneously in sea water and future work will need to involve appropriate controlled laboratory test system now being developed (Beardmore 1979).

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* Includes results of work supported by the EEC environmental research programme.

Contractor: Ministry of Agriculture, Fisheries and Food,
Directorate of Fisheries Research

Contract No: 168-77-1 ENV UK

Project leader: Dr R A A Blackman

Title of project: Assessment of long-term sub-lethal effects of pollution on selected marine organisms

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Objectives of the research

In the initial feeding experiment with Crepidula fornicata described in the final report of our work under contract No. X11/238/75E only 50% of those animals receiving the highest feeding ration (1×10^9 algal cells/g flesh wet weight/day) bred. A second feeding experiment had therefore to be conducted at closer ration intervals of $\{0, 1, 2.5, 5.0, 7.5 \text{ and } 10\} \times 10^8$ algal cells/g flesh wet weight/day, but in all other respects experimental conditions were unchanged. The following indices were measured:

(growth and condition)
a) total live weight
b) flesh wet weight
c) flesh dry weight
d) shell length
e) % flesh wet weight/dry weight;

(biochemical composition of flesh)
f) protein
g) carbohydrate
h) lipid
i) ash;

(physiological parameters)
j) oxygen consumption
k) NH_3 excretion (as ammonia);

(reproduction)
l) numbers of larvae produced per female
m) number of females to reach spawning
n) time intervals between spawnings.

Materials and methods (2nd feeding experiment)

Measurements a-i above were made on individual sacrificed animals (3 pairs of animals/treatment every 3rd week for the duration of the experiment) using the following methods: protein (Biuret), carbohydrate (Dubois et al, 1956), lipid (Barnes, Barnes and Finlayson, 1963). Oxygen consumption was measured using a Radiometer PHM 71 oxygen meter and nitrogen excretion (as ammonia) by the method of Liddicoat, Tibbitts and Butler (1975).

Results

Fig 1 shows a graph of % increase in mean live weight with time for the various feeding rations. Only the 3 highest rations showed a clear increase, and only pairs receiving these rations bred. In addition, the percentage of ash; flesh dry weight:wet weight; flesh dry weight to total live weight; shell length increase and dry flesh:shell weight showed a clear distinction between the upper and lower three rations.

Among the various tissue component analyses only % carbohydrate showed any clear distinction between feeding rations, again between $\{5-10\}$ and $\{0-2.5\} \times 10^8$ algal cells/g live weight/day. Of the physiological measurements made, the rates of oxygen consumption and of nitrogen excretion (as ammonia) also showed differences at this level of feeding, but the O:N ratios were virtually constant throughout.

The numbers of larvae released and the time to reach the various stages of development are shown in Table 1.

Thus the maintenance food ration first allowing reproduction, under our laboratory conditions, was close to 5×10^8 algal cells/g flesh wet weight/ day and this ration was chosen in a subsequent experiment exposing the animals to mercuric chloride at nominal concentrations of 3, 10 and 33 ppb (parts per 10^9) of mercury and a control, but otherwise under the same experimental conditions.

Materials and methods (Toxin experiment)

The materials and methods used follow those for the previous feeding experiment with Crepidula, described in the final report for contract number X11/238/75E.

The methodology for maintaining constant mercuric chloride concentrations in the toxin experiment presented many problems. These were largely overcome by pre-dosing the cultures of food algae at the required nominal concentrations of 3, 10 and 33 ppb (parts per 10^9) Hg, by making up the food rations in the reservoirs with water at these mercury concentrations and by renewing the water in each recirculating holding tank system every 7 days after careful cleaning of the tank surfaces. The total mercury concentration in the food-algae presented, the filtered holding-tank water, the suspended material and bottom detritus in the holding-tanks was monitored twice each week. No distinction was made between organic and inorganic mercury in analysis.

The previous feeding experiment had shown that many of the indices of growth and condition that were measured gave similar results or did not distinguish between the effects of the different feeding rations. In this toxin experiment therefore, the following indices were selected for measurement:

a) Change in total live weight
b) Change in dry flesh weight
c) Increase in shell length
d) $\frac{\%\ \text{dry flesh weight}}{\text{total live weight}}$

Results

Despite all the precautions taken, the actual mercury concentrations in the holding-tank water remained very low compared to the nominal dose, but remained constant within acceptable limits over the period of the experiment (112 days). The actual levels were:

Nominal concentration	Measured aqueous concentrations ppb Hg		Suspended solids ppb Hg	
	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	<0.04	-	< .02	-
3 ppb Hg	0.25	0.15	0.06	.004
10 ppb Hg	0.42	0.18	0.23	0.02
33 ppb Hg	1.00	0.03	0.87	0.06

In summary, the results show that 1.0 ppb of mercury as mercuric chloride reduces the growth of sub-adult Crepidula under these laboratory conditions, while at 0.25 ppb the condition of the animals after breeding is also reduced, compared to controls. Reproduction was affected down to 0.25 ppb, since fewer pairs bred more than once. Although the numbers of larvae released by those still breeding show little significant change, the viability of those larvae is reduced in terms of both the percentage successfully settling and the length of time to reach peak settlement.

Acute toxicity

To complement this toxin experiment the acute toxicity of mercuric chloride to adult and larval Crepidula has been measured using the constant-flow apparatus described by Connor and Wilson (1972), while monitoring actual tank water concentrations of mercury. The results give 96 hr LC50s to adults and larvae of 330 ppb and 60 ppb respectively. However, the 96 hr EC50s for cessation of swimming and feeding actively of the larvae were 6 ppb and 10 ppb respectively. Hence the levels of mercuric chloride which prevent larval activity are higher than those inhibiting adult growth and breeding success during chronic exposure. Testing for the acute effects of a pollutant on the larvae would not predict those levels likely to cause significant sub-lethal effects on the adult population.

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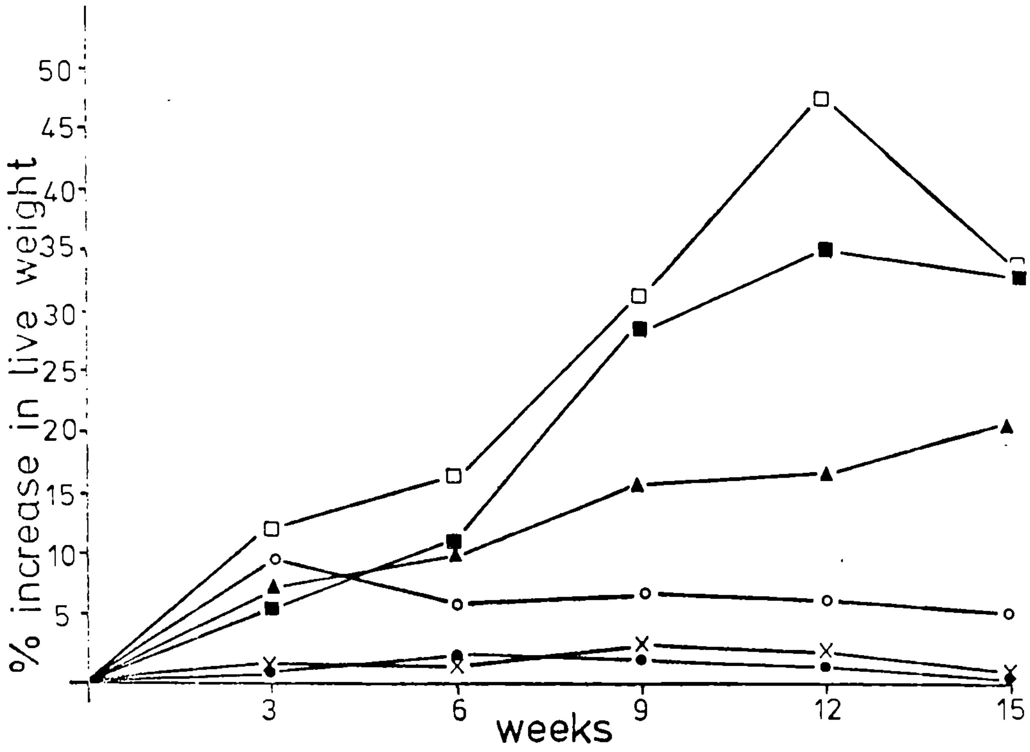

Fig 1. Percentage increase in live weight of animals in different food regimes of starvation (●) 1 (x), 2.5 (o), 5.0 (▲), 7.5 (■) and 10.0 (□) (x 10^8 algal cells/g flesh wet weight/day).

Table 1. Mean data for first and (third) spawnings of <u>Crepidula f.</u> subjected to nutritive stress.

Response	Treatment (10^8 cells/g. flesh wet weight/day)		
	5	7.5	10
Nos larvae produced	2888 (2552)	2506 (3325)	2753 (3062)
Nos of capsules produced	31 (31)	31 (32)	34 (31)
Larvae/Capsule	98 (79)	80 (111)	93 (93)
Larvae/g. of total wt. of each pair	2017 (1461)	1761 (2075)	1897 (1887)
Larvae/g. of total wt. of each female	2742 (1992)	2499 (3120)	2512 (2702)
Incubation times (Spawning to release) in days	16.20 (15.25)	15.57 (15.67)	16.62 (15.50)
Days to 1st spawning from start of experiment	40	24	28
No of pairs to reach 1st spawning	12	14	18
No of pairs to reach 3rd spawning	5	11	10

Contractors:	The Natural Environment Research Council, The Dunstaffnage Marine Research Laboratory, P.O. Box No. 3, Oban, Argyll, Scotland. Universite Aix-Marseille II, Station Marine d'Endoume, 13007 Marseille, France.
Contracts No.:	225-77-1 ENV UK 221-77-1 ENV F
Contract Leaders:	A.D. Ansell. H. Massé.
Title of Project:	Comparisons of the effect on benthic invertebrates from the Mediterranean and from northern European waters of temperature changes associated with thermal pollution.

Objective of the research

The aim of this programme, which was an extension of one begun under the first phase of the Environmental Research Programme, was to study, mainly by a series of laboratory experiments, the effects on selected invertebrates species of the types of thermal change usually associated with the discharge of cooling water from electricity generating stations in tidal waters. Under two contracts, studies have been made of species from inshore sandy sediments from the coasts of Scotland and from the French Mediterranean coast, an important aspect of the work carried out being a collaboration to examine how far geographical considerations affect the conclusions reached from experimental studies on the possible effects on the invertebrate fauna of discharges of heated water into the coastal zone.

The programme of studies proposed initially was designed to establish 1) the ecophysiological basis of observed population changes in certain intertidal and subtidal invertebrates in areas subject to heated water discharge, 2) the sublethal effects of temperature changes especially in relation to factors affecting distribution and productivity of the benthos and 3) the upper lethal limits of temperature tolerated by individual species. The main emphasis under the previous contracts was placed on determinations of lethal temperature limits, although valuable data was also obtained on the sublethal effects of temperature on the burrowing response of many of the species examined, and some studies were begun on the sublethal effects of temperature on reproduction and on energy requirements. Under the present contracts,the emphasis has been changed to extend the observations made on sublethal effects of temperature change with particular reference to gastropods of the families Nassaridae and Naticidae, which occupy a scavenging/predatory role in the inshore sand environment, and to bivalves, which are a major primary consumer as well as being the main prey of the naticid gastropods.

Results

1. Thermal tolerance of adult molluscs.

A limited number of determinations of LT_{50} and BT_{50} values were made with the aim of completing comparisons made during the previous contract period, between the same or closely-related species from Mediterranean and N. Atlantic waters.

For Tellina fabula from the Mediterranean, LT_{50} values between 26° and 29°C were found for different acclimation temperatures and for different exposure periods, confirming that, as in northern waters, this species from depths of c 8 m in the Mediterranean is less tolerant than the related Tellina tenuis which lives at shallower depths (LT_{50} between 30° and 32°C). Tellina fabula from the Mediterranean, however, has a LT_{50} value 1.5° - 2.5°C higher than T. fabula from northern waters, confirming previous comparisons. A similar comparison was completed for Nassarius reticulatus from Mediterranean and N. Atlantic populations. Compared with N. reticulatus from Scottish waters the LT_{50} and BT_{50} values for Mediterranean animals was consistently 2° - 2.5°C higher for exposure times greater than 12 hours.

These results for Tellina fabula and Nassarius reticulatus help to confirm the general conclusion reached earlier, that, when populations of the same species from Mediterranean and Scottish waters are compared,the southern populations show a higher temperature tolerance, but with a difference in LT_{50} values for the same acclimation temperature of about 2°C or less.

The effect of differences in age of individuals on LT_{50} and BT_{50} values was tested for Donax vittatus from Scottish populations by comparing values for animals from 4 year groups within the population. There was a clear difference in LT_{50} and BT_{50} values for animals of different ages, in contrast to the result reported earlier for D. trunculus from the Mediterranean, which showed no significant difference in LT_{50} for young or old groups, although there was a difference in the burrowing response. In general the life span of bivalves is shorter in the Mediterranean, and the two groups of D. trunculus used represented only 2 year groups with a difference of c 1 year in average age. The result for D. vittatus suggests that age, as distinct from size, may influence LT_{50} but further comparisons for other species are needed to confirm this conclusion.

2. Influence of salinity on the thermal tolerance of adult molluscs.

The influence of salinity on thermal tolerance was tested for a limited number of species from the Mediterranean and from Scottish waters. The Mediterranean species all showed little significant difference in LT_{50} for salinities within the range normally experienced (c 22 - 38‰), and this was true also for Tellina tenuis from Scottish waters which showed no significant difference in LT_{50} in the range 20 - 40%.. D. vittatus from Oban showed a greater response to salinity with LT_{50} and BT_{50} values being maximal at c 35% and reduced at lower salinities.

In European coastal waters there is a gradient of mean salinity from north to south; mean salinity ranges from c 32 - 33% in Scottish waters to c 37 - 38% in the northern Mediterranean, and the comparisons made hitherto between Mediterranean and Scottish populations have taken no account of this difference in mean salinity between the two areas. The present results, however, confirm that salinity differences of this order are not generally a factor in influencing the results obtained. In the case of the two Donax species, the greater sensitivity to salinity of D. vittatus reflects and reinforces the differences found in thermal tolerance: D. vittatus, where it occurs together with D. trunculus, is distributed mainly subtidally, whereas D. trunculus is distributed in the intertidal. The high thermal tolerance and lower sensitivity to salinity change shown by D. trunculus both reflect this distribution pattern.

3. Thermal tolerance of larvae of benthic invertebrates.

Determinations of LT_{50} values for larvae have shown that LT_{50} decreases with increase in time of exposure in a similar pattern to that described for the adults. In contrast to the adults examined however, there is little difference in LT_{50} for a given duration of exposure whatever the development temperature, indicating that for the larvae there is little acclimation effect.

Comparative measurements have been made of thermal tolerance for larvae of Nassarius reticulatus from both Mediterranean and N. Atlantic populations. The LT_{50} values found for the Mediterranean larvae were higher than those found for the N. Atlantic population, especially for exposure times less than 6 hours; but, with the exception of exposure times of less than 3 hours, the difference in LT_{50} did not exceed 2°C.

In a more extensive series of experiments the thermal tolerance of larvae of the polychaete Scololepis from Mediterranean and N. Atlantic populations were compared. In this case, also, the thermal tolerance of the larvae showed no significant effect of acclimation (spawning) temperature, and the results of determinations with larvae of N. Atlantic animals fell within the range of variance of determinations made with Mediterranean larvae.

4. Comparisons of thermal tolerance of larval and adult benthic invertebrates.

Where determinations of LT_{50} values have been made with both adults and larvae of the same species it is apparent that the larvae are as resistant to high temperatures as the adults, and, in some cases, more resistant. For the Mediterranean species tested, the LT_{50} values found for adults and larvae were very close, while, for Nassarius reticulatus from N. Atlantic populations, for periods of exposure greater than 24 hours the larvae were slightly more tolerant than the adults.

5. Influence of temperature on reproduction of gastropod molluscs.

The temperature range over which capsules are produced has been determined for five species of the gastropod family Nassaridae: four from Mediterranean, and one, Nassarius reticulatus, common to both Mediterranean and N. Atlantic waters. For this latter species observations have been made with animals from both N. Atlantic and Mediterranean populations. In both areas, egg capsules were produced in constant temperature cultures at 10°, 15° and 20°C. At 25°C in N. Atlantic animals egg capsules are very rarely produced and development at this temperature is frequently abnormal. For Mediterranean individuals, the same phenomenon is found between 25° and 30°C. At 10°C, Mediterranean animals will spawn, but the initial stimulation requires a thermal shock, whereas N. Atlantic animals spawn readily at 10°C and in the field begin to spawn under normal ambient temperature conditions when the temperature first exceeds 8° - 9°C.

A similar difference is found when the duration of development of the larvae within the egg capsule is compared for N. reticulatus from N. Atlantic and Mediterranean populations. At 15°C, the duration of development is essentially identical for animals from both populations. Above 15°C, the duration of development is shorter for Mediterranean than for N. Atlantic animals. At 25°C, for N. Atlantic animals the duration of development is greater than at 20°C, for those eggs which develop normally, while for Mediterranean animals the duration of development is further reduced at 25°C. Below 15°C, the duration of development is shorter for N. Atlantic animals.

In Polinices alderi, where a similar comparison has been made, the rate of development for N. Atlantic animals was slightly greater at 15° and 20°C than for Mediterranean animals, but at 25°O the rate of development increased for N. Atlantic animals while continuing to decrease for Mediterranean animals.

The most complete data on temperature effects during the life cycle are now available for Nassarius reticulatus. For animals from Scottish waters LT_{50} and BT_{50} values of 28°C were found for periods of exposure to high temperatures greater than 24 hours. Below this temperature the animals survive and burrow, but normal gametogenesis and spawning have been observed only in the range 10° - 20°C, i.e., the upper limit for successful reproduction lies in the range 20° - 25°C. Development of the eggs within the spawned egg capsules will take place in the temperature range 5°- 25°C although at both extremes of this range there is interference with the normal development and many egg capsules do not develop normally. Finally, the hatched larvae have an LT_{50} value of 29°C. Thus the most critical part of the life history for N. reticulatus, in relation to temperature for survival, is the young larva developing within the egg capsule, however, the temperature range for successful production of egg capsules is less. The most critical stage of the life cycle therefore appears to be the stage of gonadal development and spawning by the adult, and in animals exposed continuously to high temperatures (20°C) egg production is reduced or absent. Below this temperature, however, the rates of egg production and development vary. Maximum rates of egg production over short periods with well-fed animals were found at 20°C, and at this temperature the rate of development of larvae in the egg capsules was also greatest. The greatest fecundity over the breeding cycle in well fed animals was also found at 20°C.

Similar considerations apply to the Mediterranean animals except that here the relationships are shifted slightly towards higher temperatures. LT_{50} and BT_{50} values for adults are c 2°C higher, LT_{50} values for larvae are c 1° - 2°C higher, and there is a shift of approximately 2°C in the temperature range over which normal reproduction will occur affecting both the upper and lower extremes. The rates of development of the hatched larvae are also affected. Thus the slight adaptation noted earlier in thermal tolerance between northern and southern populations of the same species, appears to extend to all aspects of reproductive activity.

6. Influence of temperature on predator/prey relationships.

Aspects of the predator/prey relationship between gastropods of the family Naticidae and their bivalve prey have been investigated using the gastropod Polinices alderi from populations in the Mediterranean and from Scottish waters. The initial series of experiments involved keeping small numbers of animals in cultures at constant temperature, and these were maintained using the locally most abundant prey organisms at densities which were sufficiently high to prevent prey density becoming a limiting factor. At Oban, Tellina tenuis, and in Marseille Venus gallina, Spisula subtruncata and Donax trunculus were used as prey. Rate of growth, rate of egg collar production, rate of feeding (number of organisms bored/unit time) and rate of food consumption (weight of bivalve tissue consumed/unit time) were examined, and the accumulated data are now being used to examine in detail the energetics of the processes involved, including the energy requirements for maintenance, somatic growth, and reproduction, and the efficiencies of food conversion involved at different temperatures.

The rate of growth of P. alderi in culture, as indicated by increments in shell height and total fresh weight was variable, both between cultures and between individuals within a culture. At Oban, the rate of shell growth was initially greater at higher temperatures and there was a clear relationship with size; higher growth rates occurring in smaller animals. Later the rate of growth was reduced in all cultures and the relationship to size was less apparent. During the first period no egg collars were produced, but the latter period coincided with the production of egg collars in at least some of the cultures (those containing both males and females). A similar reduction occurred in all cultures though the initial sizes and the sizes reached prior to the reduction in growth rate varied over a wide range. As a result, there was also a wide variation in the apparent maximum size towards which individual animals grew in culture.

This effect on maximum size is even more apparent when the Mediterranean and N. Atlantic animals are compared: in the Mediterranean animals the rate of growth declined more rapidly with increase in size and the maximum size attained was much smaller than for the N. Atlantic animals. The effect of this difference is to change the relative requirements for somatic growth and reproductive output in smaller animals, but whether this difference between Mediterranean animals and N. Atlantic animals is genetically determined or induced by differences in diet is not yet clear, although preliminary experiments have been made with transferred animals, and with different diets in attempts to resolve this problem.

The feeding rate, and rate of food consumption by P. alderi in cultures showed a similar pattern to growth rate, with higher rates associated with periods when egg collars were produced, reflecting the energy requirements for gametogenesis and spawning. There was a general tendency for feeding rates to increase with increase in temperature, but with relatively little change in the range 15° - 20°C in the maximum rate of feeding. In N. Atlantic animals, the rate of egg collar production also showed an increase with temperature in the range 5° - 15°C but was reduced at 20°C. At 20°C egg collars were only rarely produced. In the

Mediterranean animals, the numbers of egg collars produced was insufficient to show any temperature relationship.

Respiration rate of Mediterranean and N. Atlantic animals increase in the range 10° - 20°C, and the measurements made suggest that when animals of similar weight and tissue content are compared there is no significant difference between the two groups.

Preliminary calculations of growth efficiencies show that the net growth efficiency in animals not producing egg collars declines with increase in size, and that this relationship is not significantly changed with temperature in the range 10° - 20°C. The effect of reproductive activities, and of differences in diet, resulting from changes in prey availability or in species composition of the prey, are being investigated.

7. Seasonal cycles of bivalve molluscs.

The seasonal cycles of the bivalves studied in Scottish waters all show common factors, of which the major elements are, a) a rapid increase in tissue weight during the spring, accompanied by proliferation and maturation of the gonad, b) a summer period of reproduction and growth during which there may be one or more peaks of spawning activiy, c) an inactive, non-reproducing period during the winter when no growth occurs, d) the accumulation of large reserves, particularly of glycogen, especially towards the end of the reproductive period, and e) reliance on utilisation of these reserves during periods of the year when metabolic requirements cannot be met by feeding. All the species examined (*Venus striatula*, *Tellina tenuis*, *Donax vittatus* and *Spisula subtruncata*) live for several years (4 or more) and a similar cycle is repeated in each successive year of life. The cycle is apparently determined by interaction of temperature and food supply, modified by the physiological state of the animals, especially the reproductive state, which in turn may be intrinsically determined, so that temperature may affect the animals differently at different stages in the cycle.

The cycles for the Mediterranean species examined (*Donax trunculus*, *Spisula subtruncata* and *Venus gallina*), when compared with these, illustrate two ways in which these factors are modified geographically. *D. trunculus* and *V. gallina* represent species of more limited geographical distribution replacing the related northern species, and in these cases the seasonal cycle shows some features which are closely similar to those of the northern species; in particular the reproductive period is limited to the summer months, but with a relatively shorter inactive period. The major increase in tissue weight coincides with proliferation of the gonad in the spring. The Mediterranean species show lower seasonal fluctuations in the rate of change in tissue weight, and this is also reflected in generally lower levels of reserves, e.g., of glycogen. The growing season however, is extended and hence the overall annual production is greater, although the average life span in the Mediterranean species is reduced.

The results for Spisula subtruncata illustrate a further extension of the geographical adaptation of the seasonal cycle, since for this species in the Mediterranean the life span is reduced to 1 year or less. Two main recruitments occur each year, and in each, growth in size is accompanied by gonadal maturation, so that changes in tissue weight, etc., are related to size rather than to seasonal change. In contrast, in Scottish waters, this species has a life span of several years, and shows a seasonal cycle cimilar to that for the other species examined. A further general difference between Mediterranean populations and N. Atlantic populations of such species as Spisula subtruncata which have a wide geographical distribution, seems to be a reduction in maximum size in the southern populations, similar to that already noted for the gastropod Polinices alderi.

8. Enzyme studies.

Samples from the routine collections of Donax vittatus have been used to make an investigation of the conditions for extraction and estimation of enzyme activities in excised foot, adductor muscle, mantle and gill tissue. The techniques development have been applied to assessment of the capabilities of these tissues for utilisation of alternative metabolic pathways, and seasonal fluctuations of enzyme activities have also been investigated. So far these studies have been confined to the northern species, Donax vittatus, but they will be extended to compare the related southern species, D. trunculus.

A preliminary comparison of isoenzymes in the gastropod Nassarius reticulatus from Mediterranean and N. Atlantic populations examined after a long period of acclimation at constant temperatures of 10°, 15°, 20° and 25°C has also been made, using electrophoresis techniques.

The enzyme studies carried out so far are still in the development stage as far as comparisons of Mediterranean and N. Atlantic species are concerned and will not therefore, be discussed in detail. The studies have the eventual aim of comparing the metabolic basis of adaptation to temperatures within the physiological range, and at near lethal temperatures, and of examining the modification of such metabolic adaptation both seasonally and geographically.

Discussion

The main emphasis in this contract period has been on providing information on the sublethal effects of temperature within the physiological limits defined by the previously determined LT_{50} and BT_{50} values for various species, with particular reference to one element of the benthic food chains of inshore sandy sediments; that involving bivalve molluscs as primary consumers and the gastropod molluscs as predators and scavengers. The results extend those previously reported, by providing information in two main areas, a) temperature effects at different stages of the life cycle, and b) the effects of interaction of temperature with other factors on rate processes with determine the productive and reproductive potential of the animals.

The main conclusions which arise from the present observations in these areas may be briefly summarised:

a) The most critical phase of the life cycle in terms of restriction of the temperature range lies in the processes of gametogenesis and spawning.

b) Within the physiological range limited by LT_{50} values the species examined from inshore sandy environments show relatively little temperature acclimation effect, i.e., rate processes increase uniformly with temperature until near lethal temperaturez, with little evidence of any "plateau" in the rate/temperature relationship, as occurs in certain rocky-shore species.

c) When rate processes are compared for the same species from the different geographical areas, there is similarly little evidence of temperature acclimation in the results, the rate/temperature relationships being similar in Mediterranean and N. Atlantic animals, with the exception of rates at or near lethal temperatures, which reflect the slight shift (c 2°C) found in LT_{50} and BT_{50} values.

d) Temperature effects on processes within the physiological range are modified by the nitritional and physiological state of the animal, especially in relation to gametogenesis and spawning. This is clear both in experimental laboratory studies (with naticid gastropods) and in relation to the natural seasonal cycle (with bivalves). This interaction is also a factor producing geographical differences,since the natural seasonal relationship between temperature and food supply differ in northern and southern areas. Examples of the complex effects of the resulting adaptations are, the plasticity in maximum size in both gastropods and bivalves, with resulting implications on production and energetics; and the geographical variation in life span, so that age and size effects may be expressed differently in the two areas.

In relation to increases in temperature caused by the discharge of cooling waters into the marine environment the present results indicate that, at sublethal levels, the first effect of temperature increase is likely to be seen in reproductive processes, involved in the successful development and spawning of eggs. Where food limitation is also a factor affecting the population, the effect of temperature increase will be to accentuate the effects of nutritional stress, leading to a complex of responses arising from the relationships between somatic growth, reproduction, and the storage and utilisation of reserve materials, and resulting in such diverse effects as reduction in the rate of somatic growth, in reduction in the maximum size reached, reduction in fecundity, and reduction in the levels of stored reserves. In northern populations, such interaction will be more critical during those stages of the seasonal cycle when food supply in relation to the animals requirements is limited, that is, during the summer, when the combined energy requirements of gamete production and somatic growth may exceed that available from feeding activity. In southern populations such seasonal effects may be of less significance since food supply in these areas appears to show less seasonal fluctuation, but, since many Mediterranean populations are functioning normally nearer to their upper thermal limits, any seasonal effects are most likely to be expressed during the summer period.

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Contractor : Department of Agriculture and Fisheries for Scotland
Contract n° : 226-77-1 ENV UK
Project leader: A. D. McIntyre
Title of Project: Controlled Ecosystem Studies of Marine Communities

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Objective of the research

The objective of the research was to improve the design of underwater enclosures and use these to study a range of problems related to pollution stress on marine communities and to the management of commercial fish populations. Both the pelagic and the benthic systems would be used.

Materials and methods

The approach was to carry out a series of controlled ecosystem experiments (CEEs). The basic experiments were done in large plastic bags suspended in the sea in water of 30 m depth. The bags are constructed of reinforced PVC and were 5 m in diameter and 20 m in length, enclosing about 300 m^3 of sea water. They are suspended by individual flotation collars designed in the laboratory's engineering department and power to run the monitoring equipment was provided by 600 m of armoured cable from the shore to two rafts moored near the enclosures. A specially constructed wave break was designed to protect the installations from excessive wave action and this was positioned between the equipment and the open sea.

In addition to the floating enclosures, a number of benthic chambers were designed and constructed at the laboratory. These consisted of perspex cylinders, 2.2 m in diameter, which are settled into the bottom, enclosing an area of sediment with associated fauna in which long-term experiments can be conducted.

The deployment and servicing of the underwater installations as well as the underwater experimental manipulation was done by a team of divers, and additional surface back-up was provided by a group of chemists and biologists allocated to the project.

In the 1977 experiments, bags were dosed with North Sea oil at a concentration of 100 µg/l to study the effect on the planktonic ecosystem over an extended period, and to follow the vertical transport rate of oil distributed through the water column to the bottom sediment. During the experiment the parameters measured in the water column on a routine basis included chlorophyll, organic carbon and nitrogen, temperature, salinity, primary production, microbial rates of hydrocarbon mineralisation, phytoplankton size spectra, hydrocarbon concentrations, and the amount of material settling out from the bottom of the bags. In addition, the structure of the zooplankton population was determined and changes in it followed by means of net hauls.

A second oil experiment was done to investigate the transport of oil from a surface slick to the bottom sediment under conditions of natural weathering and also when treated with dispersant. This contrasted with the first experiment in which oil was dispersed in the water column to simulate subsurface effluent discharge.

Finally, in 1977, after trials with the collection of ripe herring and the fertilization and transportation of eggs, a study was made of the growth of herring larvae from eggs placed in the bags after fertilization.

During the 1978 series of experiments, this research was continued and extended. The two strands of the successful 1977 experiments were brought together by adding developing herring eggs to bags treated with oil, thus adding a higher trophic level to the stressed food chain. These 1978 experiments are particularly relevant to conditions in the North Sea, since oily water effluent from one of the production platforms was used and the full food chain - plants, grazers and predators - was included. Finally, preliminary work was done to integrate the underwater benthic chambers to the bag experiments by adding sedimentary material from the bags to the chambers.

Results

The initial object of the work was to develop and test large-scale experimental facilities for ecosystem manipulation. This has been fully achieved. We can

(a) use four 300 m^2 floating plastic enclosures for periods of up to four months without significant water exchange

(b) maintain stable populations of phytoplankton, zooplankton and fish larvae for periods of 3-4 months.

(c) by mechanical stirring inside the bags, achieve vertical water mixing rates comparable to those measured in the open sea

(d) extend the work into the bottom sediments by using underwater chambers.

The salient features of the results were the effects of oil on the microbial population, which increased markedly, and on the zooplankton. There was a decline in the calanoid population following the addition of oil but this decline did not prevent the production of almost equal numbers of eggs in the oiled and in the control bags almost coincident with the addition of oil. This egg production was reflected in the control bag by an increase in copepod nauplii some ten days later and by an increased calanoid copepod population about thirty days after the peak in egg production. However, in the experimentally contaminated bag the peak in egg production was never translated into either nauplii or mature copepods.

In the experiments with herring, approximately equal numbers of artificially fertilized eggs (40,000) were placed in each of two enclosures. Both larval populations suffered considerable mortality on hatching (which may be a reflection of what happens in the natural environment), but in spite of large differences in initial mortality rates in the two enclosures, the mortality rate in both populations eventually stabilised at about 5% per day. Differences in growth rates and in morphological characteristics were found to be associated with the differences in larval density. The larvae in the less dense population were on average larger and heavier for a given age. Furthermore, the range of sizes of larvae in the more dense population became progressively greater with age. In spite of this, it was apparent that the heaviest individuals in both populations had similar growth rates suggesting that the maximum growth rate in both bags was limited to the same extent despite the great difference in larval abundance. It should be noted that preliminary results from the autumn 1978 experiment suggest that food may have had a much greater effect on limiting larval growth in the second year. Length-weight data, and various measurements of larval condition provide no evidence of starvation among any of the larvae, even in the case of the smallest individuals at the end of the experiment. Analysis of the gut contents of the larvae revealed that the relative abundance of items in the diet was very different from their abundance in the entrapped zooplankton population. However, until more is known about the microdistribution patterns of the larvae and the zooplankton, little can be said about larval food selection. Using growth and mortality data it was possible to calculate the food consumption of larvae in the bag. For example, at first feeding, a larva is estimated to need 20 μg dry weight of food daily. From a consideration of the energy content of the various food items it was concluded that the occasional large item in the diet (eg a copepod or a lamellibranch veliger) played a very important part in providing the larvae with the amount of food required.

Finally, with reference to the studies of oil movement through the environment, it was of interest that a high proportion (about 50%) of oil added to the water column in the bags reached the bottom and was collected there. In contrast, when oil was added as a slick, less than 1% was collected from the bottom of the bags in seven days, even when chemical dispersants were used which produced high concentrations of oil in the top few metres.

Conclusions and comments

This report describes two series of experiments carried out over several months during two years. The second series used autumn spawning herring and therefore had to be done at the end of 1978, so that a considerable volume of data are still available for further assessment. It is already clear however that the high levels of oil which may be found in surface waters under slicks could have adverse effects on the food of fish larvae and the bag experiments allow this to be followed through to the larvae themselves which should help with assessments of the effects of spills The bags have also been particularly useful in demonstrating differences in the availability of oil to different parts of the ecosystem depending on the manner of input, and this is relevant to decisions about the use of dispersants and the disposition of effluent sources.

Extension of this research could include not just how the whole system (microorganisms-phytoplankton-zooplankton-fish larvae) react to stress, but also how they recover when the stress is removed. The stress could include not only that from pollutants such as oil, but also from low or high nutrients or from predators.

Publications and oral communications

1. A. D. McIntyre - "Controlled Ecosystem Experiments" - oral presentation to the EEC Contact Group Meeting, Marseilles 1977.
2. Davies, J. M. et al - "Experimental studies using large scale enclosed ecosystems to study oil hydrocarbons in marine food chains"- oral presentation at symposium Research perspectives on the impact of petroleum on the northern marine environment. Seattle 1978.
3. Davies, J. M. and Gamble, J. C. - "Experiments with large enclosed ecosystems". Phil. Trans. R. Soc. Lond. B., 1979 (in the press).

Contractor:	Natural Environment Research Council
Contract No:	279-77 ENV UK
Project Leader:	Dr J. Widdows
Title of Project:	The ecological effects of sublethal stress and pollution in estuarine invertebrates

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Objective of the research

The programme at the Institute for Marine Environmental Research aims to derive practical techniques for assessing the health and condition of populations of mussels (Mytilus edulis) in relation to environmental conditions, including both man-made and natural stressors, in terms of physiological, cytological and biochemical stress indices.

In the first phase of the Second Environmental Research Programme the main objectives have been: (a) to measure biochemical and cytological stress indices over a wide range of environmental conditions; (b) to investigate genetically and environmentally induced differences in the adaptive responses of mussels transplanted between two different environments; (c) to compare physiological measurements in the field under ambient conditions with those made in the laboratory under standardised conditions, as a means of simplifying the measurement of stress indices, and (d) to develop techniques for examining chromosomes of mussels and quantifying the degree to which different environmental conditions induce genetic and chromosomal abnormalities.

Materials and Methods

a) Mussels were collected from a wide range of environments (11 sites in the UK) during autumn-winter of 1977-78. Two general stress indices were measured: (i) the concentration ratio of two tissue amino acids (taurine and glycine) from the free amino acid pool and (ii) the latency of the lysosomal enzyme β-hexosaminidase in the digestive cells.

b) The field transplant experiment compared the health and condition of native and transplanted mussels at two sites, the Lynher estuary (S.W. England) and Kings Dock (Swansea, Wales). Mussels were transplanted between the sites in January 1978 and physiological, biochemical and cytological stress indices were determined at two-month intervals until August 1978 using a mobile laboratory. Physiological measurements included rates of oxygen consumption, feeding, excretion and food absorption efficiency. Animals were also sampled for assessment of gametogenic stage, taurine: glycine ratio, and latency of the lysosomal enzyme β-hexosaminidase. Concentrations of Cu, Zn, Cd, Fe and Pb were measured in the kidney, adductor muscle and remaining tissues. A second transplant experiment is in progress.

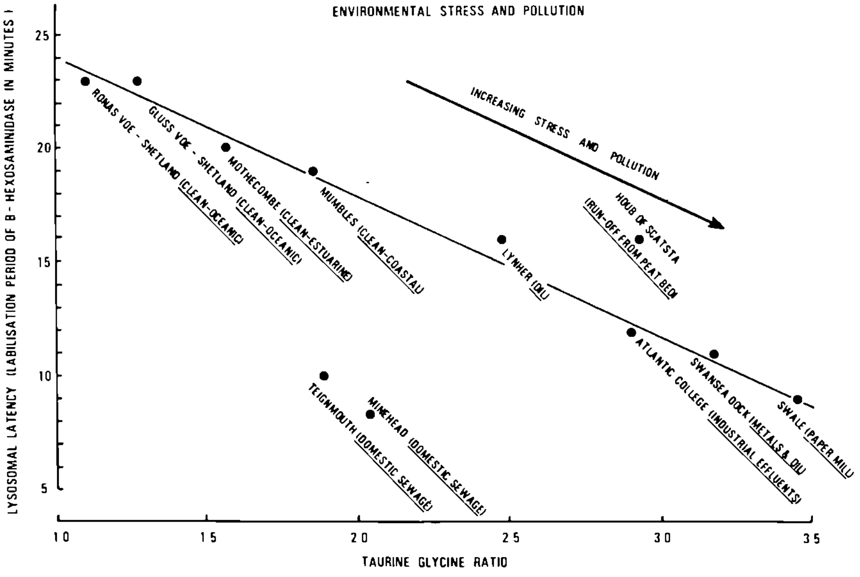
c) A comparative study of laboratory and field measurements of physiological responses was carried out in the autumn (1978). Rates of oxygen consumption, feeding, excretion and food absorption efficiency of mussels from three populations (Lynher, Cattewater and Teignmouth) were first determined in the field under ambient conditions. The measurements were then repeated within 24 hours in the laboratory under standardised conditions of temperature, salinity and food.

d) Cytological techniques used in mammalian genetics required modification before suitable chromosomal preparations could be achieved. To make them visible, chromosomes of Mytilus must first be inhibited at metaphase using colchicine, spread by treatment with hypotonic saline and selectively stained with toluidine blue or aceto-orcein. Chromosomal preparations of both somatic (gill tissue) and embryonic cells are being used to karyotype Mytilus edulis and to record the incidence of mutations or chromosomal abnormalities in 'unstressed' populations.

Results and Discussion

The taurine:glycine ratio and the latency of the lysosomal enzyme β-hexosaminidase both responded to increasing stress and pollution as shown by results from all the sites, representing a range from 'clean oceanic' to 'polluted' conditions. When the two indices are plotted against each other (see Fig. 1) they fall on or near a straight line with two exceptions (Teignmouth and Minehead), both subject only to domestic sewage which apparently affected lysosomal latency but not the taurine:glycine ratio; from this it seems that the ratio of amino acids is probably unresponsive to stress from high inputs of organic and nitrogenous material.

FIG 1 CORRELATION BETWEEN A CYTOLOGICAL AND A BIOCHEMICAL STRESS INDEX IN RESPONSE TO INCREASING ENVIRONMENTAL STRESS AND POLLUTION



Parallel monitoring of chemical and biological effects is provided by the reciprocal transplant programme (Lynher and Swansea Docks). The results suggest that the transplanted mussels adapted, at least in part, to their new environments: physiological, biochemical and cytological stress responses of both native and transplanted animals were similar within two months of the beginning of the experiment. There were, however, some environmentally induced differences between the two sites as shown by the latency of lysosomal hexosaminidase:

Site	Population	Labilisation period (min ± SE)
Lynher	Lynher-natives	23.8 ± 1.3
	Kings Dock-transplants	24.0 ± 1.0
Kings Dock	Kings Dock-natives	5.0 ± 1.0
	Lynher-transplants	12.0 ± 2.0

Concentrations of Cu, Cd, Fe, Pb and Zn were monitored in the tissues of these mussels. Different rates of accumulation and depuration in the transplanted animals showed that some metals are more labile than others. For example, Pb in the kidney showed slow and partial accumulation and depuration; the original concentrations of Cd in the kidney and body tissues were maintained, whereas Zn and Cu showed no apparent trends.

Preliminary analyses from the transplant experiments therefore suggest that recorded population differences are mainly a function of environmental factors rather than genetic or endogenous factors.

The field versus laboratory comparison will be repeated but first results show only small differences between field and laboratory measurements of animals from the same population, compared with marked differences between populations, suggesting that physiological stress responses induced by different environmental conditions are retained by the animal in the laboratory, at least for a limited period of time, and can therefore be measured in the laboratory under standardised conditions.

It is generally recognised that the mutagenic properties of environmental pollutants have a deleterious effect on the genetic material of marine organisms, and that cytogenetic techniques can provide a powerful research tool in the field of environmental toxicology. Techniques for rendering chromosomes visible in embryonic and gill tissues of mussels have now been developed in IMER. Because of the constancy inherent in genetic material and the conservative nature of the karyotype for any one species, it is possible to detect genetic damage caused by mutagenic substances from the incidence of chromosome patterns which do not comply with the genetic 'fingerprint' for the species. However, it is important first to establish the frequency with which abnormalities occur in mussel populations from 'clean' environments. When this information is available, mussels from polluted environments will be analysed.

Conclusions

Both stress indices ('taurine:glycine ratio' and 'lysosomal enzyme latency') are responsive to environmental stress and pollution. The development of cytogenetic techniques offers promise as a method for assessing the effects of environmental stress and pollution on the genetic material of an animal. On the evidence so far, differences in the physiological condition of different populations appear to be primarily the result of environmental factors.

A simplified approach to field monitoring may be provided by the assessment of physiological condition under standard laboratory conditions combined with the transplantation of mussels from an 'unstressed' population to sites along a pollution gradient.

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Bayne, B.L. 1978. The potential of bivalve molluscs for monitoring the effects of pollution. ICES Report.

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Bayne, B.L. and Widdows, J. 1978. The physiological ecology of two populations of *Mytilus edulis* L. Oecologia, 37, 137-162.

Livingstone, D.R. 1979. An empirical assessment of the tissue molar ratio of taurine to glycine and the concentration of other amino acids as an index of sublethal stress in the common mussel *Mytilus edulis* L: field and laboratory studies. Mar. Environ. Res. (To be submitted).

Lowe, D.M. 1979. Reproduction in *Mytilus edulis*: a stereological assessment (in prep.). J. Invert. Reproduction.

Lowe, D.M. and Moore, M.N. 1978. Cytology and quantitative cytochemistry of a proliferative atypical hemocytic condition in *Mytilus edulis* (Bivalvia, Mollusca). J. Natl. Cancer, Inst., 60, 1455-1459.

Lowe, D.M. and Moore, M.N. 1979. The cytology and occurrence of granulocytomas in mussels. Mar. Poll. Bull. (In Press).

Lowe D.M. and Moore, M.N. 1979. The cytochemical distributions of Zinc (ZnII) and Iron (FeIII) in the common mussel, *Mytilus edulis*, and their relationship with lysosomes. J. mar. biol. Ass. U.K. (In Press).

Moore, M.N. 1979. Cellular responses to polycyclic aromatic hydrocarbons and phenobarbital in *Mytilus edulis*. Mar. Environ. Res. (In Press).

Widdows, J., Fieth, P. and Worrall, C.M. 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol. 50, 195-207.

Widdows, J., Moore, M.N., Lowe, D.M. and Salkeld, P.N. 1979. Some effects of a dinoflagellate bloom (*Gyrodinium aureolum*) on the mussel, *Mytilus edulis*. J. mar. biol. Ass. U.K. (In Press).

Widdows, J., Phelps, D. and Galloway, W. 1979. Measurement of physiological condition in mussels transplanted along a pollution gradient in Narragansett Bay. Mar. Environ. Res. (To be submitted).

Contractor : University of Dublin, Trinity College
Contract No : 179 - 77 - 1 ENV. EIR
Project Leader : Dr. D.W. Jeffrey
Title of project : Irish Estuaries Research Programme

OBJECTIVE

The primary aim of this research programme is to design a practical scheme for the estimation of estuarine quality. We have in mind analogies with systems for classifying air or fresh water quality which are widely used administratively.

Barriers to even approaching this end have been:-

a) The low level of understanding, certainly in quantitative terms, of the nature of estuarine processes.

b) The virtual absence of true inter-disciplinary work in estuarine studies.

c) The greater intrinsic complexities of estuaries compared with lakes or rivers.

There is a growing need in Ireland for guide-lines on quality evaluation in the light of current industrial development and the introduction of "Environmental Impact Statement" methodology into planning legislation. There is also a need in Europe for quality yardsticks as goals for improvement of estuarine conditions.

METHODS

Approach

To make the inter-disciplinary approach possible a relatively simple conceptual model of estuaries has been used. Three major compartments (water column, sediment floor, organisms) are imagined as being linked through a large number of biological, chemical and physical processes (Fig.1). Generally we have sought the less transient features of the biota and most conservative environmental parameters. Data have been accumulated from a variety of field sampling and laboratory exercises. The aquisition of this data base in standard format is considered a key factor in the interpretation and evaluation of suitable quality parameters.

Team structure and administration

Three post-doctoral scientists were recruited and started work in early Summer 1977. They had previous experience in Scottish, English and Irish estuaries in the fields of zoology, botany and sedimentology. The activities of this nucleus were co-ordinated by the director, and supplemented by assistance, advice and co-operation from academic staff from several departments. Two other research workers were attracted by the existence of the programme and have contributed to its scientific aspects, a Royal Society European Research Fellow who worked on microbial aspects of hydrocarbon decomposition in Dublin Bay (12 months) and an American Visiting Professor who is currently working on environmental aspects of invertebrate metabolism and population structure (12 months). Office space for the programme is provided in one building but the laboratory facilities of several departments are routinely used. Co-ordination has been achieved by regular semi-formal conference meetings.

Technical methods

We have attempted to select, where alternatives exist, the most simple and least expensive methods. Methods have been evaluated by inter-laboratory calibration or other methods of comparison. This is in keeping with the main aim of providing a practical system of quality evaluation.

Liaison

Contact with other groups has always been of prime importance, and we have taken opportunities to liaise with estuarine scientists from several parts of Europe. Within Ireland we have also developed contacts with several local authority administrators.

Field and laboratory work programme

We have now made biological (zoological, botanical/microbiological, chemical) and environmental (sedimentology, chemistry, temperature) studies on over 50 sites from eight Irish estuaries.

This has been done to cover a range of conditions including scale, diversity, sediment type, relative size of fresh water inflow, degree of pollution from urban, industrial or agricultural sources, geographic location, and season. Examples are the complex Dublin Bay situation for which we now have a fairly comprehensive knowledge : Dungarvan (Co. Waterford)where on a small scale, industrial pollution exceeds urban : the Avoca estuary where heavy metal pollution from an inland source is combined with coarse grained sediments and a number of estuaries with slight eutrophication due to agricultural run-off.

Laboratory work has comprised detailed examination of materials brought from the field and a number of specific experimental investigations.

RESULTS

Design of a protocol for evaluating estuarine quality

We are at present writing a document which is based on the following table of contents:

1. Summary.
2. Aims and intentions - potential users.
3. Approach - Attitudes - Limitations; cost; time; knowledge. Scientific stance.
4. The Present Situation - status of environmental quality evaluation.
5. Modes of use: Synoptic quality surveys: Comparisons with regional baseline: Monitoring of whole estuaries: Formulation of policies on quality.
6. Outline of procedure - Diagram with commentary to form key to rest of description.

Technical Description

7. The Basic Survey - Review of research technique.
8. Classification of estuary.
9. Design of Basic Survey.
10. Sampling. a) Collection and storage, b) Identification methods, c) Processing.
11. Codification of data - record card.
12. Data processing - statistical and intercomparison procedures: data sorting and listing.
13. Index construction.
14. Uses of an index in policy making.
15. Case histories.
16. Guidelines for further investigation. a) Principles for continuous monitoring. b) Process analysis. c) Other analyses - when to consider difficult determinations.

17. Technical appendices - listing of analytical and other procedures.
18. Bibliography.

We wish to provide a guideline for the administrator wishing to instruct a technical consultant or a central government agency wishing to carry out a national survey.

Parts 1-6 are for the direct guidance of the administrator and also outline for specialists the limitations we have assumed and the scientific attitudes adopted. For example, we are still convinced that for the information provided, the costs involved in making detailed examinations of the water column are too great, and that the intertidal zone carries much information.

Parts 7-18 are technical information which will guide the consultant's team in the following procedures.

a) Drawing together background information: making an operationally useful classification and designing a simple cost effective survey (Basic Survey).

b) Executing the survey and presenting the results in a standard format.

c) Processing the data which will consist of biological species lists with possibly some quantitative biological information; Physical and chemical data on sediments; possibly chemical data on biota; other quantitative environmental information.

d) Interpreting the data through an index construction procedure which will permit data of the different kinds to be compared against specific bases and then amalgamated. We envisage that a quality index could be assigned to a given site, a group of zones e.g. a particular shoreline, or a whole estuary.

e) Giving indications where the basic survey needs to be supplemented by more detailed (and more costly) further examination.

At the present time we are optimistic about producing a complete "First Approximation". There have not been difficulties in designing a rational survey procedure or in deciding on most aspects of data collection. The greatest problems lie in,

a) the universality of scientific interpretation.
b) providing the best form of index for the various forms of inter-comparison.

There will also be a number of minor operational problems which only field testing will reveal.

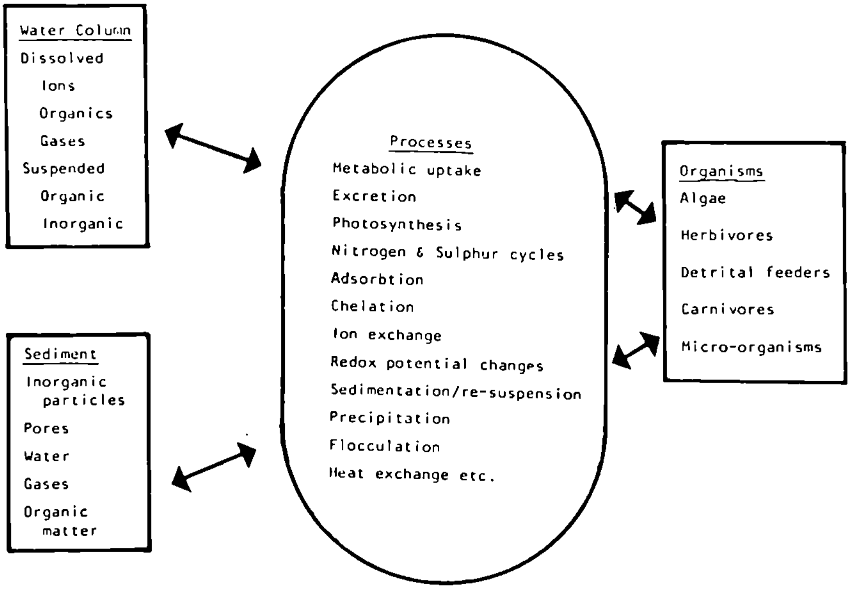
The detailed results of specific field exercises will be given in the final report.

CONCLUSIONS

1. Within the limitations of scientific knowledge of estuaries we believe that we are close to producing a viable system for practical quality evaluation and inter-comparison. It would be of great value to continue this programme for a further year of field testing and modification as outlined in our original research proposal.

2. Whilst we have deliberately concentrated on a system of evaluation which does not need a conscious method of inter-comparison with other estuaries, (this is built into the index procedure), it is realised that the data accumulated is of substantial scientific value. If standard procedures are adopted in measurement, recording and data presentation, and a large number of sites eventually surveyed then an estuarine data base of high quality and large size would result. Proper use of this as a resource would lead not only to a better understanding of estuarine science but also to possibilities of better quality evaluation by a central agency. This University would certainly be in a position to develop and administer such a scheme if funds were available.

FIG. I - A concept of functional compartments in an estuary linked by processes.



Contractor : The Royal Veterinary and Agricultural University, Copenhagen
Contract n° : 185-77-1 ENV DK
Project leader : N.O. Christensen
Title of project : Selected effects of chemical pollutants on vibrio pathogens of marine fish

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The rationale behind this project was the observation by Krongaard Kristensen that a high prevalence of Vibrios exists in marine recipients, which are polluted from carbohydrate processing plants, especially sugar factories.

Furthermore numerous reports implicate Vibrio anguillarum in disease problems in wild and farmed fish in marine environment.

During the last two years various locations - polluted and non polluted for reference - were studied. It was found that in cod there exists a special syndrome with skin ulcers with a typic cyclic course starting with papulae and vesiculae which develop in erosions and ulcers which finally may heal off. Apart from these ulcers also changes of another nature produced by mechanical lesions was found.

The typical syndrome was found in cods of the age 1½ - 2½ years and it had a varying high prevalence in polluted areas, while it was practically non existant in the reference areas. The syndrome also has its highest prevalence in late summer and authumn.

At bacterial examination of the affected cods a high prevalence of Vibrio anguillarum was found both in the ulcers and in the kidneys of affected cods. But furthermore other bacteria Aeromonas spp. etc. were found.

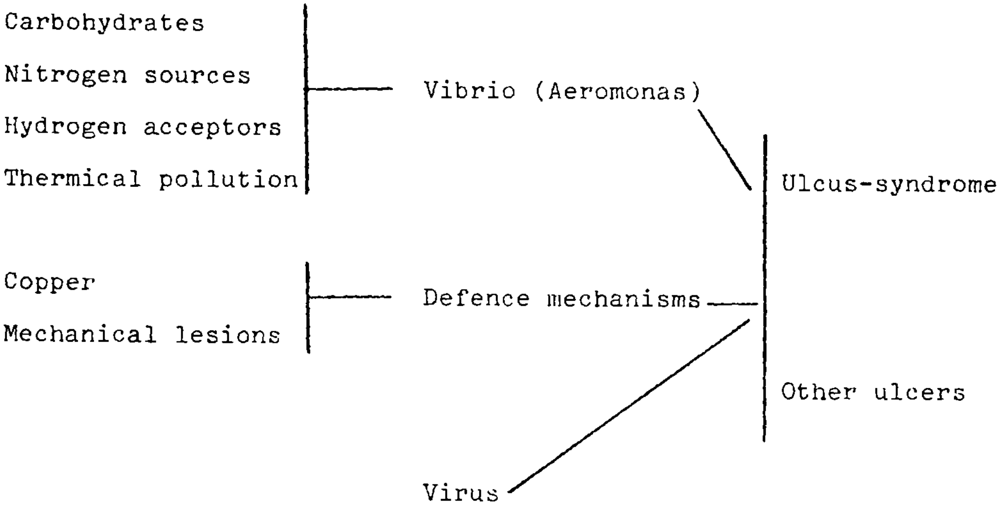
Therefore no simple correlation between the disease and Vibrio exists.

Concerning the prevalence of V.anguillarum there was a typical response to the outlet of carbohydrate in the marine recipients.

Vibrio anguillarum was not found in the mucus from cods in the reference locations and in feces of cod and in crabs the prevalence was lower than in polluted areas.

A considerable heterogeneity of the Vibrio anguillarum strains was found especially among those isolated from the environment.

Based upon the studies a working hypothesis was elaborated, which takes in consideration a complex nature of the syndrome with the following determinants.


Carbohydrates
Nitrogen sources
Hydrogen acceptors
Thermical pollution
Vibrio (Aeromonas)
Ulcus-syndrome
Copper
Mechanical lesions
Defence mechanisms
Other ulcers
Virus

TOPIC 19 : NOISE POLLUTION

Epidemiology

Contractor: University of Mainz

Contract n° 135 - 76 - 12 ENV D

Project leader: Barbara Griefahn

Title of project: Effects of noise on sleep and psychological performance.

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1. Objective of the research

Within a projected field study the hypothesis has to be proven that nightly occurring noises lead to a reduction of sleep, a decrease of psychological and physical performance, and finally to functional as well as organic diseases.

This pilot study deals with two main problems:

- though habituation takes place, noise affects sleep and performance even after years of exposure
- with increasing noise levels performance as well as sleep disturbances become greater

2. Method

2.0. The study consists of two parts; in both of them the following parameters are recorded:

1. physical parameters: noise level dB(A)
   temperature (max and min)
   real time
2. physiological parameters: 2 EOG
   1 EEG
   1 FPA (fingerpulse-amplid)
   1 MOT (motility)
3. psychological paramaters: 4-choice reaction time
   (10 minutes in the evening and in the morning)
   sleep questionnaire
   (4-choice and simple reaction time alternately during the laboratory work)

2.1. Field study

The sleep of subjects living in streets with high traffic density is recorded in their own flats during 12 consecutive nights each.

- subjects: 20 Ss (10 female, 10 male, 25-63 years), normal hearing, healthy
- experimental design:
  - control condition: nights 1-5, 11 and 12, sleep under normal conditions
  - experimental condition: nights 6-10

    noisy condition: open windows for 10 Ss (5 female, 5 male, 27-53 years) normally sleeping with windows closed

    quiet condition: earplugs for 10 Ss (5 female, 5 male, 25-63 years)
- recording time and recording place: may - october 1978
  Essen / FRG

2.2. Experimental study

The sleep of subjects sleeping in the laboratory is to be recorded during 12 consecutive nights each. Road traffic noise is applied by loudspeakers with 4 different intensities.

- subjects: 32 students (16 female, 16 male, 20-29 years), normal hearing, healthy
- experimental design:

	nights				
	01 02 03	04 05	06 07	08 09	10 11 12
8 Ss	A	C	B	D	A
8 Ss	B	D	A	C	B
8 Ss	C	A	D	B	C
8 Ss	D	B	C	A	D

A, B, C, D indicating different noise levels ranging from 34 to 65 dB(A) indoors.

- recording time and recording place: 22.1.79 until 8.6.79 (scheduled) in the sleep laboratory in Mainz

3. Analysis

The data analyzed so far are those from the field study.

- physical parameters: noise level (L_{eq}, L_1, L_{10}, L_{50}, L_{90}, L_{99})
  temperature (maximum and minimum)
- physiological parameters: the EEG/EOG recordings had been analyzed by an automatic hardware-system built by A. Kumar, University of Amsterdam. The output data had been used for a preliminary calculation. A more detailed analysis with smoothed data is not yet finished.
- psychological parameters: reaction time during the 4-choice test in milliseconds, sleep quality as judged by the subjects themselves, and the number of estimated awakenings

4. Results

The average values for noisy and quiet conditions had been calculated for each subject separately. on the basis of these data the Wilcoxon test had been carried out. The most significant results are presented in the following table:

N - Q	earplugs	windows open	female	male	20 Ss
L_{eq}	-	.01			
L_1	-	.01			
Total sleep time	.01 (+)	.01 (-)	-	-	-
Stages 1 & 2 (min)	.01 (-)	.01 (+)	-	-	-
Stage 1 (min)	.01 (-)	-	-	-	-
Stage 1 REM (min)	.05 (+)	-	-	-	-
Time awake (%)	-	.05 (-)	-	-	.05 (-)
Stages 1 & 2 (%)	-	.05 (+)	-	-	-
Sleep latency	.05 (+)	-	.05 (+)	-	.05 (+)
4ch I	.01 (+)	.01 (-)	-	-	-
4ch II	.01 (+)	.01 (-)	-	-	-
4ch I+II	.01 (+)	.01 (-)	-	-	-
Sleep quality	-	-	.05 (+)	-	-

(+): hypothesis confirmed (-): hypothesis to be rejected

5. Conclusions

The data analyzed so far indicate a great discrepancy between those subjects wearing earplugs and those sleeping with windows open in nights 5 - 10.
Regarding the results of the latter subgroup our hypothesis should be rejected (they sleep more in noisy nights and have a shorter reaction time), whereas the hypothesis seems to be confirmed for the subgroup wearing earplugs; those subjects have a significantly shorter reaction time and a longer sleep duration during the quiet period. But, summarizing both these results it has to be stated that the total sleep time increases with the duration of the test series. This has to be interpreted as an habituation effect to the experiment; the decreasing reaction time during the test series indicates a practice effect.

Nevertheless, the hypothesis is partly confirmed for the female subjects where - calculated for those sleeping with windows open and those sleeping with earplugs combined - the estimated sleep quality is significantly less in noisy nights. In addition (and this is also significant for all 20 subjects combined) sleep latency is significantly longer in nights with higher noise levels.This may cause the subjective feeling of a decreased sleep quality.

Though a second and more detailed analysis has to be carried out, the first analysis does not show an impairment of performance. Regarding this parameter it is supposed that sleep disturbances can be compensated for some time. Thus, it may be that the experimental condition (5 nights) is too short. Consequently, the projected investigation should include subjects living in streets with different noise levels. The range of noise levels should be as great as possible (what is naturally limited by social factors etc). Moreover, for measuring performance a test instrument less dependent on practice effects should be applied.

Contractor : Institut de Recherche des Transports - Centre d'Evaluation
et de Recherche des Nuisances, Bron, France
Contrac n° : 149-77-1 ENV F
Project Leader : Michel VALLET
Title of the project : Joint european study of the effects of noise on
sleep and psychological performance

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1 Objective of the research

It consists of studying the effects on sleep of long term of people living near an urban motorway. In all perception problems an habituation takes place, generally with a physiological or psychological cost.

If the effects of noise induces modification on the sleep pattern the study will allow to evaluate the acoustic level not to be exceeded to protect the welfare or the health of the population.

2 Method

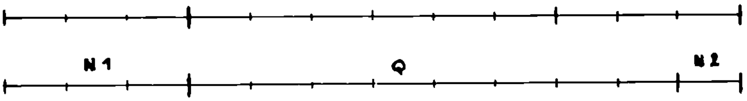
The differents associated teams have decided to carry out an "in situ" experimentation, and not in a laboratory where people don't sleep in normal conditions.

- Locality and subjects

Subjects are carefully selected (no sleep trouble, audiogram, psychological tests). Two subjects were recorded in Paris (circular road) 18 have been recorded in Lyon near A42 expressway (6 middle age, 12 over 50 years).
18 subjects are couples and are sleeping together. They are exposed to traffic noise for 3 or 5 years.

- Experimental Design

Subjects are recorded 12 consecutives nights, after 3 nights of adaptation to electrodes. Recording start with usual condition i.e noisy condition (N1) :
3 nights. They change of bed room (with the bed, light, temperature are controlled) to sleep in quiet condition (Q)
5 nights : (6 subjects have been recorded 8 nights in quiet condition as to observe a long recovery period). Subjects come back in noisy condition (N2) at the end of the period : 3 nights. Blocks start on Monday or Tuesday.



- Registration

2 EEG, A EOG, EMG, ECG
Simple reaction time ; subjective sleep quality
indoor noise level in dB(A) and for 30 nights in dB
temperature maximum and minimum.

- Analysis

EEG, Visual sleep scoring. A comparison with automatic systems has been tried (Cambridge and TNO-Amsterdam)

EEG. Mean heart rate by cardiotachometer
Noise : BK 4426 : L1, L10, L50, L90 Leq by 10 minutes periods
Reaction time : means of 2 blocks of 5 minutes.

3 - Results

12 nights for 10 subjects have been analysed, i.e. 120 nights.

Mean Noise levels : noisy conditions : Leq (recording period) : 39 to 47 dB(A)
quiet conditions : Leq (recording period) : 29 to 33 dB(A)
difference between noisy and quiet conditions : 9 to 19 dB(A)

Sleep pattern disturbance

- The total sleep time (TST) is reduced of 30 mn in noisy condition : 6 subjects out of 10.

  2 others subjects present no change, but the time past awakened decreases (10 to 20 mn)

  2 others subjects present an increased duration, but the timpe past awakened decreases (20 mn). See figures 1 and 2.
- The latency of the first sleep period is reduced in quiet condition (7ss out of 10).
- The latency of the first period of REM sleep (Dream period) is very shortened in quiet condition (8ss out of 10).

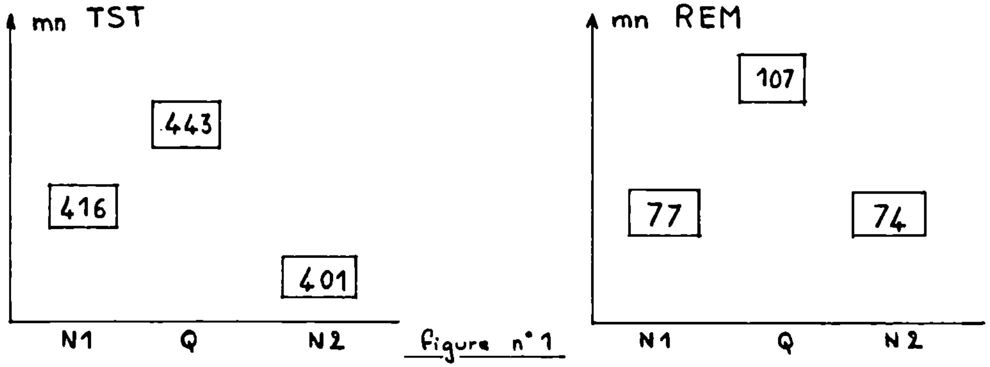


figure n°1

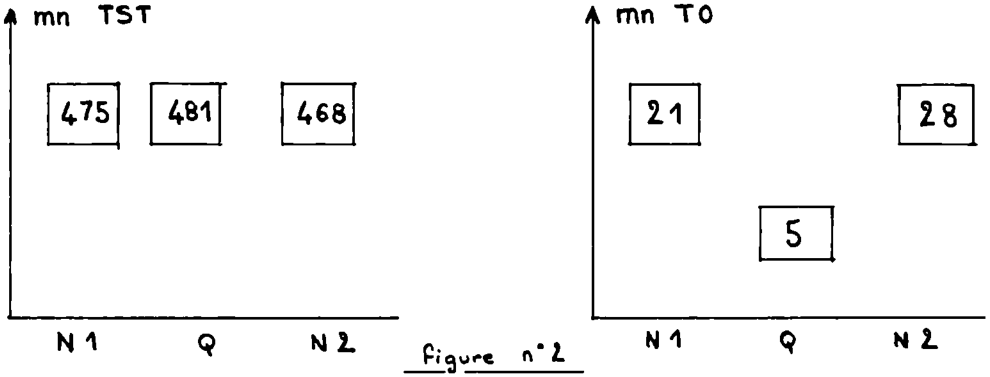


figure n°2

Heart Rate

Significant correlation appear between noise (L1, L50, Leq) and mean heart rate, calculated on 10 mn basis. There are no datas enough to conclude firmily.

Psychological assessment

In quiet condition the simple reaction time becomes shorter : 7 subjects out of 10.

The subjective assessment, by an after night questionnaire gives no significant difference between the two condition, because the initial trouble due to the change in place for bed-time.

4 - Conclusions

It is clear that the noise induces some more sleep disturbances, after five years of traffic noise exposure and that the adaptation is not achieved.

The sleep disturbance allows several electroencephalographic and vegetative signs. The datas'analysis must be specially careful, because if we sometimes find all the aspects still present in our subjects, the most part of them present only 1, 2 or 3 of theses disturbance symptons. One subject presents a remarkable adaptation.

The second phasis will be assign to a complete analysis of the whole collect datas and more particularly those which have been partly deal with : the noise effect on the heart rate and the analysis of the noise immediate effects on sleep.

Then, we will examine the relations between human responses and the noise index so as to suggest an index and an acoustic level which must not be exceeded in order to preserve a suitable sleep to the population.

These propositions to the legislator if the results are concluded, could concern as well a total index on the whole night period, a time during which a certain noise level must not be exceeded, or also a peak level.

Communication : Acoustics Congress 1979 Southampton - I.S.V.R.
Psychophysiological effects on sleep from motorway or aircraft noise.

Contractor : Gezondheidsorganisatie TNO on behalf of the
Instituut voor Milieuhygiëne en Gezondheidstechniek TNO
Delft, the Netherlands

Contract n° : 260-77-1 ENV N

Project leader : A.A. Jurriëns

Title of project : Effects of noise on sleep and psychological performance (joint European study)

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1. Objective of the research

To study physiological effects and psychological after-effects of traffic noise during sleep with people at home.

2. Materials and methods

2.1. General

Subjects in different age groups and of both sexes, having passed a screening on health aspects, were asked to sleep periods in a relatively noisy and in a relatively quiet condition. Each night acoustical and physiological signals were recorded and each morning and evening some psychological data were registered. From all registered material a number of variables was selected by the joint teams, which variables were analysed in terms of differences between condition averages.

For aspects of analysis and evaluation a subcontract was concluded with the Laboratory for Psychophysiology of the University of Amsterdam.

2.2. Situation

The subjects lived along the highway The Hague - Rotterdam, at Rotterdam and Delft. A change in noise condition was realized by fixing double glazing or (acoustically) opening windows.

2.3. Subjects

The subjects can be classified as follows.

	men	women	total
about 20 years	2	2	4
about 30 years	2	2	4
about 50 years	1	3	4
total	5	7	12

2.4. Scheme

Because of the large variation of the variables, to be expected within a condition, a scheme was preferred with relatively many nights per condition. An experimental period of 10 nights per condition was chosen, divided into blocks of 5 nights. For practical reasons incidentally more nights and/or more smaller blocks were employed. The subjects started with their normal condition and each block usually started at sundaynight. The distribution of subjects over condition sequences is as follows.

NOISY - QUIET : 5
QUIET - NOISY : 4
QUIET - NOISY - QUIET : 3

2.5. Registrations

acoustical : Indoor noise level in dB(A)

physiological : EEG, EOG, ECG, EMG, motility, respiration

psychological : reaction time, 10 minutes test in the morning
scores on subjective sleep quality and mood in the morning
score on well-being by day in the evening

2.6. Analysis

The EEG was, together with the EOG, analysed by an automatic sleep stage scoring system, developed at the Laboratory for Psychophysiology of the University of Amsterdam, monitored by oscilloscope and delta intensity graph. The final results were derived from a sleep pattern plot, obtained after software smoothing of the sleep stage scores and corrected with the aid of monitoring outcomes.

From the ECG were calculated the mean heart rate and coefficient of variability of the heart rate per night and per minute. The latter values were correlated with synchronous values of L_{eq} per minute and the number or percentage of nights with a positive correlation coefficient was used as a variable.

The reaction times each morning were analysed in 2 blocks of about 5 minutes and the sum and difference of the block means were the variables considered.

Finally, the subjective scores were expressed as a percentage of the maximum score.

3. Results

3.1. Summary

The following table summarizes the results in terms of the number of subjects with a positive and with a negative difference between the averages of the conditions noisy and quiet. Between brackets the number of significant cases is indicated.

	N	+	–
TOTAL SLEEP TIME	4	0	4(1)
SLEEP LATENCY	4	1(1)	3(1)
REM LATENCY	4	1	3
STAGES 1+2 (MIN)	4	0	4(2)
STAGES 1+2 (% TST)	4	0	4(2)
STAGE 3 (MIN)	4	4	0
STAGE 3 (% TST)	4	4(2)	0
STAGE 4 (MIN)	4	3	1
STAGE 4 (% TST)	4	3(2)	1
STAGES 3+4 (MIN)	4	4	0
STAGES 3+4 (% TST)	4	4(1)	0
STAGE REM (MIN)	4	1	3
STAGE REM (% TST)	4	3	1
STAGE W (MIN)	4	4	0
STAGE W (% TST)	4	4	0
BARYCENTRE REM	4	1	3
BARYCENTRE 4	4	2	2
CHANGES TO W	4	2	2
MEAN REACTION TIME :			
WHOLE TEST	11	7(1)	4(1)
2ND HALF - 1ST HALF	10	6	4
SUBJ. SLEEP QUALITY :			
11-ITEM SCALE	12	5	7
CONTINUOUS SCALE	12	3	9(1)
MOOD DISTURBANCE	12	7(4)	5(1)
WELL-BEING BY DAY	12	5	7(3)
HEART RATE :			
MEAN PER NIGHT H_m	4	3	1(1)
COEFF. VAR. $V_m = {}^{m}/H_m$	4	2	2
POS. CORR. H, L_{eq}	4	35(23)$^{1)}$	35(29)$^{1)}$
POS. CORR. V, L_{eq}	4	54(44)$^{1)2)}$	17(7)$^{1)2)}$

N : NUMBER OF SUBJECTS ANALYSED
+ : DIFFERENCE BETWEEN AVERAGES NOISY - QUIET POSITIVE
- : DIFFERENCE BETWEEN AVERAGES NOISY - QUIET NEGATIVE
1) : TOTAL NUMBER OF NIGHTS FOR ALL SUBJECTS AND BOTH CONDITIONS, HAVING A POSITIVE (+) OR NEGATIVE (-) CORRELATION COEFFICIENT
2) : DISTRIBUTION OVER SIGNS SIGNIFICANT

Where the total number of analysed subjects is large enough to test, no significant distribution over the two sign possibilities is present. However the with L_{eq} correlated heart rate variability does have a significant distribution of nights.

3.2. Discussion

Because only 4 subjects have been analysed so far (2 with a noisy - quiet scheme and 2 with a quiet - noisy scheme), drawing conclusions on EEG-variables becomes precarious. Further analysis was delayed awaiting a comparison of the automatic sleep stage scoring systems used by the teams. This comparison, with the aid of the same also visually scored nights and meant to investigate the possibility of an ultimate common analysis of all nights of the 4 teams, could reveal that each system shows different typical differences with visual scoring.

Realizing these reserves, the here presented results show, concerning the noisy condition, the following tendencies.

1. Less total sleep time.
2. Both in minutes and in percentage, more time in stages 3+4 and W, and less time in stages 1+2.

All 4 analysed subjects show a majority of nights, having a positive correlation coefficient between the coefficient of variability of the heart rate and L_{eq} per minute, added for both conditions. For 3 subjects this majority is significant.

Added for all subjects, as presented here, the distribution over positively and negatively correlated nights is significant too. Correlation of the coefficient of variability of the beats interval with the standard deviation of the noise level gives even more pronounced results.

There are indications that the results of the reaction time test are influenced by the condition sequence due to declining motivation during the experimental period. The supposition that looking at differences between the second half and the first half of the test would obviate influences like this, seems not to be confirmed.

Concerning all 4 subjective variables, most subjects show a deteriorating mean score during the noisy condition. Here too are indications that the condition sequence plays a role, except for mood disturbance.

4. Conclusions and additional comments

An extensive field study like this (240 nights) requires much effort to develop partly novel experimental procedures and analysing methods and to reach a sufficient common approach for all teams.
The obtained experience in these respects is an important result in itself. But as a consequence the for this project available time permitted only a limited exploitation of the vast amount of data. Nevertheless the here presented partial results contain interesting indications that this approach could adduce new evidence of the assumed detrimental effects of noise during sleep, necessary for establishing acceptable noise levels at night.
Tendencies were found that sleeping at home with traffic noise leads to less total sleep time, to shifts in EEG-based sleep patterns, to stimuli induced increases of heart rate variability, positively correlated with stimulus level, to deteriorated subjective ratings and possibly to deteriorated performance.

It is clear that this project should be continued, particularly to analyse the data extensively. This further analysis should cover :

- a completion of the results on the here selected all night variables for all subjects.
- an extension of all night results to other variables like characteristics of respiration and body motility.
- a mutual correlation of the most relevant of these physiological, psychological and acoustical variables within experimental periods.
- an examination of possible influences of for instance sex and age.
- a study of transient effects of noise on EEG, ECG, respiration and body motility, in relation to characteristics of noise levels, night time and characteristics of sleep pattern.
- a study of other EEE-descriptors per night or per sleep cycle like delta and spindle activity.

Contractor :	Medical Research Council, APU, Psychophysiology Section, Cambridge, U.K.
Contract n°	183 - 77-8 ENV UK
Project leader :	R.T. Wilkinson
Title of project :	Effects of a 10 dBA reduction in traffic noise on sleep, on subjective report, and on performance the next day

OBJECTIVE OF RESEARCH

This study is concerned with whether heavy traffic noise can impair people's sleep at night, and hence their performance and well-being the next day. The question has been examined by installing double glazing in the bedrooms of volunteer subjects and recording various physiological parameters which are thought to reflect the quality of sleep. In addition, performance tests and questionnaires about the quality of sleep are administered in the morning. For comparisons to be made these measures are taken before, during, and after the 2-week period of double glazing.

MATERIALS AND METHODS

Subjects. Fourteen subjects have been recorded so far. Nine of these records are presented here, 2 females and 2 males under 45 years of age, and 3 females and 2 males over 45. Of the 5 subjects not presented here, 2 were pilot runs not to be included in the main data, 1 gave too many faulty records, and 2 remain to be analysed.

Locality. The subjects were recorded in London. Typically, they lived alongside major roadways (North Circular Road and Western Avenue).

Conditions. Seventeen nights were recorded over a 5-week span. No data were recorded on weeks 2 and 4, which served as adaptation periods for a change of the experimental condition. Five nights in the first week were recorded in the normal noise condition. Double glazing insulation was then installed. On the third week (the "quiet" condition) 7 nights were recorded, and at the end the double glazing was removed. In the final, fifth week (the second "noise" condition) recording took place over 5 nights.

Physiological and noise recordings. These comprised the following channels: 2 EEG (C_3-RM; C_4-LM), 1 EOG, 1 ECG, and 1 signalled awakening.

Analyses. The automatic sleep analysis system consisted of a pre-processor (alpha, spindle, delta, abnormal, and REM detectors) and a minicomputer for the scoring of the sleep records. As well, the minicomputer tabulated noise levels expressed in dBA.

Noise. Both analogue and dBA noise levels were recorded.

Subjective tests. Two sleep questionnaires were employed: 1) The Stanford Sleepiness Scale (SSS) was administered before subjects went to bed and immediately upon awakening, and 2) a locally designed questionnaire designed to assess the quality of sleep, the quantity of dreaming, awakenings during the night, and sleep latency, was administered in the morning.

Performance tests. After the night's sleep, four performance tests were administered: 1) Unprepared Simple Reaction Time 2) Four Choice Serial Reaction Time 3) Auditory Short-Term Memory and 4) The Wilkinson Vigilance Test. The first three lasted 10 minutes each; the last 1 hour.

RESULTS

The analysis of the data for the presentation of the final report at the end of the first two years' contract will not be complete until the end of June, 1979. The present summary of the position must be regarded as an interim one and not necessarily indicative of what will emerge in the ultimate analysis.

The results are summarised in Table 1. In noisy conditions as compared with quiet (bedroom windows double glazed) the following significant results are apparent so far:

1) The number of minutes spent in Stage 4 sleep was reduced in 8 out of the 9 subjects (p = .04, Sign Test).
2) The start of the first Rapid Eye Movement Stage occurred earlier.
3) There were more minutes spent in Rapid Eye Movement sleep.
4) There were fewer minutes spent in "Wake" Stage sleep.
5) There were fewer changes to awakening.
6) Seven out of 8 subjects reacted more slowly in the Simple Reaction Time Test after the noisy nights (p = .07, Sign Test). The same is true for the Four Choice Reaction Time Test for 8 out of 9 subjects (p = .04).

CONCLUSIONS

Stage 4 sleep (predominance of high amplitude, low frequency, 'delta' waves in the EEG) is thought to represent a deep and useful form of sleep, first because arousal thresholds are high, and second, because this kind of sleep favours the production of growth hormone, necessary for body building. The increase in Stage 4 sleep following 10 dBA noise reduction produced by double glazing suggests that under normal noise levels these people were unable to take as much delta sleep as they required.

This may have reduced the recuperative power of their sleep, for there was a corresponding impairment of daytime performance when no double glazing was available: reaction time was slower, although short term memory was unimpaired.

The finding that Rapid Eye Movement sleep occurred earlier in the night, and was of greater duration on noisy nights than on quiet ones, suggests that this pattern of EEG and EOG physiology is a sign of disturbed sleep quality.

So far it has been possible to analyse only broad aspects of the data, averaging over subjects and noise episodes. A deeper analysis is projected in which more attention will be paid to individual patterns of sleep, and how they respond to noise. Also the effects of individual, transient traffic noises will be studied with particular reference to their immediate impact on sleeping physiology.

A further aspect of the analyses will be the correlation of concurrent trends in physiological, behavioural, and subjective measures as sleep varies from night to night within subjects. The aim will be to discover those physiological measures of sleep which relate to behavioural efficiency during the day and the subjective feeling of having slept well.

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Table 1. Differences between Noise and Quiet scores averaged over all subjects	Noise - Quiet	Wilcoxon	
		T	Sig.
Sleep physiology			
Total sleep time (min)	-14.5	11	NS
Sleep latency (min)	- 2.4	18	NS
REM latency (min)	-13.7	7	*
Sleep Stage 1 (min)	- 2.6	13	NS
Sleep Stage 1 (% total sleep time, TST)	- 1.0	10	NS
Sleep Stage 2 (min)	-12.0	11	NS
Sleep Stage 2 (% TST)	0.1	21	NS
Sleep Stage 3 (min)	- 2.3	17	NS
Sleep Stage 3 (% TST)	2.2	19	NS
Sleep Stage 4 (min)	- 1.9	9	*
Sleep Stage 4 (% TST)	0.4	19½	NS
Sleep Stage REM (min)	5.6	13	NS
Sleep Stage REM (% TST)	0.6	17½	NS
Wake Stage (min)	- 7.6	6	**
Wake Stage (% TST)	- 1.3	14	NS
Barycentre of REM (% sleep period elapsed)	- 1.0	22	NS
Barycentre of Stage 4 (% sleep period elapsed)	2.1	14	NS
Changes to wakening	- 0.7	5	**
Performance			
Simple Reaction Time (RT): Whole test (msec)	11.4	5	*
Simple Reaction Time: 2nd-1st half (msec)	4.2	7	NS
Four Choice RT: Whole test (msec)	13.4	2	***
Four Choice RT: 2nd-1st half (msec)	4.8	14½	NS
Vigilance: Correct Detections (5 subjects only)	- 2.7	3	NS
Vigilance: False Alarms (5 subjects only)	- 0.9	5	NS
Short Term Memory: Lines Correct	0.2	20	NS
Questionnaires			
Subjective sleep quality (% best ever)	- 6.3	10	NS
How you feel now (% as good as ever)	- 1.7	16	NS
Quantity of dreams (0=none; 7=most ever)	1.8	17	NS
Sleep latency (min)	- 1.8	19	NS
No. of wakenings	0.1	18	NS
Average period awake (min)	3.8	14	NS
Pre-sleep SSS rating (1=active; 7=almost asleep)	0.1	19	NS
Post-sleep SSS (1-7)	0.0	16½	NS
Noise			
Noise level in bedroom (dBA)	10.1	0	***

Note: Significance levels (two-tail) are shown as follows:
* p < .1; ** p < .05; *** p < .01; NS Not Significant

RESEARCH AREA 2 : ENVIRONMENTAL INFORMATION MANAGEMENT

TOPIC 21 : ECDIN PROJECT

Contractor: Bundesforschungsanstalt für Landwirtschaft
Institut für Bodenbiologie, Braunschweig

Contract No. 186-77-1 ENV D

Project leader: Professor Dr. K.H. Domsch

Title of Project: Effects of environmental chemicals on microorganisms.
Degradation of environmental chemicals by soil microorganisms

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Introduction

The question if pesticides can cause deleterious effects in the biosphere has been studied intensively for a number of decades. The present project " Effects of environmental chemicals on microorganisms " was set as a task to collect, register, and select the numerous and dispersed data of publications relating to the litosphere and to analyze their effects. The program was extended by uptake of a second project "Degradation of environmental chemicals by soil microorganisms " . Besides collection of single information, it was the objective to establish parameters for data analysis to allow published data to be treated under equal conditions. The input of data in the ECDIN data bank was the overall goal of the program. Therefore, the establishment of sets of parameters for data analysis was as well a prerequisite for the development of a suitable format for data sheets (see Table 1 and 2).

Methods and Results

The potential biological consequences which may exist by pesticide use are not clear, since it presents a problem to decide whether a pesticide can be grouped as 'critical' or not.

Before an attempt can be made to predict the harmful side-effects of a compound in a particular soil, the relative importance of various environmental conditions as well as the evaluation of the relative importance of expected effects must be assessed. The following sets of parameters were found to be suitable and were chosen for this research program:

I. Pesticide side-effects

a) Soil conditions:

Total carbon content, total nitrogen content, O_2-partial pressure, water regime, soil temperature.
Since natural environmental factors can influence microbial populations or metabolic functions in soil (= 'natural stress-effects'), it is necessary to have knowledge of the type of soil conditions existing before pesticide application.

b) Effects on microbial populations:

"no-effect concentration", indicates the highest dosage of pesticide tested (irrespective of the practical application rates) at which "no-effect" results were obtained.

"maximal depression", indicates the greatest difference between the treated and non-treated samples in experiments in which reversible side-effects were observed.

"maximal delay", indicates the maximum time-lag of test parameters of experiments in which reversible side-effects were observed.

"deficit", indicates the residual deficit (depression) if the test parameter in the treated sample had not reached the level of the non-treated sample at the end of the experiment.

c) Influence on soil metabolic functions:

CO_2-production, O_2-uptake, ammonification, nitrification, organic matter decomposition, soil enzymes.
Negative effects of pesticides should find their expression at least in one of those metabolic functions cited above. Finally,

the "ecological significance" of a side-effect is judged by comparing the magnitudes of depression or delay caused by a chemical to those caused by naturally occurring stress situations in the _in situ_ soil environment. In nature, maximum depressions of soil processes to the extent of 90 % are frequently observed. In view of this, depressions caused by chemicals should not be overevaluated. Considering the rather long doubling-time of microbial populations under natural soil conditions, a maximum delay of 30 days is considered as normal in the present evaluations. Up to 60 days delay is regarded as "tolerable" and more than 60 days is "critical".

The ecological significance of the "deficit" depends on the simultaneous evaluation of its magnitude and the total duration of the experiment; additional criteria are the amount and primary function of the chemical applied, and the role of the organism or process in the soil environment. In general, deficits of up to 15 % are regarded as negligible irrespective of their duration; deficits greater than 15 % are regarded as tolerable if it has not been proven that they last longer than 60 days, or if information from other sources ("No-effect" data) do not support the assumption of a significant influence; deficits lasting longer than 60 days, at a level of $\geq$ 50 %, are regarded as critical.

Microorganisms considered are bacteria (including rhizobia, other N-fixing bacteria, ammonifiers, nitrifiers), actinomycetes, fungi, alga, and protozoa. Only publications with data from work done under field or _in situ_ conditions were evaluated while side-effect studies under pure _in vitro_ conditions have not been taken up.

II. Pesticide degradation

In order to be able to characterize a pesticide as "critical" or not its decomposition rate is one of the most important aspects in determining its behavior in soil. Degradation of a compound may be immediate, slow, or incomplete with decomposition products which may be more hazardous than the original compound.

a) Experimental conditions:

soil type under field conditions, pesticide concentration applied, pH, temperature, O_2-regime, water regime of soil or culture fluid, additional carbon sources other than compound.

b) Single organisms or mixed populations with potential to actively degrade pesticides:

percentage degradation at a given time, metabolites produced. It is known that quite a number of microorganisms are able to break down pesticides in soil. Extracting data on degradation ability of microorganisms may lead to groups of organisms which may be most versatile. For degradation studies data from work done under field conditions, under in situ, and in vitro conditions have been taken up. Data from reviews have not been extracted for this purpose.

The literature of the past three decades was searched with respect to the topics and parameters cited above. From ca. 2000 publications so far registered, approximately 250 single pesticides were extracted and listed by their common names in order to avoid synonymy. Of those the following compounds have been analyzed by the parameters described above:

Aldicarb
Allylalcohol
Ametryne
Barban
Benomyl
Bentazon
Carbaryl
Dieldrin
Diuron
Ethylene oxide
Formaldehyde
Malathion
Methoxychlor

Paraquat
Paraquat-Dichloride
Parathion
Pentachlorophenol
Propham
and Thiram.

The results obtained were transferred onto data sheets (Table 1 and 2) and passed on to ECDIN.

The present work has been extended up to the end of 1980 and will be continued with a number of chemicals which are of particular interest.

SIDE-EFFECTS OF CHEMICALS ON SOIL MICRO-ORGANISMS

DEPT. OF SOIL BIOLOGY

Chemical:

Date:

Organisms (Popul. Function)	Test param.	Field/ Lab. Condit.	Time (Days)	Effects				Ecol. signif.	Chemical			Ref. No.
				Max. depr. %	Max. delay	Deficit %	No-eff. conc.		Orig. conc.	kg (a.i.)/ ha	Purity	

Table 1.

MICROBIAL DEGRADATION OF CHEMICALS

DEPT. OF SOIL BIOLOGY

Chemical:

Date:

Organisms	Field Pot Lab.	Time (Days)	Init. conc. a.i.	Intermed./Final conc. % Dissip./Degrad.	Metabolites	Experimental conditions						Ref. No.
						pH	Temp. °C	O_2 regime	Water regime	O.M. % Tot.-C% Tot.-N%	Nutr. source	

Table 2.

Contractor:	Technische Universität München Institut für organische Chemie
Contract no.:	310-77-5 ENV D
Project leader:	Professor Dr. Ivar K. Ugi
Title of project:	Prediction of environmental properties on the basis of a mathematical model of constitutional chemistry

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A mathematical model of constitutional chemistry has been developed by Ugi et al., which provides a vehicle for numerical coding and computer manipulation of chemical structures and reactions. In addition, the model defines fundamental algebraic properties of representations of chemical structures and reactions.

In the present work two major applications are being studied: correlation of substructures with properties ("effects") of chemical substances, and secondly, algorithms for representing radical reactions. Both these applications have great importance for environmental studies, both in classifying available information and in making predictions about the probable behaviour of "new" xenobiotics in the environment.

The radical reaction predictor program is an attempt to extend a previously developed program that handles non-radical "octet chemistry" reactions. The reaction sequence was split up into consecutive steps of initiation, propagation and termination. Each of these steps executes its own reaction operators and selection modules. The selection module of the initiation step is driven by tables of radical formation schemes vs. conditions. These tables were employed by generator modules for sum reactions in radical chemistry. While this approach was investigated and implemented, experience showed that although feasible, it would be inferior to a different method, which had been first applied to synthesis planning and which uses mechanistic steps, combined with evaluation procedures for instable (possibly hypothetical) intermediates instead of full reactions. The full reaction approach has consequently been given lower priority.

Major progress was achieved in the development of a substructure-activity correlation program. Substructures are defined as contiguous parts of molecules, that can be obtained by repeated removal of atoms and bonds. This is comparable to the concept of subgraphs in graph theory. An important type of queries to an information handling system is: "Which of the members of the data base have a certain substructure in common?" Combined with information about the members of a data base that exhibit a given effect, this provides knowledge about a potential correlation of substructures with a given effect. A more general type of query is: "Which substructure(s) are common to such members of the data base, that exhibit a given effect?" We call such a common "active" substructure an effector. Knowing effectors for certain effects enables to make predictions about effects exhibited by a less well investigated chemical structure. In general, however, there may be more than one effector known to exhibit an effect, and, more important, several effectors may be interdependent in either a synergistic or an antagonistic manner.

We have developed and implemented a data structure which allows to handle this type of queries. Molecules (parent structures) and substructures are represented in two separate parts: bond skeleton and atom vector. This structure not only economizes on storage requirement and execution time, but also allows queries with incompletely defined atom vectors.

Algorithms required for these queries involve extensive graph manipulations. We have split up the program system in two phases, one that does not require user interaction and executes fragmentation and generation of a linked fragment file, consisting of substructure descriptions and father-son pointers describing the paths of fragmentation ("contained-in"-pointers, "containing"-pointers). The remaining part of the program can thus be executed with response times acceptable for interactive use, and consists of programs that follow up or down the lines of pointers from given structures to common substructures or from substructures to parent structures.

The direction from parent to son is taken when looking up substructures that occur more frequently in molecules tagged with a given effect ("active" molecules) than in others.

An important feature of the retrieval system, which has been implemented in Pl/1 on IBM/360 type machines, are its statistical evaluation functions. In the process of following up the paths of substructure (father-son) pointers, for each substructure we compute the number of "active" parents that are linked to it. This value is first weighted with the total number of parents of this substructure. This number has been generated during the fragmentation process described above. These values are combined with the total number of "active" molecules and the total number of all molecules in the data base to yield a statistical merit value for each substructure of active molecules. Substructures with merit values between 0.4 and 1.0 are considered potential effectors, unless they contain or are contained in a substructure with a better merit value.

Ideally, the substructures thus obtained would be contained in all "active" molecules and in no "inactive" molecules. In terms of graph theory, these substructures would be roots of "minimal trees", the leaves of which are all the "active" molecules in the data base.

In the majority of practical cases, this minimal tree does not exist. By means of the statistical evaluation process as described, one or more trees are found, that are acceptable approximations of such (ideal) minimal trees.

With these tools at hand, we now are engaged in the development and implementation of algorithms that support queries involving interdependent effectors. It now appears that synergistic interdependencies can be handled by an extension of the independent retrieval operators, antagonistic interdependency seems to require a small intermediate data set of blocking effectors which then will have to be combined with the "minimal tree" for the positive effectors.

Publications

1.) W. Schubert, I. Ugi
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2.) I. Ugi, J. Brandt, J. Bauer, J. Friedrich, J. Gasteiger, C. Jochum and W. Schubert
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3.) J. Friedrich, I. Ugi
Informal Commun. Math. Chem., in press.

Contractor : Association pour la Recherche et le Développement en
Informatique Chimique (ARDIC)
25, rue Jussieu, 75005 - PARIS
Contract n° 286-77-1 ENV F
Project Leader : Prof. J.E. DUBOIS
Title of project : Progress in Applications of the DARC System to Structural Data Collected for the ECDIN Project

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The Environmental Chemical Data Information Network (ECDIN) project fits in the framework of research on the environment of the member countries of the European Economic Community and is being conducted in order to constitute an environmental chemicals data bank. Environmental chemicals are defined as products which man can introduce into the environment in sufficient amounts to be harmful to man, living beings and the environment.

A team from the Joint Research Center (JRC) in Ispra, Italy, was put in charge of accomplishing this project, whereas the EEC member countries were in charge of awarding research contracts to certain public or private institutions within the EEC in order to ensure the various data-collection and -processing tasks, as well as the development of suitable software (particularly for retrieval).

Thus, the Association pour la Recherche et le Développement en Informatique Chimique (ARDIC) was assigned the task of realizing software to be used in the experimental phase of the ECDIN project for the implementation of the structural data base (SDB) containing a description of chemical substances for which useful information has been gathered by the JRC in Ispra.

Therefore, a pilot SDB with 3419 chemical structures has been realized using different software based on the principles and concepts of the DARC* system.

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* DARC : Description, Acquisition, Retrieval, Computer-aided design.

This software essentially accomplishes the description, codification, interrogation and display of chemical compounds. It specifically authorizes the use of the chemist's natural language, i.e. the structural or sub-structural diagram of a molecule, to interrogate the chemical compound data bases, and thus requires absolutely no coding.

The 3419 chemical structures (originating from a CAS* file of 3520 substances) have been translated into DARC code by the same software used in creating the DARC structural reference data base (SRDB) from the CAS registry structure file (4.5 million structures from 1965 to 1978).

The CAS→DARC automatic translation software has been used to process all but a small number of structures (e.g. π-coordination compounds) which have purposely not been processed because CAS structure representation conventions do not lend themselves to application of the general interrogation software.

Structures can also be coded automatically by direct input on a graphical data acquisition device conceived by ARDIC engineers called the TOPOCODEUR. Currently, two TOPOCODEUR prototypes are in use for constituting data banks containing several thousands of chemical structures. The codes obtained by the two procedures (direct input or transcoding) are identical for a same structure. It is therefore possible to constitute unredundant files of chemical compounds.

Structural codes and structural access keys (SAK) are organized into a structural data base (SDB) according to a scheme adapted to chemical structures. The SDB can be interrogated in interactive mode regardless of its volume, which must be compatible with the computer resources in use. This interrogation is carried out using graphical or alphanumerical terminals connected to a network such as TRANSPAC or EURONET.

Question formulation and interpretation, and answer display, all rely on graphic software specifically developed to deal with chemical structures. In particular, structural diagram display is achieved by algorithmic calculation of atom coordinates from the DARC structural code.

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* CAS : Chemical Abstracts Service.

Realization of the SDB associated with the ECDIN pilot project has now been completed. The JRC team from Ispra in charge of the project has witnessed the DARC structural interrogation system in operation, both here in Paris on the CIDA's* PDP-11/35 computer and in Ispra on the JCR's IBM 370/155 computer where the pilot project is implemented. This system constitutes a specific tool which enables optimal use of the structural sets incorporated into the chemical data banks either in the field of bibliographic files or in the fields of pharmacodynamics, patent rights, physical properties,..., or environmental data, as demonstrated by its application in the ECDIN project.

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* CIDA : Centre d'Informatique et de Documentation Automatique.

Contractor: University of Pisa, Italy.
Contract no. 266-77-1 ENV I.
Project leader: A. Abbondandolo
Title of project: Collection and evaluation of data on mutagenic effects of environmental chemicals

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Objective of the research

A large variety of genetic systems is available at present for assessing the mutagenic activity of chemical substances. Different organisms, ranging from viruses to mammals, and genetic end points, extending from a single base-pair substitution in DNA to gross alterations of the whole genome, can be detected through the use of a number of *in vitro* or *in vivo* experimental procedures.

Such an heterogeneity in the quality of information demands a corresponding, articulated expression of existing data when the mutagenic activity of chemicals has to be documented.

The necessity of a comprehensive format for collecting the information on the various aspects of genotoxic activity of chemicals was recognized in the context of the ECDIN (Environmental Chemicals Data and Information Network) project for a data bank on environmental compounds. Objective of the present research was the development of such a format, together with a testing exercise of its correspondence to the aims of the ECDIN project, through a literature search on a limited number of chemical compounds.

Results

The general scheme developed for the collection of mutagenicity data is illustrated in Table I. The main features of the scheme are the following:

(a) The type of genetic effect (genetic end point) induced by the chemical is reported under the first column of the table. It seemed appropriate to give this information with priority, since "mutagenicity" is a broad term requiring specification.

(b) Since a different weight may be given to data obtained in different test systems, the information on the organism and genetic system used for assessing the genetic effect is also reported.

(c) An effort was made to establish a hierarchy to which conform for the enumeration of data pertaining to the various test systems. This classification concerns both the type of genetic effect and the test organisms, roughly arranged in order of increasing evolutionary complexity from viruses to mammals.

(d) Under the results, the qualitative information on the presence or absence of mutagenicity is given first, by the symbols + or -. Quantitative data follow in a separate column.

The tables containing the detailed data on mutation experiments are completed by a summary and a list of references.

Summaries are structured in a form easy to read and their purpose is to give a straightforward answer to the questions "is the substance mutagenic" and "in which test system was mutagenicity assessed or disproved".

With the aim to test the format illustrated above, mutagenicity data on the following chemical substances were collected and arrenged according to the proposed scheme:

Compound	C.A.S. Reg.No.	No. of references
1. Cyclophosphamide	50-18-0	84
2. Procarbazine	671-16-9	9
3. Mitomycin c	50-07-7	55
4. N-nitrosodiethylamine	55-18-5	28
5. Vinyl chloride	75-01-4	25
6. Aldrin	309-00-2	5
7. Dieldrin	60-57-1	12
8. Endrin	72-20-8	2
9. Telodrin	297-78-9	0
Mercury compounds:		
10. Butyl mercuric bromide		
11. Ethyl mercuric chloride	107-27-7	
12. Ethyl mercuric hydroxide		
13. Hexyl mercuric bromide		
14. Mercuric bichloride	747-94-7	

15. Mercuric chloride	7487-94-7	
16. Mercuric nitrate	10045-94-0	
17. Mercury	7439-97-6	
18. Mercury orange		
19. Methoxyethyl mercuric chloride	000123-88-6	
20. Methyl mercury chloride	124-38-9	
21. Methyl mercuric dicyandiamide		
22. Methyl mercuric hydroxide	1184-57-2	
23. Phenyl mercuric acetate	62-38-4	
24. Phenyl mercuric hydroxide	000100-57-2	
Total mercury compounds		19

Cadmium compounds:

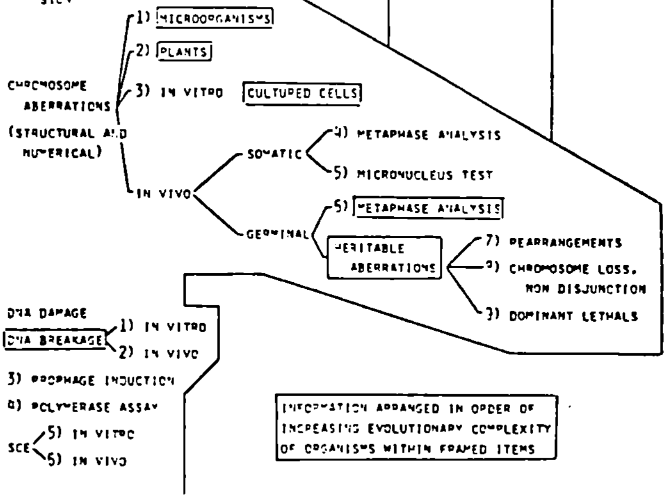
25. Cadmium	7440-43-9	
26. Cadmium acetate	543-90-8	
27. Cadmium bromide	7789-42-6	
28. Cadmium chloride	10108-64-2	
29. Cadmium iodide	7790-80-9	
30. Cadmium nitrate	10325-94-7	
31. Cadmium sulfate	10124-36-4	
Total Cadmium compounds		30

For all compounds, the basic source of literature information was the list of references provided by EMIC (Environmental Mutagens Information Center, Oak Ridge), whose collaboration is greately acknowledged.

TABLE 1

GENERAL SCHEME FOR THE COLLECTION OF MUTAGENICITY DATA

GENETIC END POINT	ORGANISM AND GENETIC SYSTEM	EXPERIMENTAL CONDITIONS	RESULTS		REFERENCES
			QUALITATIVE	QUANTITATIVE	
1) POINT MUTATION 2) RECOMBINATION 3) CHROMOSOME ABERRATIONS 4) DNA DAMAGE 5) OTHER RELATED EFFECTS	ORGANISM: 1) SPECIES 2) STRAIN GENETIC SYSTEM: POINT MUTATION E.G.: - FORWARD AND REVERSE MUTATIONS IN MICROORGANISMS; - PETITES; - SEX-LINKED RECESSIVE LETHALS; - SPECIFIC LOCUS TEST RECOMBINATION E.G.: - MITOTIC CROSSING-OVER; - MITOTIC GENE CONVERSION	1) IN VITRO ± METABOLIC ACTIVATION; IN VIVO 2) DOSES: IN VITRO, mM OR μMOLES / PLATE; IN VIVO, mg/Kg 3) PURITY OF CHEMICAL, SOLVENTS, EMULSIFIERS 4) OTHER METHODOLOGICAL DETAILS (DOSES: SINGLE OR FRACTIONATED; ROUTE OF ADMINISTRATION; SAMPLING OF GERM CELLS; SAMPLE SIZE, ETC.)	1) EFFECTIVE OR NOT EFFECTIVE 2) DOSE-RESPONSE RELATIONSHIP 3) INFLUENCE OF EXPERIMENTAL CONDITIONS	1) INDUCED FREQUENCY 2) KINETICS OF INDUCTION 3) LEC OR LED (LOWEST EFFECTIVE CONCENTRATION OR DOSE)	INDICATED BY NUMBERS



INFORMATION ARRANGED IN ORDER OF INCREASING EVOLUTIONARY COMPLEXITY OF ORGANISMS WITHIN FRAMED ITEMS

Contractor:	The Chemical Society
Contract no:	163-77-1 ENV UK
Project Leader:	J. E. Blackmore
Title of Project:	Development and enhancement of the Environmental Chemical Data and Information Network exchange format, and pilot testing of this format. Supply of compound identification data.

Objective of the Research.

1. To maintain and develop the computer file format, suitable for the exchange in machine readable form of data on chemical compounds, already developed for ECDIN under contract 092-74-5 ENV UK. The developments of the format should allow for the handling of all the present data elements covered by the present ECDIN list of Attributes and all modifications and extensions required by ECDIN in the future.
2. To test the format by:
   - (i) the supply of certain data on compounds specified by ECDIN to the network in exchange format.
   - (ii) by developing software to convert up to three other machine-readable files into exchange format to facilitate the acquisition of data in acceptable form.
   - (iii) by converting data from other UK contractors into exchange format and supplying the data to the network.
3. To supply in exchange format, CAS Systematic Nomenclature and other data for up to 1000 compounds including those for the data in 2(iii) to be commonly agreed. This information will be obtained by making use of the Chemical Abstracts Service Registry System.

Full details of the objectives may be found in Ref. 1.

Materials and Methods.

1. A study on the problems of standardisation of data content during file interconversions through the exchange format was made. This was to determine whether it was feasible, in addition to the exchange format design criteria of
   - (i) during file conversion, no information may be lost or degraded,
   - (ii) unique and unambigous descriptions for each compound and data element,
   - (iii) that the exchange format require the minimum of effort on the part of the users of the file,

it was possible to design a general solution which allowed standardisation of the possibly different data content in a given data element in several files. A general study as well as studies of specific data elements were made.

2. A study of possible bibliographic description standards that might be used in the ECDIN file in order to improve consistency of citation was undertaken.
3. An update in Standard File Format (SFF) of the CAS Registry data supplied under contract 092-74-5 ENV UK (the ECDIN-I file) was obtained which contained alterations to the records for 3006 out of the 3516 compounds including some additional data elements. Modifications to the CAS SFF-to-exchange and exchange-to-ECDIN input format programs were made to accommodate the new data elements.
4. The ECDIN-I file data and the file of European Customs Union List (GUD) (ECDIN-II) data were merged.
5. A study of the computing efficiency of the exchange-to-ECDIN format conversion suite following its use in the processing of CAS data was made.
6. Software was developed to convert CAS data in Standard Distribution Format (SDF) to exchange format.

Results.

1. A report on the study of standardisation of data content was produced (Ref. 2) which concluded that standardisation was not possible in general terms as each individual had its own characteristics. For standardisation to take place it was therefore necessary to study each data element from each network file on an individual basis.
2. A report on bibliographic standards was produced (Ref. 3) which listed some of the most common standards and recommended that the UNISIST Standard be adopted.
3. The update to the ECDIN-I file was merged with the original file and the resultant file was processed through the revised CAS SFF-to-exchange and exchange-to-ECDIN input format programs. The file now has over 30,000 names for the 3500 compounds.
4. After merging the ECDIN-I and GUD (ECDIN-II) files printouts ordered by ECDIN Number and by GUD Number were produced. The file contains data on 9209 compounds for which 489 compounds have not been assigned CAS Registry Numbers.
5. The study of the exchange-to-ECDIN input format conversion suite concluded that the efficiency of the program could be improved if it was split into four programs. The new programs, when written were 90% more efficient.
6. The programs for CAS SDF-to-exchange format conversion were written and successfully tested.

Conclusions and other comments.

1. During the contract period ECDIN have been changing their data base management from SIMAS to ADABAS. The implementation of ADABAS will mean that new programs from exchange-to-input format will need to be written. It has not been possible to proceed with these as specifications for the new files have not been received from ECDIN. The contract has therefore been extended until December 31st 1979.
2. A paper on the work performed under the previous contract detailing the development of the exchange format was written and published (ref. 4).
3. The objectives defined above have been met, with the exception to 1 as noted in 1 above, 2(ii) where only one other file has been converted, and 2(iii) where no data has been received for testing.

References.

1. Contract between the European Economic Community and the Chemical Society 163-77-1 ENV UK.
2. Problems of Standardisation of Data Content during file Interconversions through the Exchange Format in the ECDIN Network by David J. Proctor and Alan Robson. Ref E2. 771000/1.
3. Bibliographic Descriptions by Alan Robson. Ref E2. 781100/1.
4. Development of an Exchange format for the European Environmental Chemical Data and Information Network (ECDIN) by David J. Proctor, Alan Robson, Margaret A. Veal, J. Howard Petrie and William G. Town. Information Processing and Management Vol. 14 pp 429-443.

Contractor : Imperial Chemical Industries Ltd, Brixham Laboratory
Contract No : 180-77-1 ENV UK
Project Leader : Mr C R Pearson
Title of Project : Assessment of data on the distribution and effects of chemicals in the Aquatic Environment

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The objective of the Research was to assemble references from the literature on effects of chemicals in the aquatic environment, to extract relevant data, to index and cross reference it, and to submit it for storage in ECDIN in agreed formats. The initial compounds were those on the ECDIN priority list of 100 ; but excluding PCB, DDT and metal compounds.

This work would be carried out in co-operation with ECDIN at Ispra, and with the other input contractors, notably MBA Plymouth. For this reason, the professional staff involved have attended 5 full meetings of the joint team, in addition to several with MBA only.

Formats have been agreed for Tabular Presentation of data on effects on aquatic organisms and on accumulation, metabolism and excretion in aquatic systems. Data in these fields have been presented for 40 compounds.

Formats are being prepared for Biodegradation, Abiotic Degradation, and effect on Sewage Treatment Processes.

References : Brixham Laboratory Reports Nos : BL/B/1919
BL/B/1926

Contractor : Natural Environment Research Council - Marine Biological Association of the United Kingdom, Citadel Hill, Plymouth PL1 2PB, Devon

Contract n° : 181-77-1 ENV UK

Project Leader : Allen Varley

Title of project : Contribution to the ECDIN pilot study - Elaboration and testing of a draft format for ecological data, and collection of data on fate and effects of chemicals in the marine and estuarine environment.

Objective of the research

The work was concerned with the collection and presentation of information and data on the fate and effects of chemical substances in the aquatic environment. The objectives were (a) to design and test formats in which the data could be tabulated in a structured and standardised manner, and (b) to collect data from the scientific literature on particular compounds in the marine and estuarine environments, and to structure and submit the data in the agreed formats to the Commission for computer processing.

Materials and methods

The resources of the Marine Biological Association of the United Kingdom (MBA) Library and its Marine Pollution Information Centre were used for access to the scientific and technical literature. The MBA Library contains one of the world'd major reference collections in marine biology and ecology, oceanography, fisheries and related subjects; the Marine Pollution Information Centre was established by the Natural Environment Research Council in 1970 and since that date has made every effort to collect and index the world literature of marine and estuarine pollution, providing specialised information services. A particular strength of the MBA is in its access to the non-conventional, technical and report literature which is not widely distributed and which is not adequately covered and indexed by the major abstracting journals and information systems.

The scientific literature was reviewed, international criteria documents were examined, and experts consulted, to determine the factors which should be included in tabulations and presentations of data and information relevant to the marine and estuarine environments for the following ECDIN fields:

Dispersion and Transformation

- Dispersion pathways
- Abiotic degradation
- Biological absorption, metabolism and excretion
- Biological retention and accumulation

Effects

- Effects on aquatic organisms
- Effects on reproduction including teratogenicity
- Carcinogenicity
- Mutagenicity
- Effects on aquatic plants
- Effects on aquatic microorganisms
- Effects on ecosystems

Information and data was collected from the literature on five representative compounds (Ethylene glycol, Formaldehyde, Hexachlorobenzene, Malathion, and NTA). Data for each field was set out in a variety of ways, illustrating possible methods of presentation. Following discussions with Commission staff and with the freshwater data contractor, and bearing in mind the needs of potential users, factors and draft tables were agreed, and it was recommended that as little free text as possible should be used. A programme of data and information collection on compounds on the Hundred Compound Priority List was then put into operation.

For each chemical compound the literature was searched extensively and thoroughly, and relevant data and information was extracted. The resources of the MBA were used, together with the major and specialised abstracting journals and services. A computer terminal was used to undertake on-line interactive searches of computerised bibliographic data bases, current literature was monitored to ensure that the most recent and most up to date information was captured, and documents not available locally were purchased or borrowed. The MBA's previous experience that treatment of the marine sciences by the large bibliographic data bases is inadequate was confirmed. In general these systems cover less than half of the relevant literature; however they were useful as a check to ensure that comprehensive coverage was achieved, and a number of new references were retrieved.

No problems were experienced in collecting the bibliographic information and in extracting the data from the literature. The formatting and tabulation of data on effects (ECDIN Category 9) presented no major problems once the initial research had been completed, though some difficulties were experienced in devising and developing suitable formats for dispersion and transformation data (ECDIN Category 8). Some re-structuring of certain fields was found necessary.

Results

Suitable formats were successfully designed and tested, and used for submission to the Commission of tabulated and standardised data and information on 43 compounds from the Hundred Compound Priority List (see Appendix 1). Despite detailed and extensive searches of the literature, no relevant information was found for 11 compounds (see Appendix 1). This was a smaller proportion than had been anticipated. For some subject fields there was little or no information relating to the marine and estuarine environments (i.e. Dispersion pathways, Abiotic degradation, Carcinogenicity, Mutagenicity, Effects on ecosystems). A comparison of sources of freshwater and marine/estuarine information on the compounds Aldrin, Dieldrin and Endrin showed that overlap is minimal, and confirmed that normally scientific papers deal with either the freshwater environment, or with the marine/estuarine environment, but seldom with both.

For the Dispersion Pathways field it was not possible to tabulate the information and data, and this had to be expressed in the form of a free text summary. Because of the large amounts of data on levels of certain pollutants in the environment, it was agreed that time would not be devoted to this, and data was extracted and submitted on only two compounds – Aldrin and Lindane.

The presentation of data on metals necessitated considerable research because they do not degrade, and some modifications and changes to formats originally designed were found necessary. Particular problems identified and examined included the representation of the thermodynamic activities of compounds; the accumulation of pollutants on to and into food before uptake by organisms; and the behaviour of metals. Topics identified for further study, or upon which preliminary studies were undertaken included methods of summarising and condensing data; biomagnification in a food chain; updating; standardisation of terminology, and taxonomic and geographic indexing.

Conclusions

Surveying the scientific literature was time-consuming, but there are no short-cuts because experience and comparative studies confirmed that environmental data concerning seas and estuaries is not covered adequately by existing data banks and compilations, or by bibliographic information systems and abstracting services. A cause for concern is that many of the international and national criteria documents are inadequate, and at times inaccurate, in their treatment of the marine and estuarine environments. For the majority of potentially hazardous compounds there are gaps in knowledge, and little or no basic scientific research appears to have been undertaken, for example on carcinogenicity, mutagenicity, and effects on ecosystems.

Regular feedback was received from ECDIN staff, and contact was maintained with other ECDIN contractors and consultants. Frequent discussions with members of the MBA Plymouth Laboratory scientific staff, and in particular the advice of the marine chemists and biochemists proved valuable, and illustrated the advantages of undertaking the work in a marine scientific research establishment.

Appendix 1

Compounds for which data and information was submitted

Acetic acid
Acetone
Acetophenone
Acrylonitrile
Aldrin
Allyl alcohol
2-Amino ethanol
Aniline
Anthracene
Arasan
Benzene
Benzidine
Benzoic acid
Benzo(a)pyrene
1-Butanol
Butyl cellosolve
Butyraldehyde
Carbaryl
Carbon tetrachloride
Chlorobenzene
Chlorophenol
m-Cresol
Cyclohexane
Cyclohexanol
Cyclohexanone
o-Dichlorobenzene
1,1-Dichloroethane
1,2-Dichloroethane
Dichloromethyl ether
(2,4-Dichlorophenoxy)acetic acid
Dieldrin
Dimethyl sulphate
Dioxane
Diuron
Ethanol
Ethylene glycol
Fenchlorphos
Formaldehyde
Hexachlorobenzene
Lindane
Malathion
NTA
Paraquat

Compounds for which no relevant data and information was found

Allyl chloride
m-Aminophenol
Bis(2-chloroethyl)ether
1,3-Butadiene
2-Butanone
n-Butyl benzoate
m-Chloroaniline
4-Chloro-1,2-dinitrobenzene
Di-allate
Diethylamine
Difluorodichloromethane

Contractor :	Stanford Research Institute, Croydon, UK
Contract n° :	259-77-1 ENV UK
Project leader :	V. von Schuller-Götzburg
Title of project :	Investigation and analysis of data gathering methodology and collection of chemical economics data for ECDIN

OBJECTIVE OF THE RESEARCH

The aim of the project carried out by SRI International under the above-mentioned contract has been:

1. To investigate the problems that must be solved when expanding the data base of the Environmental Chemicals Data and Information Network of the European Communities (ECDIN) by supplying economics data on additional chemicals, using a structured approach to facilitate the collecting effort and to meet the basic requirements of task and problem oriented use of the data;

2. To collect such economics data for two groups of compounds that are determined by different selection criteria, such as a substituent (chlorinated organic chemicals), and a chemical relationship (aromatics).

The results of the research were intended to provide the Environmental Chemicals Data and Information Network of the European Communities (ECDIN) with additional information on the feasibility of and problems connected with a systematic expansion of its data base, and to supply it with data such as production, consumption, uses and bulk displacement for the chemicals described above. Considering the financial limitations of the project, the main emphasis was to be laid on those compounds that are of major interest from the environmental point of view.

MATERIALS AND METHODS

The project work carried out under the above-mentioned contract consisted of two parallel research efforts:

1. A forward tracing effort on chlorinated organic chemicals, carried out in order to provide ECDIN with another practical example for this type of basic approach, in addition to supplying data on chemical compounds of general environmental interest.

2. The collection of data on production processes, producers, capacities (if feasible) production, consumption, uses, and bulk displacement for a set of compounds pertaining to the benzene, toluene and xylene derivatives.

Each of the two parallel research efforts essentially consisted of two major phases:

1. The identification and evaluation of the existing data, the selection of the most appropriate data sources, and the decision on the most effective way of using these data sources in order to obtain the best possible results.

2. The preparation and verification of the data on the different compounds to be incorporated into the final report.

The first phase was initiated by screening the data bases and other pertinent sources of information developed by and available to SRI. Among others, information from several continuing multiclient SRI programs, such as the Chemical Economics Handbook Program, the Process Economics Program, the World Petrochemicals Program etc. was used to identify the pertinent data available, check their quality, and evaluate their usefulness for the project. It soon became evident that practically no official or other published data on most of the chemicals covered by the project were available. It, therefore, was necessary to try to obtain the data required for the study from the producer or user companies. For various reasons, this was a very difficult and time-consuming endeavor that considerably delayed the project work. After repeated contacts and discussions with trade associations and individual companies, a mutually agreeable solution was found to the problem. For those chemicals, on which no data were available from publications or from industry, SRI experts are preparing qualified estimates.

In the second phase, the best data or estimates available for each chemical compound covered by the project are being compiled, and verified where this is possible. These data will be incorporated into the final report in the form of small dossiers for each individual product.

RESULTS

The results of the research project will be the data on the two groups of chemicals covered by the project that are being developed and provided to ECDIN in the form of small dossiers by SRI, and that will be incorporated into the ECDIN data bank; and the systematic approach that was developed by SRI to prepare and evaluate these data and to organize the dossiers. No publications have been prepared by SRI within this contract.

CONCLUSIONS AND ADDITIONAL COMMENTS

The problems and difficulties that SRI has encountered when developing the data for this project have shown clearly that there is quite a concern in industry that the information it supplies to environmental data banks might be used against it. SRI's experience with this project leads to the conclusions that:

a. more extensive efforts will be required in the years to come to develop techno-economic data on chemicals that are not published, nor easily available.

b. a clarification by the authorities concerned about the purpose, future use and limitations of environmental data banks might dissipate some of industry's fears, and thus, increase its willingness to cooperate in these research efforts.

Contractor: The United Kingdom Atomic Energy Authority, Atomic Energy Research Establishment

Contract No: 296-77-3 ENV K

Project Leader: Mr EE Finnecy

Title of project: Contribution to the ECDIN pilot project - Elaboration and testing of a draft format for data on waste disposal.

Objectives

Phase 1. Harwell is required to draw up a draft format for information on disposal of wastes from manufacture and use of chemicals. The format will specify phases of manufacturing processes from which wastes arise, their composition and environmental target.

Phase 2. Waste disposal data are to be collated for a number of chosen compounds. Choice should take into account their importance related to production and use, degree of hazard and environmental impact.

All stages of the work require collaboration and agreement with the Commission.

Method and Rationale

Phase 1. The SIMAS Format

Ispra proposed a provisional wastes disposal input format as a field in the existing USE (Use and Disposal) category of the ten main categories of the ECDIN data bank.

It was apparent on considering this draft format that some of the most important sources of environmentally significant compounds would be missed. Up to this time ECDIN dealt solely with the production of pure chemicals. Environmentally significant wastes may arise from pure chemicals production but may also arise during the manufacture of other products in what we have called "secondary products industries" eg coke, steel, leather, petrochemicals industries. Thus phenol is introduced into the environment as a waste in significant quantities during coke production and by the oil refining industry and not significantly by the manufacture of phenol.

Thus wastes from all industries not making pure chemicals, eg service industries, need to be considered. These were incorporated into a new category called the "Waste Management Information Category". The category was divided into appropriate fields and subfields and the case for their inclusion presented. The new format was approved by Ispra (1).

ADABAS Format Revision

A meeting was convened at Ispra to discuss the changes required by their implementation of the new ADABAS retrieval system (3). Our proposed format was impossible to reconcile with the ADABAS system and the basis for a new format was then worked out and agreed.

Harwell subsequently modified and extended this format, within the ADABAS constraints, so that it could answer typical questions on wastes management. We analysed over 1300 such questions asked of our Wastes Management Information Bureau, the national advisory centre for the UK. We also analysed 264 questions listed in a study for the Commission on potential user requirements for ECDIN (5), 7% of questions being on wastes disposal. These questions were concerned with the types of waste related to a particular industrial category; the disposal method and consequent waste environmental location related to specific industrial wastes and vice versa.

We have stored information in a "Waste Description File" and a "Waste Treatment File". The type of information stored is indicated in Figs. 1 and 2. Many terms are written in full but in practice will appear as accession numbers. Dictionaries with related accession numbers have been compiled for various terms eg treatment processes. Nodes of contact provide means of correspondence between these and other ECDIN files. The nodes of contact include waste, waste class and standard industrial classification numbers.

Waste Description File

The waste must be described sufficiently precisely to determine its treatment. The simplest case is for a given concentration of a single waste of a specified physical and chemical form. However for a complex waste stream, treatment and disposal will be further influenced by the presence of other components. Other useful data are indicated in Figs. 1 and 2.

Waste Treatment File

Again choice of disposal method may be simple, or it may be relatively complex requiring a sequence of several different treatments prior to ultimate disposal, each producing its own waste stream. Each entry in the file is distinguished by a treatment process sequence numbered sequentially, and including the ultimate disposal method and recovery options. Output wastes from each stage are identified by an output waste number preceded by the treatment sequence number. The output wastes are also entered in the Waste Description File. Assessment of the environmental impact of the wastes is aided by data on wastes quantity, composition, transport and final place of disposal.

Phase 2. Selection criteria for Environmentally Significant Compounds for Further Study

ECDIN has no stated rationale for the selection of chemicals of the greatest environmental significance (4). Therefore we thought it would be profitable to derive a simple decision logic for quick selection.

Selection criteria were based on an assessment of environmental harm caused by any chemical. This may be estimated by how much is released to the environment, how widespread the release and how intense the reaction on the target system. These criteria were quantified and used to examine available data on several hundreds of compounds, including a provisional list supplied by Ispra. 140 compounds were selected using these criteria and submitted to Ispra (2).

At this point Harwell were asked to consider environmentally significant wastes rather than environmentally significant chemicals. This was due to the change over from SIMAS to ADABAS and ECDIN's progression to the concept of an information network making greater use of existing data banks. We have now selected a list of environmentally significant wastes and are awaiting their approval by Ispra.

Conclusions We believe that other industries apart from those producing pure chemicals should be considered by ECDIN as many of their wastes are environmentally important.

We have examined potential questions and uses of ECDIN related to wastes management to aid satisfactory format compilation. Two formats for waste disposal have had to be prepared suited to SIMAS and ADABAS respectively.

We have identified criteria for the selection of environmentally significant chemicals and wastes and have selected 140 significant chemicals.

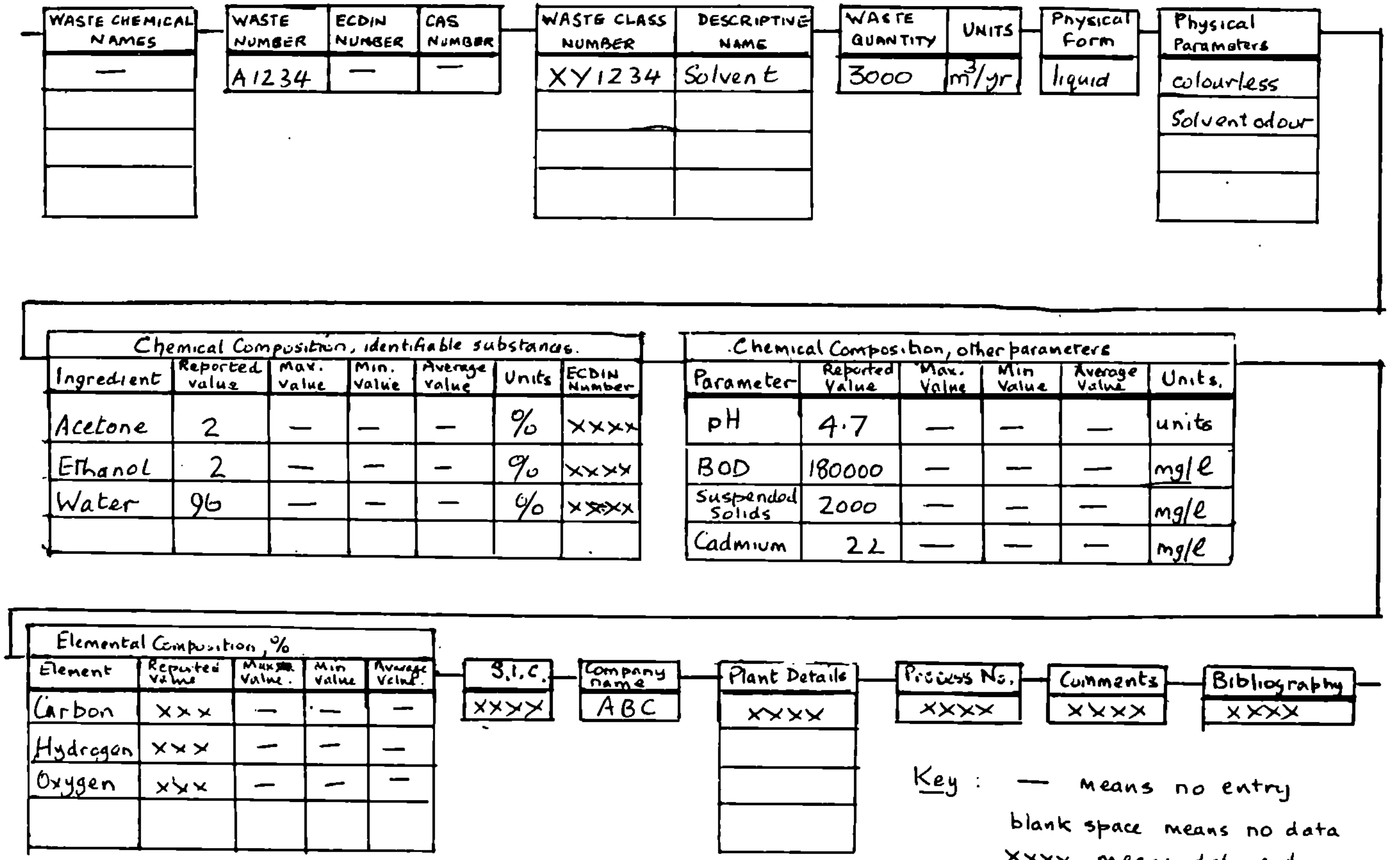
Comments We have applied for an extension to our contract time due to the delay and complications caused by the change over to the ADABAS programme. We are awaiting approval by Ispra of the ADABAS format and list of wastes.

References

1. 1st Periodic Report, 16th December 1977, (submitted to Ispra).
2. 2nd Periodic Report, March 1978, (submitted to Ispra).
3. 3rd Periodic Report, November 1978, (submitted to Brussels).
4. Environmental Chemicals Data Information Network - Progress Report, (July 1976).
5. Bletchely AD, Report on a Study on the Apparent Needs of Possible Customers in the United Kingdom for an Operational ECDIN, European Economic Communities, (October 1978).

FIG. 1. WASTE DESCRIPTION FILE

(Simplified and Indicative)



WASTE CHEMICAL NAMES
—

WASTE NUMBER	ECDIN NUMBER	CAS NUMBER
A1234	—	—

WASTE CLASS NUMBER	DESCRIPTIVE NAME
XY1234	Solvent

WASTE QUANTITY	UNITS
3000	m^3/yr

Physical Form
liquid

Physical Parameters
colourless
Solvent odour

Chemical Composition, identifiable substances.						
Ingredient	Reported value	Max. value	Min. value	Average value	Units	ECDIN Number
Acetone	2	—	—	—	%	xxxx
Ethanol	2	—	—	—	%	xxxx
Water	96	—	—	—	%	xxxx

Chemical Composition, other parameters					
Parameter	Reported Value	Max. Value	Min Value	Average Value	Units.
pH	4.7	—	—	—	units
BOD	180000	—	—	—	mg/l
Suspended Solids	2000	—	—	—	mg/l
Cadmium	22	—	—	—	mg/l

Elemental Composition, %				
Element	Reported Value	Max. Value.	Min Value	Average Value.
Carbon	xxx	—	—	—
Hydrogen	xxx	—	—	—
Oxygen	xxx	—	—	—

S.I.C.
xxxx

Company name
ABC

Plant Details
xxxx

Process No.
xxxx

Comments
xxxx

Bibliography
xxxx

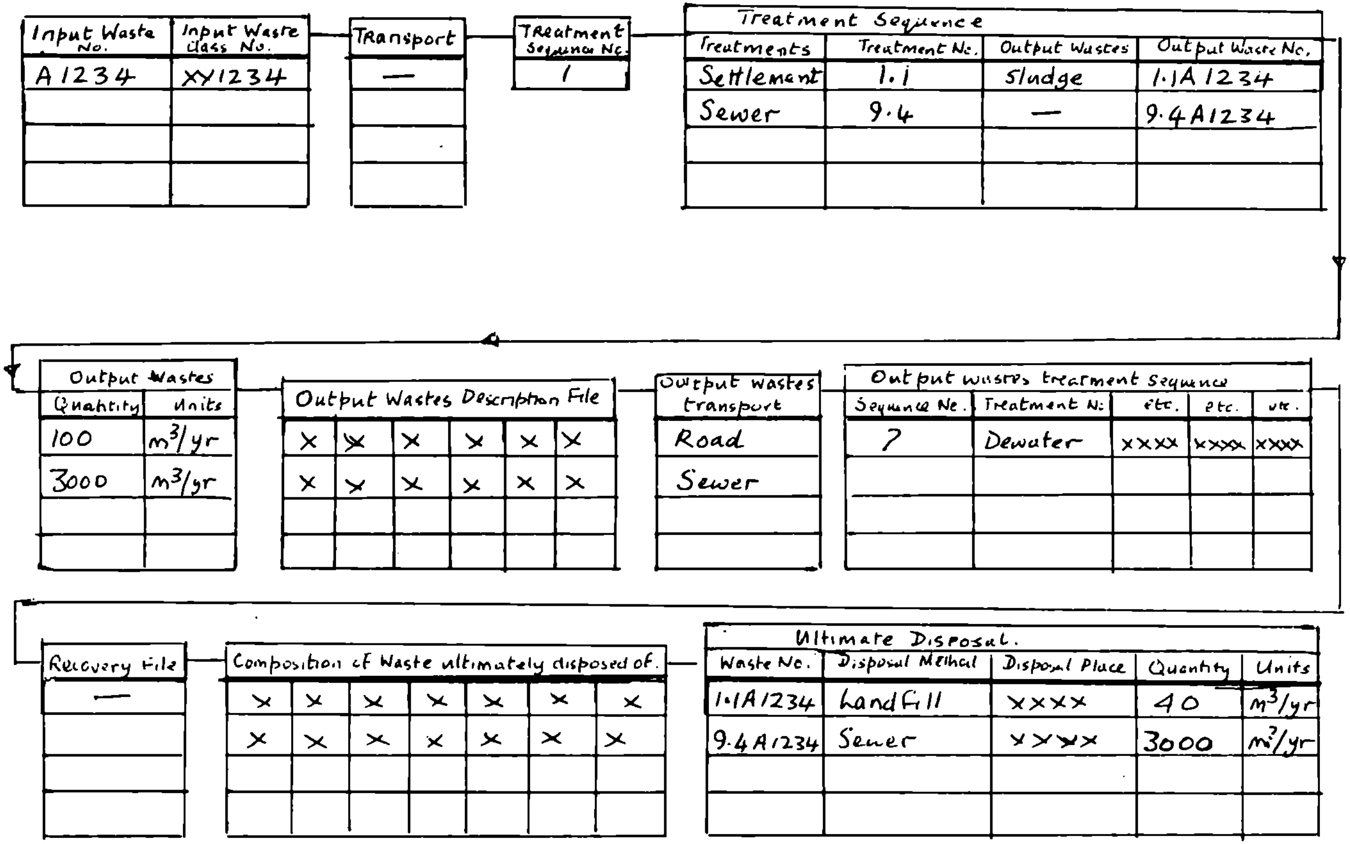
Key : — means no entry

blank space means no data

xxxx means data entry

FIG. 2. WASTE TREATMENT FILE

(Simplified and Indicative)



Input Waste No.	Input Waste Class No.
A1234	XY1234

Transport
—

Treatment Sequence No.
1

Treatment Sequence

Treatments	Treatment No.	Output Wastes	Output Waste No.
Settlement	1.1	Sludge	1.1A1234
Sewer	9.4	—	9.4A1234

Output Wastes

Quantity	Units
100	m^3/yr
3000	m^3/yr

Output Wastes Description File

x	x	x	x	x	x
x	x	x	x	x	x

Output wastes transport
Road
Sewer

Output wastes treatment sequence

Sequence No.	Treatment No.	etc.	etc.	etc.
7	Dewater	xxxx	xxxx	xxxx

Recovery File
—

Composition of Waste ultimately disposed of.

x	x	x	x	x	x	x
x	x	x	x	x	x	x

Ultimate Disposal.

Waste No.	Disposal Method	Disposal Place	Quantity	Units
1.1A1234	Landfill	xxxx	40	m^3/yr
9.4A1234	Sewer	xxxx	3000	m^3/yr

RESEARCH AREA 3 : REDUCTION AND PREVENTION OF POLLUTION AND NUISANCES

TOPIC 31 : WATER POLLUTION ABATEMENT

Contractor :	INRA, Science du Sol, Paris
Contract n° :	254-77-1 ENV F
Project leaders :	P. Dutil and J.C. Muller
Title of project :	Limits of soil purifying capacity and hazard of pollution in the application of waste water from potato starch and beet sugar factories

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1 - OBJECTIVE OF THE RESEARCH

To establish the physico-chemical mechanisms concerning the problems of the evolution of chalky soils, an experimental equipment of lysimeters has been set up in genuine chalky soils in the laboratory I.N.R.A. in CHALONS-sur-MARNE (France). Five lysimeters are reserved to the study of pollution problems stated by the application of waste water from starch and sugar-beet factories.

2 - DESCRIPTION OF THE LYSIMETERS AND METHODS

The equipment is located in the middle of the experimental fields of the laboratory, nearing the meteorological Station wich enables to know the climate environment.

The soil is a brown rendzina lying on cryoturbated chalk.

The lysimeters comprised soil monoliths of 8 m^3 (2 x 2 x 2 m) isolated in their original state. Samples taken systematically from drainage water and solution collectors placed at differents levels from the soil surface to the water table to be followed continously and the nutrient balance to be calculated for a top-soil and sub-soil two meters deep.

Two lysimeters are reserved to the application of "waste water from potato starch factory", two other for "waste water from beet-sugar factory" and one is used as a "control".

Two differents ways of application of waste waters are used : one of the lysimeters received only one time waste waters and the other every two years.

The "application" lysimeters don't get any mineral fertilizer. The "control" lysimeter is fertilised normally according to the same crop planted over the lysimeters : sugar-beet, wheat, potatoes, alfalfa.

Since this equipment has been progressively set up between 1974 to 1977, only one application lysimeter of potato starch waste water receveid two applications. The other lysimeters has been in working order under the application system only from 1977.

Consequently this report only deals with the results obtained on the "control" and "application" lysimeters of potato starch waste water.

The residual water from potatoes starch factories had a high organic nitrogen and potash content corresponding to 700 kg/ha/N and 1200 kg/ha/K_2O to an application of 80 mm.

3 - RESULTS

Transfer of organic compounds - Within the 24 hours which follow the application, the water drained is one a more very important when the soil is at field capacity. Organic soluble carbon and nitrogen which have moved through the fissure porosity of the chalk lead to an increase of C.O.D. and of the organic nitrogen of the draining water. The comparaison of quantities brought by the application and drained away 24 hours after enables to define a transfer rate = drained amount/ brought amount. The value of which gives the percentage of solution moving through the fissure porosity of the chalk.

The extrelly small results of the transfer rate, inferior to 1 %, show the minor importance of chalk fissure porosity of the soil filter towards organic compounds.

Transfer of mineral compounds. The proportion of the losses by drainage and the bringing by fertilizers, the rain, and or, the waste waters, definite the rate of transfer of mineral compounds to the deepness.

For the period 1973 to 1977, the rate of transfer are the following one :

Compounds / Origine	N	Cl	SO_4	P_2O_5	Ca	Mg	K	Na
fertilizers and rain	0.16	0.62	0.77	0.0	1.27	3.64	0.04	0.83
waste water	0.30	1.43	1.04	0.0	1.79	1.79	0.07	4.07

For all the elements except magnesium, the transfer rate of elements brought by waste waters is superior to the one of fertilizer and rain. The losses are superior to the bringings, in so far as sodium, calcium, chloride and sulphate are concerned. A little quantity of nitrates moved away. Magnesium and potassium are slightly exported and mineral phosphore remains the same.

4 – CONCLUSIONS

We may draw the following conclusions from the measured carried out between 1973 and 1977 :

- The state water in the soil reveal two phases throughout the year : one draining phase from 15 december to april 15, one no-draining phase for the rest of the year. The winter-draining phase represents 90 to 95 % of the yearly draining. Consequently the main part of losses in mineral elements is done during this period.
- The concentration of the draining of the "control" lysimeter has not varied much since the beginning of the experiment. Then we may conclude that it has reached a stationary state. In opposition in the "application" lysimeter, the concentration in mineral elements have been increasing during the experiment. The stationary state had not yet been reached.
- The water-balance shows that the whole quantity of water had not make restitution to the water-table. Only 52 % of the water brought by application contribute ot the filling-back.

- In spite of high applications of organic matter and organic nitrogen, the transports downwards are limited. The transport by the draining waters represent at the most 7 % of the C.O.D. and organic nitrogen.

- The transfer rate of mineral elements shows that some of them are strongly retained by the soil : phosphorus and potash. For these two elements, the transfer rate have pratically been the same since the beginning of the experiment. Just a little quantity of nitrogen remains. The transfer rate remains the same in the "control" lysimeter (0.15). On the opposite, under the effet of successive applications it had strongly increased, from 0.14 in 1973 to 0.30 in 1977. Chlorides and sulphates are slightly retained in the case of normal fertilization rate, they are no longer retained in the case of application of waste-water from starch potatoes factory.

As a conclusion, the application of waste-waters provokes an increase of the transfer rate of mineral elements proportionally to the variable amount of these elements.

The reaction to draining is not immediate. To get a stationary state and to determine the duration of remaining in the lysimeter, we need to carry on the experiment for several years.

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- MULLER J.C. 1975 - Sol système épurateur : Epandage d'eaux résiduaires de Féculerie en sol de craie. Etude en cases lysimétriques. Résultats des campagnes 1973-1974 et 1974-1975 - Trvx. Stat. Science du Sol. CHALONS. Public. n° 36.
- MULLER J.C. 1977 - Epandage d'eaux résiduaires de Féculerie, Etude en lysimètre sur sol de craie non remanié. Science du Sol 1977, n° 3, 167-182.
- GRADOS D. et MULLER J.C. 1978 - Sol système épurateur : Epandage d'eaux résiduaires de Féculerie en sol de craie. Etudes en cases lysimétriques : II - Résultats des campagnes 1975-1976 et 1976-77. Trvx. Stat. Science du Sol CHALONS. Public. N° 64.

Contractor : SETUDE 27 Bd des Italiens PARIS

Contract n° : 308 - 78 - 1 ENV F

Project Leader : Cyril GOMELLA

Title of project : DESINFECTION DES EAUX RESIDUAIRES URBAINES PAR L'OZONE

An experimentation was initiated by SETUDE on behalf of European Communities Committee to develop an efficient technique for disinfection of sewage, which would involve minimum nuisance to the environment.

In this work, well - known pollution features were taken into consideration. Data obtained with ozone as disinfecting agent were compared to chlorine disinfection data.

1 - OPERATING CONDITIONS

Tests were carried out on urban sewage at COLOMBES purification plant, where a biological treatment brings the purification quality up to level IV.

Ozonization pilot equipement comprised four cylindrical vessels in series, receiving the effluent from the purification plant either directly or through a sand-filter.

Waters to be treated were flowing downwards the ozonation wessels, while ozonized air was injected from the bottom.

Chlorination was performed in one a batch system, along with the same waters.

After being treated with ozone or chlorine the waters were neutralized with sodium hyposulfite and then analysed.

2 - DEVELOPMENT OF AN ECONOMIC TECHNIQUE USING OZONE FOR DISINFECTION

The purpose was the reduction of coliforms by a factor 10^{-4}, which is generally taken as a satisfactory figure in urban waste water treatment.

We looked to determine minimum values for contact time and ozone residual required to obtain the specified reduction level of coliformes. It has been found that a 10^{-4} reduction factor of coliforms is achieved under the best conditions with a 9 minute contact time between water and ozonized air : in a first stage, ozone residual is progressively brought up to a desired level and then maintained at this level.

Treatment rates and efficiency values are still under examination; but the following statements, can already be given :

a) ozone dosages varied between 6.5 and 10.8 mg/l (averagely 8.7 mg/l), with 9 minute contact time, Coliforms reduction factor varied between 5 and 5.5 x 10^{-4} (averagely) 1.9 x 10^{-4}

b) chlorine dosages varied between 10 and 25 mg/l (averagely 17 mg/l) with 13 minute contact time, Coliforms reduction factor varied between 3 and 7 x 10^{-4} (averagely 4,2x10^{-4}).

With regard to coliforms, results with ozone or chlorine are not very different, and for very close results the dose of chlorine was found slightly below 2 times the dose of ozone. According to data recorded, we may finally admit that coliforms 10^{-4} reduction can be achieved at the following average dosages :

- ozone : 8 mg/l with 9 minute contact time,
- chlorine: 14 mg/l with 13 minute contact time.

Concerning disinfection of waters coming from the purification plant(and further sand-filtered), the conditions of ozone application remained the same as when waters are not filtered, i.e. 9 minute contact time comprising a first period of 5 minutes with a progressive increase of residual ozone, followed by a period of 4 minutes during which ozone residual level is maintained at a constant value 0.5-0.6 mg/l (instead of 0.6 - 0.8 mg/l when not sand-filtered).

Parallel testing using chlorine gave coliforms reduction factors comprised between 10^{-4} and 8×10^{-4} (averagely 4×10^{-4}), with treatment rates between 10 to 15 mg/l (averagely 13 mg/l) with 13 minute contact time.

Taking these data into account, it appears that treatment dosage required to obtain coliforms 10^{-4} reduction after sand filtration, are :

- with ozone : 5 mg/l and 9 minute contact time
- with chlorine: 10 mg/l and 13 minute contact time

3 - FURTHER RESULTS CONCERNING DISINFECTION WITH OZONE OR CHLORINE

Tests comparing ozone to chlorine as disinfecting agents showed the effects of both products on a number of parameters.

3.1 Pathogenous germs - Fecal contamination testing

Operating conditions of disinfection treatments were :

1st chain - Ozonization	9.1 g O_3/m^3 water, 9 minutes
2nd chain - Chlorination	15 g Cl /m3 water, 13 Minutes
3rd chain - Filtration + Ozonization	6.6 g/O_3/m3 water, 9 minutes
4 th chain - Filtration + chlorination	12 g Cl/m3 water, 13 minutes.

The first results established that, under the above conditions, Coliform and E-Coli reduction factors were a little higher than 10^{-4} with ozone procedure (with or without sand-filtration). The other indicators were sensitive to ozone action, more than to chlorine action.

. Streptococci are reduced by a factor close to 10^{-4} through ozone action, and only 10^{-3} to 2.10^{-3} through chlorine action,

. Salmonellae are reduced by a factor of about 75 through chlorine action alone (from 230 to 3) and by a higher factor with the 3 other chains,

. On the other hand, with regard to thiocyanic bacilli, all chains are equally efficient, at a high level (5×10^{-4} in waters before treatment, but not revealed after treatment).

. Reversedly, treatments are very poorly efficient with regard to clostridium spores, rejected at a factor inferior to 10^{-1}, with an advantage for ozone action.

. At last, with regard to Coliphages ozone is more efficient than chlorine, with a reduction factor 10^{-1} to 5.10^{-1} for ozone compared to less than 10^{-1} for chlorine, while salmonel phages are not seen in the waters to be treated.

3.2 EFFECTS ON OTHER POLLUTION FEATURES

Ozonization by itself permits to reduce color by 72 % (from 40 % to more than 90 %, against 19 % with chlorination.

. In the third chain (ozonization) the cumulated effects of filtration and disinfection gave a color reduction of 75 % averagely (from 60 % to more than 90 %), instead of 23 % in the fourth chain (chlorination).

. Concerning organic pollution of water, the two procedures lead to different results :

Ozonization gives a very substantial decrease of UV absorption at 254 nm (from 6 % to 32 %, averagely 19 %) whereas chlorination generates a significant and systematical increase of this characteristic (from 19 % to 67 %, averagely 32 %).

In this field the cumulated effects of sand-filtration and disinfection tend to an average decrease of 30 % in the 3rd chain (ozonization) and an average increase of 25 % in the 4 th chain (chlorination).

Important deviations are also seen in the case of oxidizable organic matters BOD and COD. Disinfection through ozone permits to achieve the purification quality level V (or even VI), especially when preceded by sand-filtration :

. By itself ozonization permits to reach an average reduction ratio of 20 % for COD (from 16 to 26 %) and 37 % for BOD (from 20 to 56 %).

. Combined sand-filtration and ozonization reduces COD by a 29 % (from 20 to 40 %)factor.

Chlorination data show that chlorine has almost no effect on COD and a slight effect on BOD (average 10 % reduction).

Concerning Total Organic Carbon, the two disinfecting agents show only slightly marked effects, with a tendency to decrease with ozone treatment, and practically no effect with chlorine treatment.

3.3 Effects on organic micropollution

Ponderal Analysis of the Chloroform Extractable Matters (by weight) gives an idea about the global results of both desinfection procedures on organic micropollution. Our tests confirmed the facts observed in the field of organic matters, i.e. a substantial decrease with ozonization and a significant increase with chlorination.

From now on, the effects of both disinfection techniques may be evaluated on a particular family of organic pollutants, volatile haloformes the analysis of which has been systematically performed, because of the medical importance of such compounds and their inherent ability to be generated in chlorine medium. Seven organo-halogenous compounds have been studied : chloroform, 1,1,1 trichloroethane, carbon tetrachloride, trichloroethylen, tetrachloroethylen, dichlorobromomethane chlorodibromomethane, five of which were found in the purification plant effluents :

- trichloroethylen, tetrachloroethylen, chloroform, 1,1,1 trichloroethane and carbon tetrachloride.

The first four compounds were much more substantially present, in that increasing order.

The global amount of these compounds does not generally exceed a hundred micrograms per liter, and except in three-high pollution cases caused by trichloroethylen and tetrachloroethylen, this amount is most often comprised between 10 and 30 µg/l.

Chlorination treatment causes the global content of these compounds to increase by 10 to 20 µg/l, this is essentially due to chloroform, increasing level but also coming from the formation of dichlorobromomethane and sometimes traces of chlorodibromomethane. This relatively moderate effect of chlorine comes from the fact that chlorine desinfection is largely inferior to break-point rates. These conditions does not promote the formation of haloforms.

On the contrary, disinfection by ozone causes a significant decrease of haloforms (about 2 to 20 µg/l). This is essentially due to trichlorethylene and tetrachloroethylen oxidation.

CONCLUSIONS

It is still too soon to draw out conclusions from a work in progress.

But from now, we may state that the time-modulated ozonization process which has been developed permits to reach the specifications required for urban waste water disinfection, preliminarily purified at level IV, under admissible economic conditions (at about 8 O_3 g/m3 as treatment rate, and 9 minutes as contact time). The same kind of effluent,preliminarily sand-filtered, requires a much lower dose of ozone (about 5 g/m3) for the same contact time.

Considering Coliforms as a base, chlorination conditions are more stringent (with a treatment rate of about 14 Cl g/m3 when the effluent is not sand-filtered, and 10 Cl g/m3 when the effluent is filtered, for 13 minute contact time) but the process seems to be more economic than ozonization.

On the other hand, data obtained on other polluting characteristics (aspect, organic matter content) promote the use of ozone, permitting to achieve purification quality at the level V, and even VI when ozonization is preceded by sand-filtration, whereas chlorination increases the formation of volatile haloforms (mostly chloroform). Finally, it seems that chlorination is slightly less efficient in the field of some pathogenous elements.

From these results, ozone treatment appears to be a little more costly than chlorine treatment, but it answers much better to the problems raised bynature protection.

Contractor: Istituto Ricerche Breda S.p.A.

Contract N° : 247 - 77 - 1 - ENV I

Project leader: dr. Antonino Aveni

Title of the project: Storage and reactivation of acclimated biological sludges

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Research program

Setting up of a storage method of high amount of biologic sludges to be utilized, after reactivation, for the rapid starting of waste water depuration plants.

Materials and methods

The main equipments used are: laboratory and semi-pilot scale activated sludge treatment plants; "SAPROMAT" respirometer; equipment for biodegradability determination; laboratory plate filter-press; laboratory centrifuge, flotation cell, freezing cell, laboratory equipments to determine various parameters for the biological sludges characterization and to follow the waste waters biological treatment.

Test methods (standard or specifically set up) were: dewatering tests by constant vacuum filtration and by centrifugation; specific oxygen uptake determination by a constant oxygen pressure respirometric method; organic substances biodegradation rate (up to 48^h) determination, at different organic load values, by a "batch test"; aerobic and facultative anaerobic biomass determination by microbiological counts; activity determination by testing the efficiency of organic substances reduction in the activated sludge treatment.

Results of the research

Three types of standard biologic sludges have been prepared using activated sludge plants fed by:

a - artificial sewage water
b - waste water of a canned tuna fish industry
c - waste water of olive-oil production, mixed with artificial sewage water

The three above - mentioned sludges have been used to carry out laboratory and semi-pilot scale tests.

Laboratory tests

Preliminary tests have pointed out that, storing a centrifuged biological sludge at temperatures of –17 ÷ – 20°C, the sludge activity is not prejudiced: the oxygen uptake is comparable to that of the fresh sludge.

It has been then set up a storage technique including a pre-treatment line of the sludge consisting of:

- sludge extraction from water depuration plant
- flotation by air
- chemical treatment by cationic polielectrolyte
- dewatering by filter-press or centrifuge

The dewatered sludge-cakes are sealed up in polyethylene bags and rapidly freezed and stored at – 20°C. Before the use, the sludge is reactivated by simple aeration after mixing with waste water.

Tests have been made with sludges dewatered at various degree (5÷15% Total Solids) and stored for several months (3÷15 months). The activity and the biodegradation ability of fresh and stored sludges have been determined. The results indicated that the storage operations determine a microbial charge reduction but the sludge activity is not compromised. The reactivated sludge gives the same high values of waste water depuration efficiency obtained with fresh sludge (fig. 1).

Semi-pilot scale tests

Sludges stored for 5 ÷ 7 months have been used to carry out waste water treatment tests.

The stored sludges have been put into the aeration tank of an activated sludge treatment plant containing the same waste water used to produce the sludges. After 15÷ 20 hours of aeration time, it has begun a regular and continuous feeding of the plant, testing the principal service parameters.

The results have confirmed what previously observed in the laboratory "batch" tests: biodegradation process rapidly begins and acts with high values of organic substances reduction efficiency, comparable to those obtained by fresh sludge testing. 1 ÷ 2 days after the plant start, BOD_5 reduction values even higher than 90% have been obtained. Sludge increase was regular. In fig. 2 results of tuna fish waste water treatment with stored biologic sludge are shown.

Conclusions

Laboratory and semi-pilot scale tests carried out with different biologic sludges have demonstrated the possibility of the storage at low temperature (– 20°C) for several months of acclimated sludges to be utilized for the rapid starting of biologic waste waters treatment plants.

The sludge reactivation can be made in the aeration tank of the treatment plant.

The tests have pointed out that the organic substances reduction efficiency reaches high values since the first days after the plant start.

To store high amounts of sludge, a pre-treatment line has been set up and tested with good results.

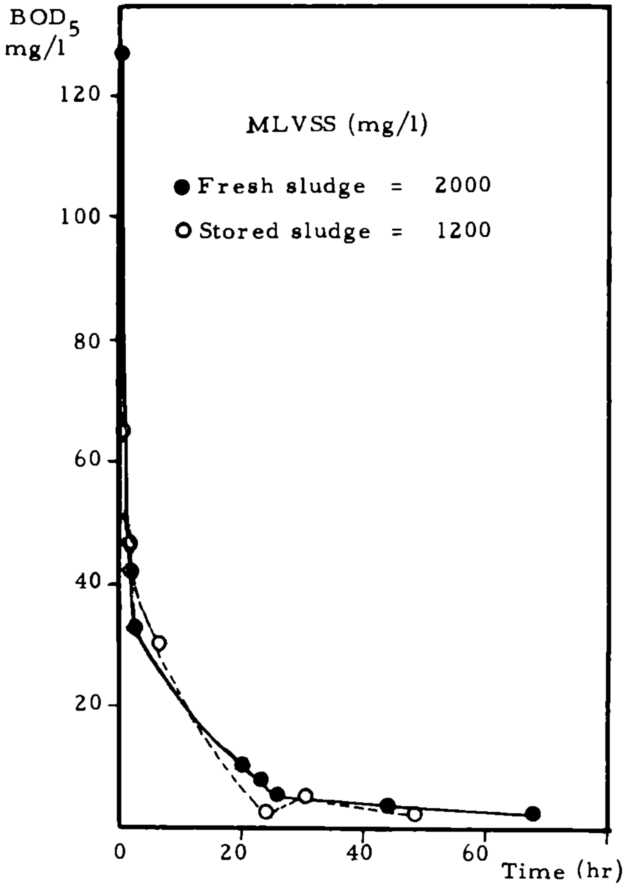

Fig. 1 - BOD$_5$ reduction in the biological treatment laboratory test of artificial sewage water by fresh (—) and stored (---) sludge (3 month)

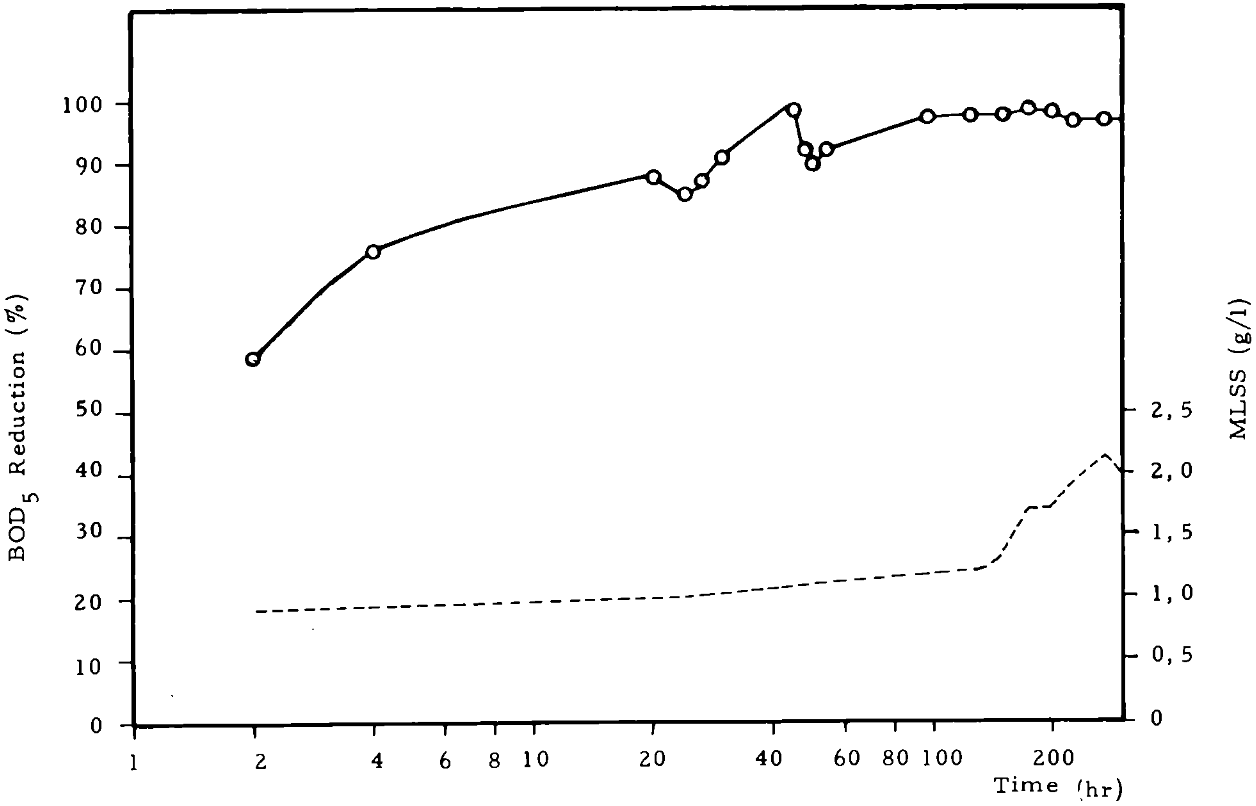

Fig. 2 - BOD$_5$ reduction efficiency (—) in the biological treatment of tuna fish production waste water using five month stored biological sludge.

Contractor : Organisation for Industrial Research TNO (The Netherlands)

Contract No.: 250-77-1 ENV NL

Project leader : Ir. H. Schukking

Title of project: Development of a new method for the dyeing of cotton and polyester by means of an engraved roll.

Objective of the research

1. A frequently applied method for the dyeing of cotton fabric with reactive dyestuffs consists of dipping, squeezing and batching the fabric at room temperature (cold pad-batch dyeing).

   In general the colour yields of this dyeing method are relatively low due to hydrolysis of the dyestuff. To obtain a high colour fastness the hydrolyzed dyestuff should completely be removed from the dyed fabric by washing with hot water.

   The chance of hydrolysis of a reactive dyestuff decreases when the rate of exhaustion of the dyestuff from the dyebath increases. The rate of exhaustion is, among other factors, determined by the liquor ratio. The lower the liquor ratio, the higher the rate. Therefore pad-batch dyeing techniques (liquor ratios 1 : 1 to 1 : 0.8) are preferable to batch dyeing techniques at long liquor ratios.

   A further reduction in the liquor ratio, which can be realized by impregnating the fabric with an engraved roll, might lead to a still faster rate of exhaustion and consequently to higher colour yields.

   Higher colour yields result in the following advantages:

   - a reduction in the water and energy consumption during the washing-off stage;
   - less pollution of the effluents with hydrolyzed dyestuff;
   - savings in dyestuff.

2. Engraved rolls can also be used for the impregnation of polyester fabrics, which are dyed by the thermosol process.
   Compared with the usual padding technique a reduction in the moisture content of the fabrics from about 70 to 40% can be realized in this way. As a consequence less energy will be needed to dry the fabrics.
   Due to the lower moisture content the chance of migration of the dyestuff during the drying stage will decrease, so the quantity of a migration inhibitor in the dyebath can possibly be reduced.

Methods

1. The cold dyeing of cotton with reactive dyestuffs

A cotton fabric is impregnated with an alkaline solution containing a reactive dyestuff. The impregnation is carried out with a pad mangle and an engraved roll.
The impregnated fabric is batched at room temperature (fixation of the dyestuff). After batching, the fabric is washed with hot water to remove non-fixed dyestuff.

2. The dyeing of polyester by the thermosol process

Polyester fabric is impregnated with a dispersion of a disperse dyestuff by means of a pad mangle and an engraved roll. After drying the fabric and baking at temperatures in the range of 190 - 230 °C for times of the order of 30 s to 2 min, non-fixed dyestuff is removed by an after-treatment with a reducing agent.

Results

1. The cold dyeing of cotton with reactive dyestuffs

The experiments have been carried out with dyestuffs containing a dichlorotriazine group. Due to the alkalinity of the dyebath the dyestuffs can partly be hydrolyzed before dyeing has started. The quantities of hydrolyzed dyestuff can differ from experiment to experiment. This must be taken into account in calculating the colour yields of the dyeings.
A chromotographic analysis method has been developed to determine the quantity of hydrolyzed dyestuff.
Cotton fabrics of 120 g/m^2 were impregnated with a pad mangle and an engraved roll. To obtain equal colour depths on both sides of the fabrics a roll with engravings of at least 180 microns should be used.

Applying the engraved roll, the pick-up of dyeliquor was two and a half times as small as the pick-up obtained with the pad mangle (liquor ratios 0.3 : 1 and 0.75 : 1 respectively).

First a series of experiments was carried out with Procion Orange MX-2R and Procion Blue MX-R as reactive dyestuffs. The compositions of the dyebaths were chosen in such a way that with both the pad mangle and the roll dyeings with equal depths of shade were obtained. So the dyeliquors used to impregnate the fabrics with the roll were 2½ times as concentrated as the pad liquors.

From the experiments it appeared that an improvement in the colour yields of 5 - 10% is obtained by using the roll instead of the pad mangle. However, this only applies to dyeings of 0.9 and 1.5% depth of shade. There is a decrease in the colour yield at dyeings in deeper shades (2.7 and 3%). This decrease is caused by the insufficient solubility of the dyestuffs at the concentrations applied (100 g/l). Addition of urea to the dyebaths to improve the solubility resulted in a slight increase in the colour yields.

Next dyeing experiments were carried out, using a number of Procion dyestuffs in concentrations of 20, 40 and 60 g/l. In contrast with the previous experiments both for the impregnation with the roll and the pad mangle dyeliquors containing the same quantities of dyestuff were used. The results of the experiments are mentioned in the table on page 4. In this table P refers to the pad mangle and R to the engraved roll. The batching times depend on the alkalinity of the dyebaths.

The dyestuff concentrations of 20, 40 and 60 g/l give dyeings with depths of shade of 1.5, 3 and 4.5% in case the pad mangle is used. Applying the roll these percentages are 0.6, 1.2 and 1.8 respectively.

It appears from the table that only in a few cases the applied reduction of the liquor ratio from 0.75 : 1 to 0.3 : 1 leads to an appreciable improvement in the colour yields (Procion Yellow MX-4G 40 and 60 g/l; Procion Brown MX-GRN 20 and 60 g/l).

Successive impregnations of the cotton fabric with a blank alkaline dyebath and a dyestuff solution, using the engraved roll, only in the case of Procion Blue MX-R lead to an increase in the colour yields from 75 to 85% (1.2% depth of shade dyeing).

Colour yields (%) of dyeings with reactive dyestuffs

Dyestuff	Method	Concentration of dyestuff					
		20 g/l		40 g/l		60 g/l	
		Batching time (h)		Batching time (h)		Batching time (h)	
		4	24	4	24	4	24
Procion Yellow MX-4G	P	82 ± 1	84 ± 1	77 ± 2	75 ± 1	74 ± 2	74 ± 2
	R	83 ± 1	82 ± 2	87 ± 2	82 ± 2	84 ± 2	83 ± 2
Procion Orange MX-G	P	78 ± 1	77 ± 1	77 ± 2	79 ± 2	70 ± 2	70 ± 2
	R	80 ± 2	81 ± 2	80 ± 2	79 ± 2	74 ± 2	73 ± 2
Procion Orange MX-R	P	86 ± 1	90 ± 2	95 ± 1	93 ± 1	94 ± 1	86 ± 1
	R	90 ± 1	92 ± 1	97 ± 2	96 ± 1	95 ± 1	88 ± 2
Procion Red MX-58	P	91 ± 2	98 ± 2	94 ± 1	92 ± 2	92 ± 2	93 ± 2
	R	92 ± 2	97 ± 2	96 ± 1	94 ± 2	96 ± 1	91 ± 2
Procion Blue MX-2G	P	96 ± 1	96 ± 1	87 ± 1	89 ± 1	84 ± 1	85 ± 1
	R	94 ± 2	95 ± 2	91 ± 1	86 ± 1	86 ± 1	85 ± 1
Procion Blue MX-R	P	68 ± 2	71 ± 2	70 ± 2	72 ± 2	61 ± 2	61 ± 1
	R	73 ± 2	75 ± 1	75 ± 1	76 ± 1	59 ± 1	63 ± 1
Procion Brown MX-GRN	P	77 ± 1	79 ± 1	76 ± 1	77 ± 1	68 ± 1	70 ± 1
	R	88 ± 1	86 ± 1	60 ± 2	80 ± 1	74 ± 1	76 ± 1

2. The thermosol dyeing of polyester fabric using engraved rolls for the impregnation

Depending on the weights of the fabrics to be dyed, use should be made of rolls with engravings of different depths in order to obtain equal colour depths on both sides of the fabrics. It has appeared that fabrics with weights up to 138 g/m^2 should be impregnated with a roll with engravings of 300 microns to meet this requirement. Higher weight fabrics (158, 168 and 212 g/m^2) are dyed unsatisfactorily with this roll.

However, if these fabrics have been pretreated with a wetting agent good results are obtained. It has appeared that, with the exception of the fabric of 212 g/m^2, a pretreatment of the fabrics investigated makes the use of a roll with engravings of 180 microns possible.

Depending on the weight and the structure of the fabrics, the moisture contents of the fabrics, impregnated with the roll of 180 microns, are 25 - 60% lower than those of the padded fabrics.

Concerning the 212 g/m^2 fabric, good dyeing results can be obtained by impregnating the fabric twice with the roll of 180 microns. Compared with a treatment on the pad mangle, the moisture content of the fabric has decreased by 85%.

The savings in energy during the drying of the fabrics correspond to the above mentioned percentages.
Drying experiments carried out in a small-scale infrared heater have shown that the use of a migration inhibitor is not necessary when the fabrics have been impregnated with the engraved roll.

Conclusions

- In the cold pad-batch dyeing of cotton fabrics with reactive dyestuffs a reduction in the liquor ratio, which is obtained by impregnation of the fabrics with an engraved roll instead of a pad mangle, leads to an appreciable improvement in the colour yields in only a few cases.

- The dyeing of cotton in deep shades results in low colour yields. In this case the impregnation with the engraved roll should be carried out with dyeliquors containing high concentrations of dyestuffs, in which concentrations the dyestuffs are insufficiently soluble.

- The thermosol dyeing of polyester fabrics leads to good dyeing results when engraved rolls are used for the impregnation of the fabrics.

  Compared with impregnations on the pad mangle the moisture contents of the fabrics are reduced by 25 - 60%. Corresponding percentages of energy can be saved during the drying of the fabrics.

  Owing to the relatively low moisture contents of the fabrics, the use of a migration inhibitor is not necessary.

Contractor :	Professor L.K. Dunican, Department of Microbiology, University College, Galway, Ireland
Contract n° :	195 - 77 - I ENV EIR
Project leader :	Professor L.K. Dunican
Title of project :	The use of nitrogen-fixing bacteria in sewage flocs to improve quality of treated water from activated sludge plants treating low nitrogen wastes

Objective of the research

This contract involved a study of the problems associated with the operation of the activated sludge process when treating wastes with low nitrogen. Wastes with high carbon and low nitrogen, e.g. papermill and creamery wastes, are imbalanced relative to the proportion of nitrogen present for optimum microbiological activity in the activated sludge process. Poor efficiency of treatment in such plants can be aleviated by adding nitrogen fertilizer to the waste. This contract undertook to study the feasibility of using nitrogen-fixing bacteria in activated sludge to fix the gaseous nitrogen required to balance the carbon/nitrogen (C/N) ratio of the waste. Two approaches were used. The first approach examined the indigenous nitrogen-fixing bacteria which are found in natural environments with high C/N ratios to see if these could be encouraged to grow in the activated sludge environment. The second approach required the use of mutation and bacterial genetic methods to make nitrogen-fixing bacteria more suitable for the activated sludge environment. To achieve these aims, bench activated sludge units were operated at a series of C/N ratios to study low nitrogen effects on waste treatment efficiency and the microbiological ecology of the process.

Materials and Methods

Experimental design of activated sludge (AS) units: six units (1 litre vol.) were set up and operated for more than six months after preliminary studies to determine the best design for such units. The units were fed a synthetic waste with varying C/N ratios (Gaudy et al.,1974, Biotechnol. Bioeng. XVI, 723-738). Most of the information in this study relates to synthetic waste fed at a concentration of 2200 mg/L COD.

Ammonium sulphate was added to the synthetic waste to give C/N ratios of 5:1, 10:1, 20:1 and one additional unit had no nitrogen added. The waste was added daily in a semi-batch type process designed to give a wastage of 25% of the biological solids per day. The units, when seeded with activated sludge solids from a domestic sewage plant and acclimatized to the synthetic waste, operated normally as would be expected with wastes of varying C/N ratios, i.e. settleability was normal, waste treatment efficiencies were normal and bulking of the AS units occurred where wide C/N ratios were used.

Performance of activated sludge: the performance of the various activated sludge units was based on the measurement of the following parameters:

pH
O_2 level
Temperature
Biological solids
Sludge volume
Sludge settleability
Sludge volume index
Nitrogen level
Nitrogen-fixation
Microbiological viable counts

Microbial ecology of activated sludge: viable bacterial counts were used to assess changes in the microbial population in activated sludge units receiving wastes with different C/N ratios. The activated sludge liquor was homogenized to break the sewage flocs using ultrasonic vibrator. The suspensions were diluted in sterile water and plated on a selection of media, <u>viz</u>. nutrient agar, McConkey agar, Burk's N-free agar and starch agar. Plates were incubated at 25°C for 4-6 days.

Nitrogen-fixation in activated sludge: the conventional acetylene reduction method was used to detect nitrogen-fixation in the activated sludge and is putative N_2-fixing bacteria isolated from wastes. Five ml samples of sludge of culture fields were incubated in a 4% of acetylene atmosphere for 1, 3 and 24 hours and assessed for ethylene production.

Results

The laboratory activated sludge units worked satisfactorily for the duration of the period after they had been acclimatized. Sewage flocs developed and as expected, wastes with high C/N ratios of 20:1 and 40:1 showed bulking. However, COD reductions of 90% were observed in all experimental sludge units. Biological solids ranged from 1700-2700 mg/L in units with wastes of 5:1 and 10:1. Biological solids were higher and more variable in 20:1 and 40:1 C/N ratios due to intermittent bulking. Sludge volume confirmed these results with compact settled sludge with 5:1 and 10:1 wastes and more flocculant bulky sludge in wastes with high C/N ratios. Over the period of 18 months, activated sludge worked perfectly with C/N ratios of 10:1 or less and imperfectly with C/N ratios of 20:1 and greater.

Microbiological studies showed that activated sludge units receiving low nitrogen inputs had slightly lower viable bacterial counts than that with a C/N ratio of 10:1. Starch agar selecting for a different component of the microbial population also showed higher numbers where the C/N ratio was 10:1. Counts on Burk's N-free agar showed the opposite effect with higher numbers at a C/N of 40:1. Growth on nitrogen free media is common in bacteria isolated from nature which favour environments low in nutrients. Work is progressing to test these isolates for their ability to fix nitrogen.

Nitrogen-fixation in activated sludge units with a C/N of 40:1 was implicated when these units often reverted from the usual 'bulking phenomenon' to more normal sludge character indicating that they were overcoming their nitrogen limitation. A mixed inoculum from an enrichment was added to duplicate activated sludge units with C/N ratios of 40:1 to encourage nitrogen-fixing bacteria in the sludge. Significant fixation of nitrogen was detected using the acetylene reduction assay in the units with C/N of 40:1. One of the duplicate units was then run for 10 weeks without any nitrogen added in the feed. The unit continued to function normally with a COD removal of 90% efficiency and with a sludge with good settling properties. Fixation was not detected in activated sludge plants with C/N ratios of 20:1 or less. When nitrogen fixing bacteria were added to these, a little fixation was noted in the C/N 20:1 unit but none in the units with C/N 10:1 or 5:1. Fixation therefore only occurs in environments with a low N level. The principle microorganism involved in fixation has been identified as *Azomonas* sp.

Experiments are in progress to generate genetically strains of these bacteria which are de-repressed for ammonia, i.e. they fix nitrogen in the presence of ammonia and therefore perform satisfactorily in C/N environments of 20:1. In addition, associated research in the contractant's research group now permits the generation of specific nitrogen-fixing activated sludge bacteria by gene transfer if the genetic improvement of Azomonas and other natural nitrogen-fixing bacteria proves difficult.

Conclusions

The results from the initial two-year study show that the concept of utilizing nitrogen-fixing bacteria to supply nitrogen in activated sludge treating low nitrogen wastes is theoretically feasible. Azomonas sp. has been shown in the laboratory studies to fix sufficient nitrogen to permit a normal activated sludge operation with wastes exceedingly low in nitrogen. It is less successful in treating wastes with C/N ratios of 20:1 which conventionally often cause problems. This partial inhibition arises from the natural repression of nitrogen-fixing enzymes by ammonia or combined nitrogen - such repression can be removed relatively easily by mutation. The on-going project therefore has two priorities: (i) the isolation and testing of depressed mutants of the nitrogen-fixing bacteria to increase the nitrogen-fixing range in environments with variable levels of nitrogen; and (ii) to test out the process under field conditions. This work is in progress.

List of project-leader's publications and communications

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TOPIC 32 : WASTE DISPOSAL

Contractor:	Forschungsinstitut für Edelmetalle und Metallchemie Schwäbisch Gmünd (West Germany)
Contract n°:	275-77-9 ENV D
Project Leader:	Dr. rer.nat. Ch.J.Raub
Title of project:	Influence of sorroundings on behavior of Industrial sludge during storage on public dumps

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1) Objective of Research

Hydroxide sludges originating in the metal finishing industry contain CN^-, PO_4^{3-}, CrO_4^{2-}, mineral oils etc. in addition to various basemetals. Up to now these sludges have to be deposited on various dumps, until new recycling procedures have been developed.

In extension of earlier work, present investigation serves to study long time behavior of these sludges, stored together with other waste on public dumps e.g. home waste, and to find appropriate testing methods about its behavior. Special care is given to the behavior of complex cyanides present in these hydroxide sludges.

Cyanides often are present as insoluble basemetal cyanides or complex Fe-cyanides (relatively nonpoisonous). The amount of soluble alkali cyanides and of other complex cyanides is rather low in general. Nevertheless by various ways a dissolution of cyanides, as well as a decomposition, can occur.

2) Materials and Methods

Studied were typical hydroxide sludges from an electroplating shop and a steel plant with a hardening division.

Experiments were made under following conditions:

a) For laboratory tests we developed a setup, in which long term elution behavior depending upon various parameters can be measured, since it was observed that some of the standard methods yield rather unreliable results.

b) A test, approximating real conditions, on an artificially built waste dump (60 l volume, mixture of hydroxide sludge and home waste) was established.

c) Experiments on an official home waste dump in specially provided areas were undertaben. In this dump sludges of an electroplating shop and of two iron-working plants were deposited and the effluent waters tested.

Advantages and disadvantages of these methods are:

Laboratory tests enable the adjustment of various parameters and an accurate investigation of elution processes, since conditions on a real waste dump are difficult to reproduce.

Tests on an artificial small waste dump can be controlled easily and as in laboratory tests the kind of sludges can be varied without complications. Results do not always refer directly to the ones observed in practical behavior.

Experiments in a real waste dump are difficult to be influenced once they are set up and in addition are time consuming. Sludges with higher cyanide concentrations can not be investigated this way.

We therefore decided that a combination of all three methods would yield the most reliable results.

3) Results

Since research is still in progress only some of the more important details shall be discussed:

1) Laboratory tests showed that elution behavior of the sludge is mostly depending upon the $CaCO_3/CO_2$ equilibrium and the ammonia concentration of the water.

   At $pH > 8.6$ the Cu-concentration of ammonia containing water increases strongly. The same is true to a certain extent for nickel.

   At $pH < 7.6$ a strong dissolution of nickel occurs. There is only a small pH-range in which copper and nickel do not dissolve. Complications arise because of the oxidation of ammonia by bacteria, which forms nitrite and nitrate and causes a reduction in pH.

   Rain water is uncritical. Fig. 1 shows the elution behavior of copper from an electroplating sludge with airated, deionized water which was replaced after 55 days by ammonia containing water.

2) Tests on the small experimental waste dump proved that copper and nickel are most critical elements. Fig. 2 shows the behavior of copper in this dump. In addition to the composition of the sludge its method of segregation is of strong influence upon elution (NaOH vs. $Ca(OH)_2$ precipitation). Sludge with a better storage behavior is precipitated by $Ca(OH)_2$.

3) Cyanide

   Up to now we have not found an appreciable dissolution of cyanide from the sludges investigated during long time storage. Only complex Fe-cyanides seem to dissolve in carbonated water of $pH \sim 10$.

Sludges from iron hardening plants seem to give away in the beginning relative high concentrations of Fe-cyanides (up to 15 mg/l) and free CN^- (~1-3 mg/l), but this values drop by a factor of 10 very soon.

4) Conclusion and additional comments

Of highest importance for dissolution of sludges are certain constituents of the leaching water e.g. NH_3 or NH_4^+ and $Ca(HCO_3)_2$. A high pH value is - in contrast to general opinion - of disadvantages to storage of nickel, copper and cyanide-containing sludges.

Special care is presently given to chromate formation from chromium (III) containing slugdes by waters of $pH \geq 9$.

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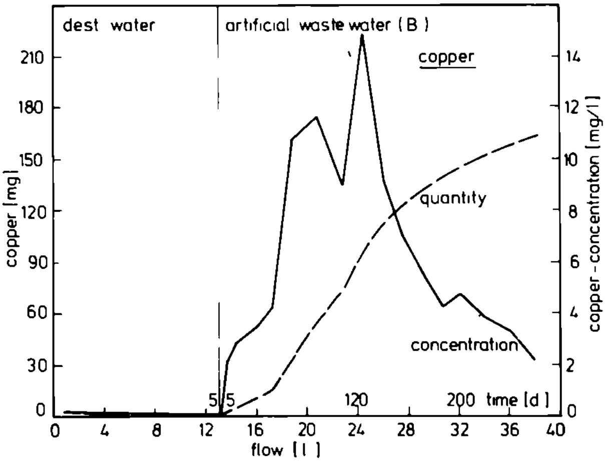
Fig. 1

Elution behavior of copper from a electroplating sludge (laboratory test)

Fig. 2.

Elution behavior of copper from electroplating sludge on a small waste dump



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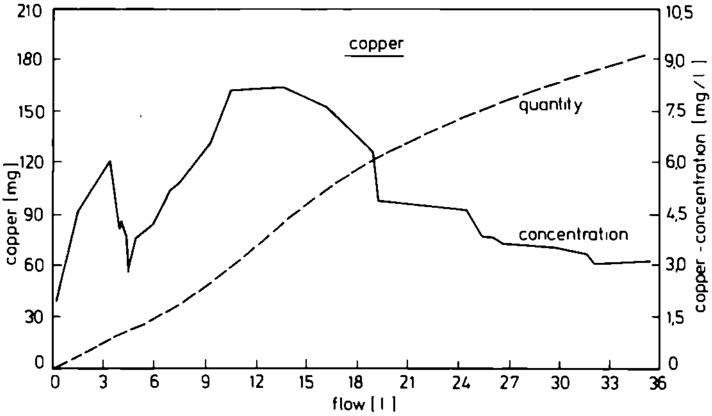

2)

Contractor : Centre d'Etudes et Recherches des Charbonnages de France
(CERCHAR)

Contract : 219-77-1 ENV F

Project Leader : R.H. BUSSO

Title of project : Methodological study of the interaction between industrial waste dumps and the environment.

- Objective of the research

The purpose of this methodological study was to find means that can be used for assessing the risk of pollution of underground water supplying populations ; with regard to the nature of the industrial wastes and the hydrogeological characteristics of the underlying ground, two typical cases were considered, viz. :

a) the "Louvroil" dump where waste products resulting from the treatment of metal surfaces are deposited on alluvial soil separated from the underground water by a layer of clay,

b) the "Vendin" dump comprising organic wastes resulting from the treatment of coal tar and discharged on silty soil separated from the underground water system by a fractured layer of chalk.

- Materials and methods (program applied)

The experimental procedure was developed in six successive stages :

1. Selection of two dump sites with the assistance of responsible local authorities.
2. Topographical surveying of the dump site, estimation of the quantities of different kinds of wastes deposited, collection of representative samples, determination of metal elements or toxic organic compounds by current (partly standardised) methods : atomic absorption spectrometry, infrared spectrometry, gas chromatography, calorimetry, DBO, etc. ; selection of a reduced number of reference pollutants to be determined in the soil and in the underground water.
3. Determination of the physicochemical and hydrogeological characteristics of the underlying ground by taking cores and drilling piezometric wells for determining the variations of the upper level of the ground water system, and taking instantaneous water samples every month .

4. Analysis of ground samples to determine the degree of pollution on the outskirts of the dump sites and at distant points for comparison with non-polluted ground.
5. Analysis of the water samples taken every month over a period of ten months, for all the piezometers used (1 at Louvroil, 6 at Vendin).
6. Interpretation of field and laboratory data.

- Results

Depositing wastes at the two dump sites concerned is now prohibited, but the quantities of pollutants deposited are rather considerable :

- at Louvroil : 6 t of toxic metallic ions : Cu, Zn, Mn, Cr, Ni, Pb and 500 t of hydrocarbons ;
- at Vendin : about 500 t of various hydrocarbon-containing substances of which some contain sulfur and cyanides.

During the 10-month monitoring period the transfer of pollutants in direction of the underground water was not alarming ; at Louvroil the ground water is polluted within 20 m of the dump site, but at a distance of 200 m where the water is pumped for urban use, pollution cannot be detected appreciably. At Vendin the ground water is polluted within 400 m, but pollution is not detectable at 850 m.

- Conclusions and additional comments

The methods used to determine the amounts of pollutants deposited and to get a first view of their transport towards underground water through the soil were satisfactory in general. The storage of the samples of contaminated soil and their analysis to determine the degree of reversibility of fixation of metallic ions or their ability to degrade organic or mineral matter should be improved.

Because of the potential risk for pollution by relatively large quantities of toxic matter still present on the dump sites, monitoring should continue over several years with elaborate means, to check that the transport of contaminants and the pollution of the soil do not increase. Samples of underground water should be taken more frequently and the amount of rainwater that falls locally and in the corresponding water basin should be recorded. Peculiar results such as high water acidity found at Louvroil and the retention or degradation capacity of the soil at Vendin should be further examined by means of more precise methods.

The very typical hydrogeological configuration of the Louvroil dump site could be the occasion for a very interesting investigation on site about the underground pollution and the transport of pollutants in connection with rainwater percolation.

Contractor: The Royal Veterinary and Agricultural University,
Agrovej 8, DK-2630 Tåstrup, Denmark.

Contract no: 218-77-1 ENV DK

Project leader: S.T. Jakobsen

Title of project: Effects of fly ashes on nutrient uptake rates, growth rate and mineral content, including heavy metals of plants

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Objective

Fly ashes deposited in concentrated amounts on small areas may give pollution problems. A better solution might be to use these wastes in smaller concentrations on agricultural areas. More information on the effect of fly ashes on yield and on quality of plants is needed.

In previous experiments, it was found that solid wastes from a destry-gas-sludge plant mixed in different soil types had a negative, a neutral or a positive effect on growth rate, nutrient uptake rates and on yiels of barley. From these experiments, it was concluded that the effects of the wastes was related to their influence on nutrient balances in soils, especially regarding the plant nutrient calcium. Fly ashes might have a similar effect.

By Ca-deficiency in plants the root system may be ineffective for a few days and the roots may become brown. New roots will develop from the ineffective roots. The new roots will work for some days, in which nutrients from the soil solution are absorbed. Then these roots also may lose their ability to take up calcium and a deficiency of this nutrient in the root will occur, because calcium is not retranslocated from the top to the roots.

Interaction between calcium and phosphate is of importance. Affinity between the two nutrients provides uptake of calcium when phosphate is absorbed by plant roots. But high concentrations of calcium in the soil solution or at the surfaces of the plant roots, may precipitate phosphates as sparingly soluble calcium phosphates. Therefore an acute stage of Ca-deficiency may occur. On the other hand, a continual movement of Ca against the root tips is needed. Fly ashes might have an influence on these reactions, because the fly ash may contain anions, which will keep cations into solution and thereby secure a mobility of cations to the roots in a period, where nitrate may be excausted from the solution.

Materials and methods

The effect of fly ashes on the nutrient balances in soils was measured in plastic pipes 40 cm in length and 8 cm in diameter. Different soils were mixed with different fly ashes and filled into the pipes. Deionized water was let into the bottom and drained off the top in portions of 100 ml. The extracts were analyzed for content of P, K, Na, Mg, and Ca.

Growth rate, nutrient uptake rates, yield and mineral content of crops were measured in pot experiments in the summer 1977 and 1978, and also during the winter 1977-78 in a greenhouse. Plant samples of barley were taken 5 times a week during a period which, for the summer 1977, lasted most of the growing period and in 1978 covered a 3 week period. The plant samples were also grown. Each year, 4-5 different soil types were used as the growth medium.

In the experiment in 1977, two sandy soils, one loamy and one humus soil were used. In 1978, one of the sandy soils was replaced by another sandy soil which had not been cultivated before and in which we knew that Ca-deficiency probably would appear in the plants unless precautions against such a deficiency were taken. That soil was used both with and without the incorporation of $CaCO_3$ into the soil.

Three fly ashes were applied:

1. Fly ash from a refuse disposal plant.
2. Fly ash from an oil-fed power station.
3. Fly ash from a coal-fed power station.

The ashes were analyzed for content of nutrients and of heavy metals.

Results

Extractions

Effects of the fly ashes employed on the nutrient balance in soil solution are given in report no. 1. Fly ash no. 1 from a refuse disposal plant had a high content of chloride, especially as sodium and potassium salts. This affected the soil solution very much and the concentrations of the chloride and cations Na, K, Ca and Mg were much higher in the soil solution during the growth period where fly ash 1 was incorporated in the soil. Fly ash no. 1 had a small increasing effect on pH in the soil solution while the effect on the concentration of P in soil solution was generally negative, even if the fly ash contained 0.9 % P.

Fly ash no. 2 had a very high content of Fe. This had a generally decreasing influence on the concentration of P in the soil solution. The activity of calcium increased, but less than with the incorporation of fly ash no. 1. The pH decreased with the incorporation of fly ash no. 2.

Fly ash no. 3 had a small increasing effect on pH as that of fly ash 1, but the effect on activity of Ca in soil solution was small and the effect on P was also less negative than that of fly ash 1.

Pot experiment in 1977

Results from the pot experiment in 1977 are shown in reports no. 2 and no. 3. The growth rate fluctuated as found earlier where the uptake of Ca was interrupted.

Fly ash no. 1 had a small effect on nutrient uptake rates and growth rates in three of soils until mid-June. From the time of earing, the growth rate in these soils was increased when fly ash no. 1 was incorporated. The increased growth rate resulted in a significant increase in grain yield.

In the humus soil from Aamosen, the effect of fly ash no. 1 was much different. Early in the growth period, Mn-deficiency was observed in all treatments, but the symptoms were especially extensive where fly ash 1 was employed and the growth rate and nutrient uptake rates decreased in relation to the other treatments. The grain yield was found, but in the loamy soil and the humus soil, significant yield reduction was found.

Fly ash no. 3 had only small and insignificant effects on growth rate, nutrient uptake rates and yield.

Supplementary pot experiment in the soil from Aamosen

The large effect of fly ash 1 on the occurence of Mn-deficiency could be explained either by a decreased availability of Mn in the soil, or by a decreased capability of the plant roots to absorb Mn from the soil. We thought that fly ash no. 1 had a disadvantageous influence on root development and root activity in this soil, which might be the cause of the yield depression. This assumption was confirmed in a supplementary pot experiment performed in a green house and it is described in report no. 4.

Pot experiment in 1978

Results from the experiment are described in reports no. 5 and 6. The following five alterations in the experimental plan influenced the results in relation to the results found in the first year:

1. The Ca-deficient soil from Sdr. Omme was included.
2. The humus soil from Aamosen was heavy fertilized by a Mn-fertilizer.
3. Five different crops were grown.
4. Two NPK-fertilizers containing different amounts of P and K were used.
5. The temperature in early spring was very low in 1978.

In the soil from Sdr. Omme a very pronounced Ca-deficiency occurred in control treatments and in treatments with applied fly ash no 2. The growth of barley was stunted. The seedlings of lettuce, carrots and tomatoes died back. Application of fly ash no. 3 increased the growth of barley and some plants of the other plant species survived. Application of fly ash no. 1 had a pronounced effect on that soil. The barley plants grew favourably throughout the season and the highest yield in the experiment was found in these treatments.Fly ash no. 1 had also a pronounced effect on growth of other plant species.

Application of $CaCO_3$ also improved the early growth of barley. But this improvement was not sustained throughout the season unless fly ash no. 1 was applied also.

In the humus soil from Aamosen the fly ashes no. 1 and no. 3 had a positive effect on the yield of barley grain. This was opposite of the results found in 1977 and it might be because no Mn-deficiency was observed in 1978.

In the second sandy soil and the loamy soil fly ash no. 1 reduced the early uptake of P and Ca and therefore also the growth rate. This was different from that found in 1977 and it is supposed to be caused by an interaction between a stunted root development owing to the low temperature in the spring and Ca-deficiency in roots. Later in the growing period the plants might grow faster where fly ash 1 was applied, but that could not surpass the early growth reduction in the sandy soil and the final yield was reduced.

Fly ash 2 reduced the uptake of P obviously because of precipitation of phoshpates by the high content of Fe in fly ash no. 2. This had both effects on uptake of P and Ca and a reduced root activity.

Heavy metals

The analyses of plants for content of heavy metals are under performance. The results will be shown and discussed in report no. 7.

Conclusion

Effect of fly ashes on nutrient uptake rates, growth rate and mineral content is dependent on their effects on the composition of the soil solution which may be tested in extract experiments as proposed in the present project.

It was found that three parameters in the soil solution were important:

1. activity of calcium

2. Concentration of phosphate
3. Concentration of other anions e.g. Cl, SO_4 etc.

The three factors are mutually dependent on each other. A high content of anions may increase the activity of calcium, which may decrease the concentration of phosphate. Later in the growing period the anions are important in maintaining an adequate concentration of cations in soil solution.

The factors are of different importance for plant growth in various soils and the effect may shift from year to year depending on climatic conditions.

It was found that fly ashes should only be incorporated in well fertilized soils without any problems in cultivation.

List of reports

Reports from the project are prepared as Meddelelser (Communications) from: The Department of Soil Fertility and Plant Nutrition. The main title of the reports are the same as the title of project:

Effects of fly ashes on nutrient uptake rates, growth rate and mineral content, including heavy metals of plants:

1. J.Mehlsen and S.T. Jakobsen (1978): Results from extraction. Meddelelse no. 1108.
2. J. Mehlsen and S.T. Jakobsen (1978): Dry matter yield as a function of time. Meddelelse no. 1109.
3. S.T. Jakobsen and J. Mehlsen (1978): Nutrient uptake and mineral content during the period of growth. Meddelelse no. 1110.
4. S.T. Jakobsen and S.W. Bille (1978): Growth rate and nutrient uptake by barley in Aamosen soil as influenced by incorporating fly ashes. Meddelelse no. 1111.
5. S.W. Bille and S.T. Jakobsen (1978): Dry matter yield of barley, lettuce, yarrow, carrot and tomato as a function of time. Meddelse no. 1112.
6. S.W. Bille and S.T. Jakobsen (1979): Nutrient uptake and mineral content during the period of growth by barley, lettuce, yarrow, carrots and tomatoes grown in soils incorporated with fly sahes. Meddelse no. 1113. (In preparation).
7. Ib Johnsen and S.T. Jakobsen (1979): Uptake and content of heavy metals in higher plants grown in soils incorporated with fly ashes. (In preparation).

RESEARCH AREA 4 : PROTECTION AND IMPROVEMENT OF THE NATURAL ENVIRONMENT

TOPIC 41a : ECOSYSTEMS ECOLOGY

Contractor : CEVENNES NATIONAL PARK

Contract n° : 220 - 77 - 05 ENV F

Project leader : E. LEYNAUD

Title of the project : Research into the organisation and evolution of the ecological unities in the Cevennes national Park with a view to establish a plan of control and management of the territory

Preliminary remark : The contract cited above not having yet reached completion, this report concerns only the partial results obtained at the biginning of 1979.

Objective of the research : The goal of the research programme undertaken by the Cevennes national Park is to furnisch the administrative council of the institution with a scientific basis for the programme of control and management of this territory.

The aims to be achieved by the pluridisciplinary studies that have been undertaken, have been defined in such a way that their results blend with a research procedure comprising the following stages :

- definition of the conservation aims to be considered bearing in mind the imperative aims imposed by the law creating the national parks and the particularities of the areas studied.
- determination of the factors which are liable to have a certain influence on the aims and an reflection on the ways and means to limit this influence.
- analysis of the evolution of the factors according to two types of scenario ; the first called "tendentials" supposing a continuation of the action of the socio-econimic agents concerned according to the logic proper to each of them, the second known as "intentionals" supposing the existence of a public determination united in an aim for a harmonious economical development of the area (1).

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(1) it should be noted that the Cevennes national Park does not own the territories over which it exerts its action ; it is merely an institution moderating the actions of the socio-economic agents and its role is to see that the conservation aims that led to its creation are respected.

- confrontation of the present situation and the simulated evolutions as represented by the above scenarios with the conservation aims.
- proposal of the measures necessary to remedy the effects thus detected, that are contrary to these aims.
- establishment of a set of measures that satisfy both the conditions necessary for the observance of the conservation aims and also the assumptions of the chosen development scenario.

Materials and methods : As the means put into operation did not permit a simultaneous study of the whole Park, this territory was divided into 4 regional unities, which differ from each other in certain of their characteristics, whether physiographical, socio-economical or cultural. To the moment, only the Mont-Lozère - North Bougès regional unit has been studied. This regional unit is composed roughly speaking of two parallel crystalline mountains ranges, stretching from west to east over a distance of 30 kilometers, and separated by the bed of the Tarn. The altitude varies from 800 m to 1 700 m. The rock is mainly granit with a little schist and some shreds of limestone sedimentary rock, The climate is severe, the winters long and the mediterranean influence manifests in the violence of some rainfalls, especially along the eastern extremeties of these mountains. The population clustered in hamlets, reached 20 inhabitants per square kilometer a little more than a hundred years ago ; it now totals only 5 inhabitants per square kilometer. The agricultural economy is turning more and more towards bovine stock-keeping (milk on the northern slopes of the Mont-Lozère, meat on its southern slopes and on the northern slopes of the Bougès). Sheep transhumance from the southern plains of the Languedoc has considerably diminished . Whortleberry picking constitutes an economical supplement . At the end of the 19th century , the state undertook the reafforestation of lands extremely impoverished by overgrazing. Another period of reafforestation occured about 1935. Finally over the last ten years, the state has replanted trees along certains streches abandonned by ovine transhumance. 35 000 hectares of this regional unit are within the Cevennes national Park ; 50 % of this surface is woodland (remains of ancient beech and fir forest, natural conquest of moors by the scotch pine, lands artificially replanted) ; 20 % is grassland and 30 % moors and scrublands.

The regional unit was divided into ecological units. The ecological unit, which is the basic spacial unit of this research, designates a particular geographical area in which the geological nature of the bedrock , the mesoclimate and the aspect of the vegetation manifest a certain homogeneity. The limits of the ecological units were determined with the aid of the geological maps, the maps of thermic classes compiled in 1973 by ROUSVOAL, the map of the aspect of the vegetation elaborated in 1974 on a scale of 1/25 000 (published on a scale of 1/50 000) by the Centre phyto-sociological and ecological studies in Montpellier, and a campagne of observation carried in the field. Streams were considered as a special ecological unit.

The list of essential conservation aims as regards the regional unit "Mont-Lozère - North Bougès" was drawn up as follows :

a/ to avoid indulgence in those practices liable to provoke an upset in the balance of nutritive elements ;

b/ to avoid impoverishment of the fauna and eventually to enrich it by reintroductions ;

c/ to maintain the quality of aquatic conditions ;

d/ to avoid modifying the caracter of landscapes that are considered remarkable ;

e/ to avoid the disappearance of sites of scientific interest.

A socio-econimic study then supplied an initial approach to the human composants liable to influence the evolution of the ecological units in the regional unit (this study listed and described the socio-economic agents that affect, either directly or indirectly , the ecological units ; it explained the way these agents acted and revealed the logic of their actions).

It was by bringing together the conservation aims and the information furnished by the socio-economic study and by the field observations, that enabled the description and delimitation of the ecological units, that the choice of composants for constructing the systems, representing the organisation of the ecological units so as to emphasize the role of the main factors influencing the conservation aims, was effected.

At this stage of the procedure, it was necessary to undertake several specialised studies in order to increase knowledge about some composants so as to be able to elucidate certain parts of the systems elaborated as described above, or else to check their validity. These specialised studies also supplied elements for explaining certain scenarios.

Then a modelisation of those parts of the systems representing the main mecanisms influencing the conservation aims was undertaken in order to simulate the different evolutions possible in the ecological units within the next 20 years, starting from a certain number of scenarios.The repercussions of these evolutions on the conservation aims were evaluated and the scenario classified according to whether they lead to evolutions compatible with the conservation aims or not.

This modelisation is based on information that has been collected and combined on a triple computarized card-index : the card-index "owners", "farmers" and "plots". The information to be marked on the card-index "plots" was chosen with regard to the models in view ; it concerns the present condition of the plots and their past condition in as much as it is liable to directly influence the possible evolutions of the next 20 years.
A set of measures meant to favour the scenarios liable to bring evolutions compatible with the conservation aims has been proposed to the administrative body of the Cevennes national Park.

Apart from the general procedure of the research, some specialised studies, undertaken to analyse certain composants in particular, used methods worthy to be noted. So specify :

- the DAGET, POISSONET (1965-68) method for the determination of the present pastoral value and the optimal pastoral value of ecological units either cropped or grazed. This method enables one to calculate a pastoral value represented by a global quality estimate (from 0 to 100) by taking into account the floristic composition noted on the spot and the relative forage values of species to be found in the bibliography ;
- an attempt to perfect a new method for a rapid determination of the potential production of ligneous plants, based on the measurement of parameters of the structure of the existing plants, effected with the aid of lineal readings.
- the use, on a large scale, of the method of sample trails in order to evaluate the influence of different variables on the distribution of certain animal species, all through the year, within the ecological units.

Results : (As already mentioned in the preliminary remark, only partial results are concerned at this stage of the research).

a/ results concerning the balance in nutritive elements :

The analysis of forages in the regional unity revealed its extreme poverty in mineral elements. It would seem that the soil was completely devitalised. It is most likely that transhumance which dates from a long time and was practised formely on a large scale, led to sheep excess-grazing and the systematic exportation of nutritive elements, through the practice known as "manuring nights". These factors can only have worsened on initial situation determined by the poverty of the bedrock in mineral elements necessary to life. The little use now made of pasture lands and their bad management used for cattle-grazing , apart from the fact that they favour the predominance of the least appetising plants, are incapable of preventing the development of ligneous species such as *Cytisus purgans* which necessitates the present practice of the frequent burning which leaves a large part of the land bare, and open the erosion. A pastoral experiment in process, should reveal if it is possible or not, apart from employing mechanical means - scything or gyropulverizing - to manage the strips untouchable by tractors (sleep slope, high rock content) as in such a way as to ensure that the herbaceous formations constitute a stable plant formation, owing to the utilisation of pasture rotation, completed, from time to time, by a burning of refusals at the end of the summer, when the herbaceous strata completely covers the soil and the biomasse of combustible materials constituted by the stems of the graminaceae is not such that the temperature reached by the fire might harm the humus. This experiment linked with the method, called "the small watershed technique" (BORMANN and LIKENS - 1977) should enable one to judge the influence of such and such a pastoral practice on the balance of nutritive elements.

b/ results concerning faunistic diversity and quantity :

Factors liable to influence the faunistic density are taking off, disturbances, modifications in the vegetation and badly managed attempts of reintroduction. Without waiting for the results of the study in progress on the role of different environmental variables on the spatio-temporal distribution of animal populations, it has been estimated that the vegetation constituted one of the most important environmental aspects for most animals. This is why a model was constructed enabling one to simulate the evolution of the vegetation under the combined actions of the farmers and the private and state foresters. The intended actions of state foresters are revealed in the forest management plans for succeeding periods of 20 years. On the other hand, assumptions had to be made on farmers and private foresters' activities. These assumptions, grouped together into scenarios, take into account :

- for farming, the present under employment of pasture-lands, the lack of successors for certain farms, systems of farming that seem the best adapted to the region in the future, taking particularly into account the poorness of forage, the narrowings to the normal developpment of the present farm-estates and to the new farmers' settlement formed by the high price of the land and of the building of modern stables, the awailability of meadows which can be mown.

- for private forestry, the location of the areas which are able to produce more than 5 m3/ha/year (limit for the intervention of the National forest Found).

c/ results concerning the quality of aquatic life :

The different surveys which have been realised, have not revealed a tangible effect of the present resinous forests upon the quality of the rivers-waters which drain them. A much more precise approach to this phenomenon should be necessary in order to finally state which effect a massive reafforestation with resinous trees in the regional unit could have upon aquatic life.

Pollution marks are quite slight and are very localized. In the days to come, only the opening of uranium mines could change the present state of things (an examination of its impact would be then necessary).

Some consequential effects of the erosion upon aquatic life have been noticed but in one single case only.

Flow disturbances, occuring during low-water times, are linked to the possible setting up of micropower-station and might be also linked to the possible draining of some wet areas and to the extension of the irrigation-practice. It is why complementary researches on the cycle of water within the regional unit should be envisaged.

The streams of the regional unit are greatly exploited by fishing (high percentage of fishmen who are strangers to this part of France) and the annual trout-catchings represent an important proportion in the "net production" ; the stocks do not seem yet to be in danger but it is advisable to be vigilant.

d/ maintenance of the features of landscapes that are considered as remarkable :

The model allowing to simulate the evolutions of the vegetation will be used linked to a study on landscapes which by the way is going to start in order to estimate the consequential effects of these evolutions upon landscapes.

e/ maintenance of sites of scientific interest :

The inventory of the remarkable botanical sites, as well as the one of wet areas and forests which are especially interesting, has been realised.

Conclusions and comments : Except some special methods that have not been very often used up till this day, especially the one which enables one to determine very quickly the ligneous potential production, or the use on a large scale of sample trails to estimate the effect of the different parameters upon the spacial and temporal distribution of the mammals' populations, the originalest aspect of the present research seems to be its attempt to make work together, on the field, from the stage of the observations-gathering, experts from all the disciplines in order to converge on the mutual elaboration of a model which enables one to simulate the evolutions of vegetation under the combined actions of the different socio-economic agents which are concerned and to estimate the consequential effects upon aims of preservation which are preliminary defined. This approach to the subject has lead every one of the disciplines to work with the same scales (time and space scales). The ecological unit has formed the spacial unit of thinking whereas the plot has been used as basis to the gathering of data (a part of a well defined ecological unit is almost in every case a set of several plots). The use of plots has allowed us to link the spacial unit to the units of decision (land-owners, farmers ans foresters). Hence the interest of the triple computarized card-index that has been realised for this research as well as for a further management.

Finally, one has to emphasize the advantage which can be found in carrying on certain studies to precise some parts of the model. In particular we think of the pastoral experiments , of the analysis of the balance of the nutritional elements in some ecological units, of the cycle of water according to the draining of wet areas and to the irrigation of some meadows which can be mown, of the effect of a large reafforestation with resinous trees on the aquatic life and above all of the extension of the studies which enables one to link, in a better way, the existence and even the importance of the wild vertebrates populations to the data of the environment.

LIST OF REFERENCES TO PUBLICATIONS ON CONTRACT RESEARCH

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. CAMUS O., septembre 1977 - Les systèmes de production agricole dans une région à basse densité de population : le Mont-Lozère et le Bougès nord.

. CURMI J., octobre 1978 - Estimation du revenu agricole des systèmes de production de l'unité régionale "Mont-Lozère - Bougès nord".

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. DEJEAN R., mai 1978 - Réflexion sur la mise au point d'une méthode pour la détermination rapide de la productivité des peuplements forestiers.

. DEVES B., octobre 1978 - Etude de la pêche dans les cours d'eau de l'unité régionale "Mont-Lozère - Bougès nord".

. FONTANEL et NEGRE, 1978 - Cartographie et inventaire écologique des zones humides du Parc national des Cévennes.

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. MACCAGNO Y., octobre 1978 - Inventaire des sites botaniques remarquables de l'unité régionale "Mont-Lozère - Bougès nord".

. MAIGNE Ph., octobre 1977 - Etude socio-économique de l'unité régionale "Mont-Lozère - Bougès nord".

. MAIGNE ET PILLET, mai 1978 - Recherche sur l'organisation et l'évolution des unités écologiques de Parc national des Cévennes en vue d'établir le plan d'aménagement et de gestion de ce territoire (objectifs et démarche générale - suite au document d'octobre 1977).

. PILLET Ph., octobre 1977 - Présentation du programme de recherche (objectifs et démarche générale).

. PLAIGE V., octobre 1978 - Contribution phytoécologique à l'étude pastorale de l'unité régionale "Mont-Lozère - Bougès nord".

To be publisched :

. DEMAISON A., - Etude de l'entomofaune et des arachnides de l'hypergaion des formations herbacées et des landes de l'unité régionale "Mont-Lozère - Bougès nord".

. KERMABON J. et JAFFUEL R., - Etude des peuplements d'oiseaux nicheurs de l'unité régionale "Mont-Lozère - Bougès nord".

. KERMABON J. et BONNET J., - Les rapaces diurnes de l'unité régionale "Mont-Lozère - Bougès nord".

. KERMABON J., - Approche écologique du peuplement en mammifères de l'unité régionale "Mont-Lozère - Bougès nord" - Application de la méthode des itinéraires échantillons.

Contractor: University of Copenhagen
Contract n^o 251-78-1 ENV DK
Project leader: F.E.Eckardt
Title of project: Use and Conservation of the Plant Cover in Greenland

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Objective of the project.

The objective of the project, as stated in the contract between the University of Copenhagen and the European Economic Community, is to provide criteria for good management of the plant cover in Greenland. To achieve this goal, research is conducted with a view to 1) *relating* CO_2-exchange of various types of vegetation to weather and plant canopy structure, 2) *identifying* limiting factors in plant dry matter production, 3) *assessing* plant cover behaviour under various grazing pressures and 4) *generalizing* experimental results so as to make them applicable to larger land surfaces. In structuring the project this way, due consideration is taken to the vastness of Greenland, the instability of its climate, the scarcety of available plant physiological data, and the fact that the arctic plant cover, in general, is highly sensible to man-induced interferences, overgrazing in particular.

Methods and Materials

In order to *relate CO_2-exchange to weather and canopy structure,* research is conducted at two levels of perception, at leaf level and at ecosystem level. At leaf level, CO_2-exchange is studied as a function of temperature, photon flux density and CO_2-concentration, and results entered into a model which permit the prediction of CO_2-absorption as a function of these factors, as well as of the diffusive resistance of the stomata. At ecosystem level, studies include measurements of, on the one hand, photon flux densities within the plant cover as a function of canopy structure, on the other, CO_2-exchange below and above the soil surface under naturally varying conditions. All data enter into a larger plant production model.

With respect to *the identification of limiting factors in dry matter production,* other than temperature and photon flux density, plant and soil samples are analysed chemically and the results compared with manuring experiments carried out by local farmer's associations. Particular emphasis is put on the nitrogen cycle without, however, going into details as regards to the physiology and biochemistry of the bacteria that effects the fixation of the gas. Great importance is also attached to the study of the phosphorous cycle. Results are added to the production model eclarged for the purpose.

The assessment of plant cover behaviour under various grazing pressures is essentially based on simulation experiments. Grazing is either simulated in actual field experiments by clipping and the effects on CO_2-absorption actually measured, or the same experiment is performed on the computer using the enlarged production model. In both cases comparisons are made at later stages with genuine grazing situations.

Generalization of results is already, to a certain extent, assured thanks to the modeling procedure. On a larger scale, however, remote sensing techniques are brought into use. It implies that smaller land surfaces are mapped from a phytosociological point of view and compared with corresponding satellite imagery. To attain this, a common computer-aided classification of spectral signatures provided by the satellite and phytosociological relevées obtained in the field, is sought.

The principle of the production model is to link plant production to plant species and climate. Plant production is calculated as the sum total of daily carbon absorption, as it depends on canopy structure, CO_2-exchange of leaves and stems and on the whole set of weather situations that exist during the growing season. Information on these weather situations are obtained from meteorological stations combined with data from satellites, and a specially built climate generator, using geographical coordinates, time, slope, azimut and type of cloud cover as input variables.

Most of the measuring equipment used is specially designed for field work in Greenland and include controlled-environment plant-chambers of different sizes, scanners for the evaluation of photon flux densities within the plant cover and a micrometeorological mast complete with sensors for the study of the vertical fluxes of CO_2 and H_2O in the lower atmosphere. Signals from the various sensors and CO_2 and H_2O analysers enter a 30 channel data-logger for final recording on cassette.

Field work is essentially confined to two areas, the Søndre Strømfjord district, just above the polar circle, close to the western edge of the icecap, and where roam herds of reindeer and muskox, and the sheep farming district close to Julianehåb, at the southern end of Greenland. In these areas, a number of dominant, or otherwise important, plant species are selected for the physiological investigations.

Results

As plant physiological studies in Greenland, for climatic reasons, are limited to a three month growing season in summer, this report only covers one field campaign in 1978, the first, which was carried out in the Søndre Strømfjord area. Research included studies of heat budget, surface temperature, carbon budget and plant cover.

Heat budget studies comprised measurements at five minutes intervals of global radiation, net radiation, heat flux in bare and vegetation covered soil, as well as of the temperature in 6 depths of the soil, during the whole summer. The ratio between latent and sensible heat flow in the air was determined by means of the Bowen ratio method in preliminary experiments.

Surface temperature of the vegetation was measured radiometrically during the overpasses of the Landsat 3. Temperatures on south slopes at noon could attain $45.1^oC \pm 2.5$ at the same time as the temperature on the north slopes remained at $13.5^oC \pm 1.9$. The thermal channel on the satellite unfortunately developed technical problems during the field campaign, but it seems now likely that some data, although of rather low quality, will be available. They may prove highly valuable for deliminating areas with, for example, early plant activity in spring. Early spring is the difficult period for most ungulates.

Most time, by far, was devoted to the study of *the carbon budget of the vegetation* as it comprised determination of the structure of plant canopies, standing crop, photon flux density within the plant cover, leaf resistance and CO_2-exchange of various plant organs, as well as of soil respiration.

Photon flux density measurements were carried out under clear sky in stands of *Poa pratensis* L., *Calamagrostis lapponica* (Wbg.) Hartm., *Betula nana* L. and *Salix glauca* L. For *Salix*, measurements were also carried out at three levels within the plant cover before and after leafing. In addition, a few measurements were made under dense cloud cover. Special emphasis was put on the determination of CO_2-exchange at leaf and twig level as a function of photon-flux density, as this information is essential for the building of the larger production model. This function was established for the following species: *Salix glauca*, *Betula nana*, *Ledum palustre* L. and *Rhododendron lapponicum* (L.) Wbg. CO_2-exchange of the whole plant cover was measured with the largest plant chamber in *Salix glauca*, *Poa pratensis* and *Calamagrostis lapponica*, with clipping of the plants at various heights serving as a substitute for grazing. Change in standing crop with time was followed from the beginning of the growing season in *Salix glauca*, *Betula nana*, *Carex supina* (Wbg.) and *Calamagrostis lapponica*.

A total of 500 phytosociological relevées were made in the field, each providing information about the phanerogams present and their abondance-dominance, as well as the altitude, slope and azimuth of the site. An area of 10 km x 10 km was mapped from a phytosociological point of view after prior, although provisional, classification of the relevées. Testing of this classification is presently being made by means of the Euclidean distance and the reciprocal averaging techniques.

As regards the mathematical modelling work, two submodels are available: 1) An irradiancy model which makes it possible, for a given time and latitude, to calculate, on clear days, the direct and indirect radiation received by a surface with a specified slope and azimuth, and for a given atmospheric absorption coefficient, and 2) A leaf model which, for a given canopy structure and a given relation between CO_2-absorption and photon flux density at leaf level, permit the calculation of the total CO_2-absorption by the canopy as a function of incoming direct and indirect radiation.

Conclusions and additional comments

Research is progressing in a very satisfactory manner and the development of the plant production model and the collection of data will continue as planned. Field work in 1979 will take place in Southern Greenland with, as participants, Lisbet Heerfordt, Holger Morell Jørgensen, and the project leader.

The project is financed by the Institute of Plant Ecology of The University of Copenhagen, the Danish Natural Science Research Council and the European Economic Community. Other, and most valuable aid has been received from the civil and military authorities in Søndre Strømfjord, Christian Rovsing Ltd., the Botanical Garden in Copenhagen, the Geological Survey of Greenland, The Commission for Scientific Research in Greenland and the Electronics Department at the Risø National Laboratory.

Publication

F.E. Eckardt - Solenergien og den fotosyntetiske produktion, in *Forskning i Grønland*, no. 1, side 8-11 (1978).

TOPIC 41b : OZONE SHIELD DEPLETION

Contractor : University of Reims, France
Contract n° : 300-77-7 ENV F
Project leaders : P. Connes, P. Jouve and G. Amat
Title of project : Fourier transform infrared spectrometer for the study of minor constituents of the atmosphere

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OBJECTIVE OF THE RESEARCH

The remote monitoring of atmospheric pollutants depends on the detection of the absorbed or emitted electromagnetic radiation associated with the pollutant of interest. Remote sensors are termed passive since they rely on natural sources of radiation.

Infrared spectroscopy is applicable to the study of all atmospheric pollutants both qualitatively and quantitatively. The most suitable atmospheric windows for optical studies are in the visible and infrared regions. The atmosphere is not transparent to ultra-violet radiation below 0.3 µm. Aerosol and nebulosity measurements are caried out mainly in the U.V.

In the visible and infrared regions the passive and dispersive method is suitable for the detection of minor constituents over long vertical or horizontal paths and also for precision measurements of these atmospheric concentrations. The most common dispersive intrument used in these regions is the grating spectrometer. In this instrument an increase in the resolution causes a rapid decrease in the luminosity. To detect pollutants which are, for example, highly active radicals, the highest resolution possible is required.

Ten years ago Pierre CONNES et al., in France, developed a new type of interferometer. Associated with this instrument is a very large étendue and the multiplex advantage. We are constructing such a Fourier Transform Spectrometer which will be dedicated to high resolution spectral studies of the atmosphere. The resolution obtainable is approximately 3×10^{-3} cm^{-1} and the spectral range is from 0.6 µm to 20 µm. All the atmospheric pollutants and minor constituents will be detectable and their atmospheric concentrations measurable.

MATERIALS AND METHODS

A high resolution Fourier transform spectrometer to be used in ground based studies of the atmosphere is being constructed. The interferometer will be used in the spectral range 0.6 μm to 20 μm. The general design of the interferometer is similar to the astronomical Fourier spectrometer designed and built by Connes and Michel (1975). In the present instrument 12 cm optics are used and the maximum optical path difference is 3m. Thus the interferometer has a maximum resolution of $3\times10^{-3}\mathrm{cm}^{-1}$ which is significantly higher than that previously available in interferometers, commercial or otherwise, used in atmospheric investigations and should permit the détection and monitoring of many atmospheric pollutants and minor constituents.

The complete optical system is mounted on an aluminium base of dimensions 3 m x 1 m approximately. The interferometer has two entrance apertures, either of which may be used. The entrance optics which consist of a concave mirror and plane mirror, direct the radiation of interest on to the beamsplitter assembly. The beamsplitter which will ideally have 50 % transmission and reflection, directs the radiation to the cats' eye retroreflectors. It is intended to use four different beamsplitters to cover the whole spectral range. The beamsplitting system actually consists of two identical beamsplitters, a splitter and a mixer, which are plane, parallel and of equal thickness.

The beamsplitting coatings and antireflection coatings are identical. However, the reflecting surfaces are on opposing sides to ensure complete optical symmetry i.e. equality of the reflection phase shifts. Also, in order to compensate for the slight lateral displacement of the rays due to refraction in the plates, the beam splitter and beam mixer will be offset by a distance given by $(n-1)/n$ t where n is the refractive index of the plates and t their common thickness.

The reflected and transmitted beams from the beamsplitter assembly are incident on the cats'eye retroreflectors, one of which is fixed while the other is movable. Cats'eye retroreflectors are used because of their insensitivity to tilt. Also they make it possible to obtain perfectly achromatic interferograms, see Connes and Michel (1975). The scanning system for the cats' eye retroreflector will use the slave carriage system proposed by Connes and Michel (1975). The system is servo controlled. The small cats' eye mirrors will be mounted on piezoelectric stacks which will be used to provide both the servo control and internal modulation.

The signal arising from the radiation of interest in the interferometer will be detected using a detector appropriate to the spectral region. For infrared measurements, in order to remove water vapour from the interferometer system, the complete optical system with detectors will be enclosed in a large vacuum chamber. As it is intended to make atmospheric observations the recording time is limited. This will necessitate using the continuous scan method and recording at frequencies <150kHz. The interferometer system will also include a stabilized Helium-Neon laser as the reference source.

A real time special purpose digital computer designed by Michel at Meudon will be used during recording of data to display slices of the spectrum for checking the resolution, signal to noise and even perhaps for detecting interesting features. The complete interferograms will be recorded simultaneously on magnetic tape or disk for detailed analysis at a later date.

As stated earlier it is intended, when the interferometer is operational, to make atmospheric observations in the range 0.6 µm to 20 µm using the beamsplitter and detector appropriate to the particular portion of the spectral region under study. Absorption spectra will be obtained using the sun as source during the day and the moon as source at night. It is also intended to observe the thermal and non-thermal atmospheric emissions during the day and night. Also the visible and near infrared emissions in the dayglow and nightglow will be studied. An initial experiment will be to observe NO_2 in the visible and to look for a diurnal variation. The latter is the subject of much discussion at the present. With the many advantages associated with this instrument, e.g. large étendue, multiplex advantage and high resolution, it is hoped that a valuable contribution can be made to the study of atmospheric pollutants and minor constituents.

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Contractor : Institute of Astrophysics
Univ. of Liège (Belgium)

Contract n°: 302-77-1 ENV B

Project leader : R.J. ZANDER

Title of project : Study of the pollution of the stratosphere by solar spectrometry

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OBJECTIVE of the RESEARCH

The main aim of the proposed research is to make infrared solar observations of very high quality and to analyse these in order to establish the presence, the concentration and the eventual temporal evolution of atmospheric gases which are either primary pollutants or species issued from the photochemical decomposition of these last ones. The observational effort which is being carried out from the ground and by balloon concerns by priority a number of molecules whose studies are likely to help to assess the preoccupations related to the ozone shield depletion problem.

METHODS and MATERIALS

The infrared solar observations have been carried out :

1.- at the International Scientific Station of the Jungfraujoch (Switzerland, 3580 m altitude), using a 7.5 meters focal length grating spectrometer operating in a double-pass configuration with a narrow intermediary slit.

2.- using a 2.5 meters focal length double-pass grating spectrometer on board of a balloon gondola which is launched from the U.S. facility of Palestine, Texas.

When equipped with modern cryogenically cooled detectors (e.g., InSb, HgCdTe, Ga-Ge bolomters), both instruments are capable of spectral resolutions of a few hundredths reciprocal centimeters over the 2 to 13 microns range; they are ideally suited for making infrared observations over narrow spectral intervals into which the species under investigation present characteristic rotational or rotation-vibrational absorption lines.

Till now, regular observations at the Jungfraujoch have allowed to establish the temporal trend of the average concentration of hydrofluoric acid (HF) and of the average column density of hydrochloric acid (HCl) above the site of observation. Measurements concerning CH_3Cl, CH_4 and H_2O are already available; few observations carried out with the 0.01 cm^{-1} spectral resolution interferometer of the Kitt Peak Observatory, Tucson, Arizona, will also be analysed in the present context.

Two balloon flights carried out in September and October 1978 have allowed us to make solar observations from about 30.5 Km altitude; many spectra have been recorded for studying the stratospheric content of HCl, HF, CH_3Cl, CH_4, CO, CO_2, H_2O, O_3, HOCl, ...

RESULTS

The most original results deduced till now from 1978 observations are presented hereafter.

Figure 1 summarizes the results about HF and HCl obtained at the Jungfraujoch. In this presentation, HF has been considered to be uniformly concentrated above 20 Km altitude, while the HCl values correspond to mean column densities for the whole atmosphere above 3.6 Km altitude.

While the tendency to an increase for both HF and HCl was noticed until mid-1978, a significant decrease has been observed in December 1978, especially for HCl; specific dynamical conditions likely to have prevailed during that period of observations need to be investigated in order to explain such a decrease. The two HF maxima (between parentheses) have been associated with the transport of air from the polar upper stratosphere towards mid-latitude lower altitudes.

Figure 2 shows the distribution of HCl deduced from our 1978 balloon observations. Data which were obtained in the afternoon till sunset indicate a decrease, during sunset, of the average content of HCl above 30 Km altitude; the broken lines (1) and (2) correspond, respectively, to high- and low sun observations; extreme modeled concentrations are given by the curves (a) and (b). Our HCl concentrations above 30 Km altitude are higher than those obtained by other groups; below 26 Km, they are significantly lower; the differences being outside of the experimental errors, the few profiles of Fig.2 tend to support the idea that HCl is highly variable in the upper stratosphere.

Our 1978 balloon results on the average concentration of HF above 30 Km correspond to $(4.6 \pm .2) \times 10^{-10}$ parts per volume; in 1976, we had deduced a value of $(3.6 \pm .6) \times 10^{-10}$ ppv.

The analysis of the stratospheric measurements regarding CH_3Cl, HOCl, CO, CO_2 and CH_4 is in progress and will be reported later.

CONCLUSIONS

The results presented here are typical of what is felt to be done regularly and over long periods of time, for a series of species, in order to establish any kind of temporal or seasonal variations. Aside of HF and HCl, the actual Jungfraujoch effort includes measurements in the 7.5 to 13 microns window, about ClO, $CFCl_3$, CF_2Cl_2, HNO_3 and CH_3Cl.

Our 1979 balloon program will be devoted by priority to the study of the total chlorine budget in the stratosphere, e.g. measuring simultaneously the concentrations of HCl, ClO, HOCl (if any), $ClONO_2$ and CH_3Cl. A second series of observations about NO, NO_2, N_2O and HNO_3 will also be attempted in relation with the catalytic cycle of the nitrogen oxydes.

This work is being supported by the Commission of the European Communities, by the Belgian Government through the University of Liège, by the Manufacturing Chemists Association, by NASA and by the Air Force Geophysical Laboratory, Bedford, Mass., U.S.A.

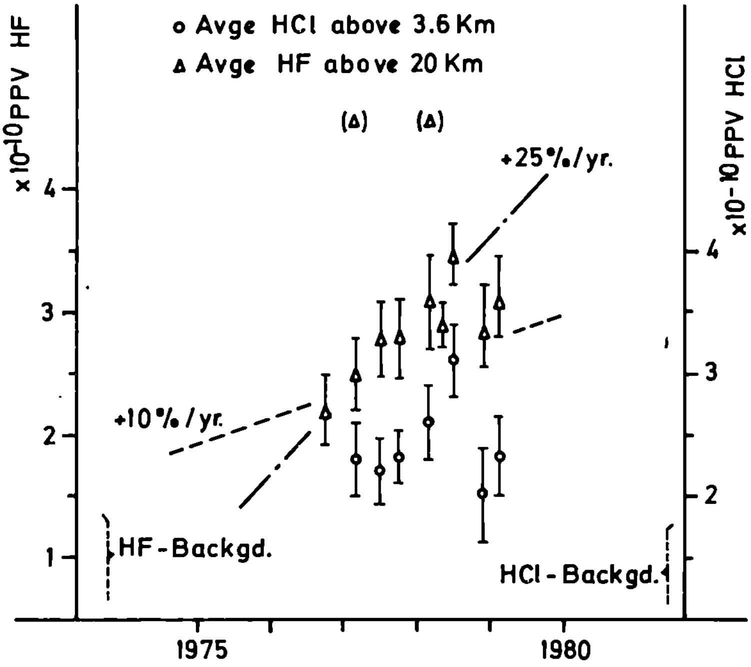

Figure 1 .

FIGURE 1.- Temporal variation of hydrofluoric and hydrochloric acids, as observed at the Jungfraujoch. Note the different ordinate scales and altitude distributions adopted. The two straight lines visualize trends corresponding to increases by 10% and 25% per year.

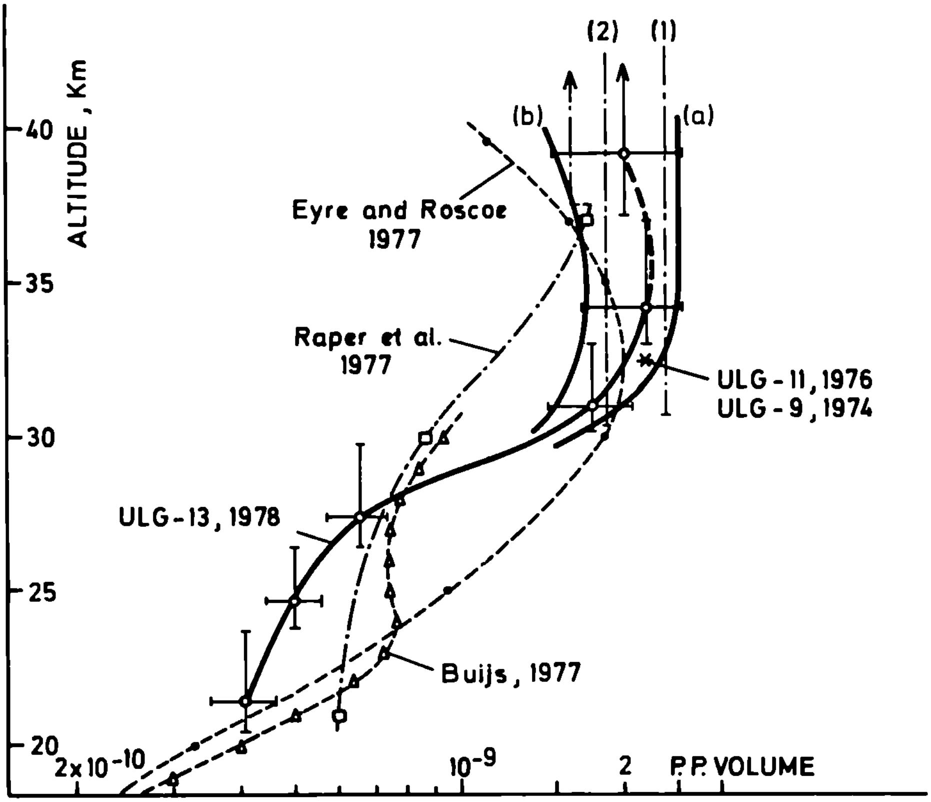

Figure 2

FIGURE 2.- Preliminary HCl distribution as deduced from our 1978 balloon observations. The range of variations from (a) towards (b) corresponds to high- and setting sun measurements.

Contractor :	Institute of Physics, University of Rome
Contract n° :	301-77-1 ENV I
Project leaders :	G. Fiocco, P. Petroncelli and A. Mugnai
Title of project :	Annual variation of the effects of solar radiation diffused by atmospheric molecules, aerosols and by the ground on the ozone photolysis

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1. INTRODUCTION

Several authors have recently studied the effect of the solar radiation diffused by the molecular atmosphere and by the ground on the photodissociation of active atmospheric species (e.g. LUTHER and GELINAS, 1976; CALLIS *et al.*, 1976; ISAKSEN *et al.*, 1977; LUTHER *et al.*, 1978; PITARI and VISCONTI, 1979), due to its relevance in stratospheric photochemical modelling. FIOCCO, MUGNAI and FORLIZZI (1978, FMF hereafter) and MUGNAI, PETRONCELLI and FIOCCO (1979, MPF hereafter) have extended these analyses by considering the effects of the diffuse radiation, respectively on the photodissociation of O_3 and of NO_2, NO_3, HNO_3 and H_2O_2, under conditions of atmospheric turbidity ranging from purely molecular to heavy particulate loads, and assuming different values of the ground albedo and of the solar zenith angle. The effects of diffuse radiation are particularly evident and are felt over a wide region of altitudes for those species, such as O_3 and NO_2, that have appreciable values of the photodissociation cross section in the visible portion of the spectrum.

The physical mechanism encompassed by the analyses of FMF and MPF results from the coupling of two factors, i.e. the ground reflectivity and the scattering and absorption by the overlaying turbid atmosphere. At stratospheric heights, for low values of the ground albedo the presence of the aerosol generally leads, bacause of scattering, to an increase of the total radiation field thus enhancing the photodissociation; on the other hand, at large values of the albedo the main effect of the aerosols is to attenuate the radiation going into and coming from the ground thus reducing the photodissociation.

The contribution of the diffuse solar radiation can be introduced in the analysis with varying degrees of complexity, depending on the amount of detail and accuracy required.

In this report, that is a summary of a paper by PETRONCELLI, FIOCCO and MUGNAI (1979), the daily-integrated photodissociation coefficients of ozone are shown as a function of the day throughout the year.

2. ANALYSIS

The theory and computational techniques utilized to obtain the direct and diffuse components of the solar flux, which combine to give the total flux, are described in detail in our publications, namely FMF and MPF. Here it will suffice to say that through a radiative transfer algorithm for a plane-parallel, absorbing-scattering atmosphere (GRANT and HUNT, 1969; WISCOMBE, 1976 a,b) we have calculated the total flux of solar radiation, $F_{tot}(\lambda,\delta\lambda)$, in the spectral interval $\delta\lambda$ around the wavelength λ and entering a sphere of unit section, as a function of the altitude, z, and of the solar zenith angle, χ.

Thus the photodissociation coefficient of O_3 in the spectral interval $\delta\lambda$, $J(\lambda,\delta\lambda)$, has been obtained as follows:

$$J(\lambda,\delta\lambda) = q(\lambda,\delta\lambda)\ \sigma(\lambda) \qquad (1)$$

where $\sigma(\lambda)$, the photodissociation cross section of O_3 averaged in the interval $\delta\lambda$, coincides with the absorption cross section of O_3 averaged in the same spectral interval and $q(\lambda,\delta\lambda)$, the average number of photons per unit time in the spectral interval $\delta\lambda$ entering a sphere of unit section, has been obtained from the flux computation as follows:

$$q(\lambda,\delta\lambda) = \lambda\ F(\lambda,\delta\lambda)/hc \qquad (2)$$

h and c are Planck's constant and the speed of light respectively. Such results are then integrated over the entire spectral region $\Delta\lambda_T$ where the ozone is photodissociated:

$$J(\Delta\lambda_T) = \Sigma\ J(\lambda,\delta\lambda) \qquad (3)$$

According to the successive degrees of detail introduced in computing the radiative field, the following values for the photodissociation coefficient can be calculated

J_o : is the photodissociation coefficient due to the direct solar flux for a purely molecular atmosphere in the absence of scattering;

J_m : is the photodissociation coefficient due to the total flux, direct and diffuse, when the effects of molecular scattering and ground diffuse reflectivity are included;

J_x : is the photodissociation coefficient taking into account the effects of the molecules, of the ground and of an aerosol load of type x(x = a,c) according to Table I in FMF.

The time integration of J performed over a 24 hour interval, taking into account the time-variation of the solar zenith angle $\chi(t)$, gives the daily integrated value

$$L_{o,m,x}(D) = \varepsilon(D) \int_{1\ \mathrm{day}} J_{o,m,x} |\chi(D,t)|\ dt \qquad (4)$$

where D indicates the particular day of the year (D = 0 corresponds to January 1 and D = 364 to December 31) and $\varepsilon(D)$ is a coefficient accounting for the variation of the Sun-Earth distance.

Elements of control of the atmospheric turbidity are the concentration of aerosols, their characteristics and in particular the imaginary part of their refractive index, all quantities subject to change in the real atmosphere. As regards the aerosol load, we have considered several cases and refer the reader to FMF and MPF.

3. RESULTS

Fig. 1 gives, at five specific heights, the values of $L_o(D)$ which are used as reference. Fig. 2 depicts, in a three dimensional presentation, the $L_m(z,D)/L_o(z,D)$ ratios for a ground albedo $\mathcal{A} = 0.3$. A large excursion of the ratios is noticeable at heights where the O_3 photodissociation is important.

The inclusion of scattering involves a small reduction of the photodissociation in the lower troposphere during winter because of extinction, but in the upper troposhere and throughout the stratosphere, where the effects of photodissociation are most important, scattering enhances photodissociation.

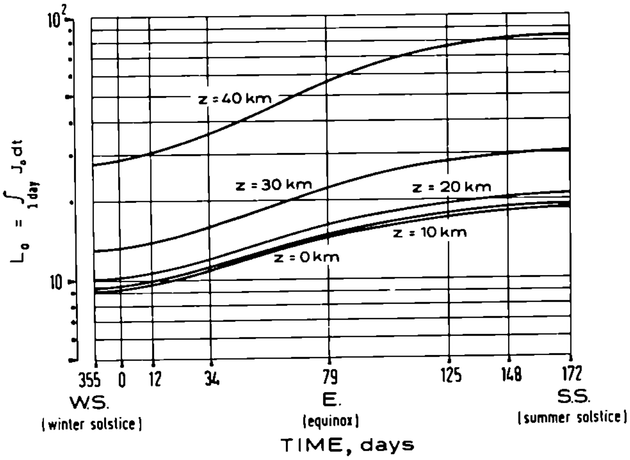

Fig. 1

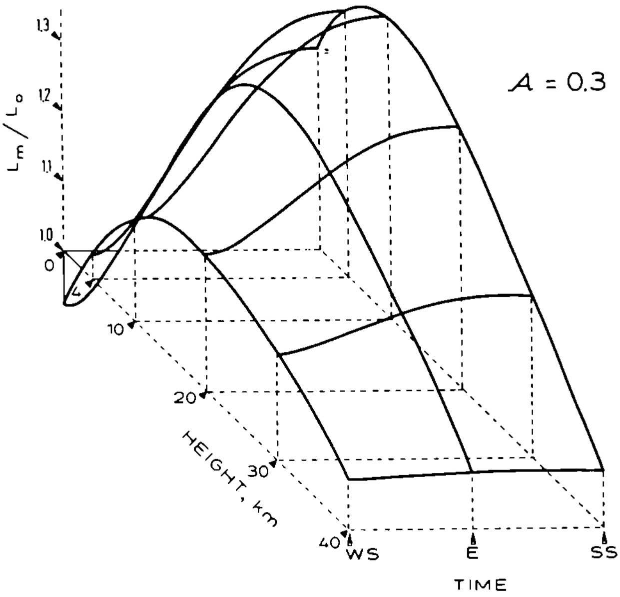

Fig. 2

The increase in photodissociation because of the solar radiation diffused by the ground and by atmospheric molecules is higher in summer than in winter. In fact, in winter, due to the large values of the zenith angle, the direct solar radiation is considerably depleted before reaching the ground, thus diminishing the contribution to the solar diffused field by the surface reflectivity and by the molecular scattering by low-altitude atmospheric layers, where most of the atmospheric mass is located.

Fig. 3 is a three dimensional representation of the ratio L_x/L_m for the two aerosol cases x = a, c and for the albedo $\mathcal{A}$ = 0.3. The ratio L_x/L_m is a correcting factor that can be applied to the previous values of the photodissociation coefficient, obtained for a molecular atmosphere, to account for the presence of an aerosol load.

The case a, representing average atmospheric conditions leads to a ratio L_a/L_m which is larger than unity throughout the stratosphere. The representative surfaces show a deep minimum down to 0.6 at the winter solstice below approximately 4 km; however, at the summer solstice this extinction effect of the aerosols in the lower troposphere is much less noticeable. The effects of the atmospheric particles on the ozone photodissociation are much more intense when a heavy aerosol distribution (case c) is considered.

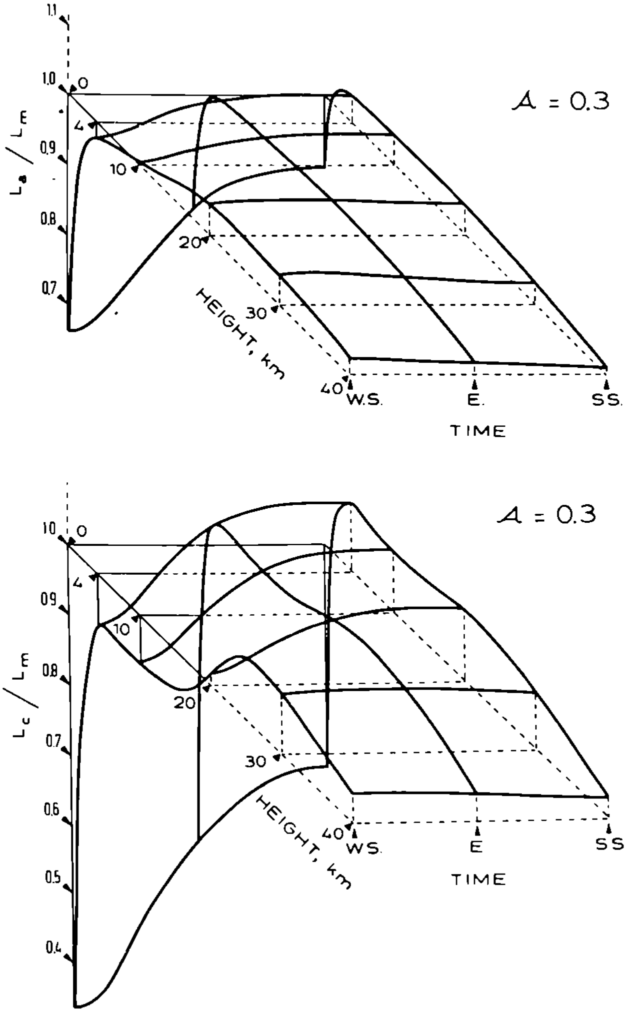

Fig. 3

4. CONCLUSIONS

The main conclusion drawn from our work is that computations of the stratospheric photochemistry aiming at accuracies of a few per cent cannot avoid taking into account the diffuse solar field to the extent of including not only the molecular contribution, but also the effects of the aerosols. The daily-integrated values of the photodissociation coefficient have a strong variation depending on height, season, aerosol load and ground albedo.

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Contractor : Institut d'Aéronomie de Belgique
Contract n° : 303-78-1 ENV B
Project leader : P.C. Simon
Title of project: Measurements of absorption cross section for halocarbon photochemical reactions

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OBJECTIVES OF THE RESEARCH

This research deals with laboratory measurements of absorption cross sections in the 175-240 wavelength range for halocarbon of industrial interest, belonging in the methane, ethane and ethylene families. Photodissociation of these gases in the stratosphere leads to the release of atomic chlorine which in turn initiates a catalytic destruction cycle for ozone. In order to evaluate correctly the chlorine production in the stratosphere, absorption cross sections which can exhibit a temperature dependance, must be measured in a temperature range corresponding to stratospheric conditions.

MATERIALS AND METHODS

The ultraviolet absorption cross sections of halocarbons have been measured as a function of temperature with a classical single beam equipment including a Mc Pherson monochromator model 225 of 1 m focal length, working under vacuum of the order of 10^{-6} torr obtained by means of a turbomolecular pump, and an absorption cell of 2 m optical path which can be evacuated down to 10^{-6} torr by an ionic pump. The light source is a deuterium lamp and the signal at the exit of the absorption cell is recorded via a solar blind photomultiplier tube type EMP 542 P-09-18 with a RbTe photocathode. The temperature regulation down to 220 K of the absorption cell is achieved by the circulation of cooled methylcyclohexane through a double jacket. Thermic equilibrium is usually obtained after 3-4 hours with a temperature gradient inside the cell inferior to 2 K at 220 K measured by means of two thermic resistors fixed on the inside walls, at the ends of the absorption cell. Gas pressure inside the cell is measured by a capacitance manometer MKS Baratron. A complete description of the experiment device has been published by Wisemberg and Vanlaethem (1978).

Gas pressure is initially measured at room temperature and its decrease is followed during the refrigeration process by the manometer. The gas temperature is finally assessed by considering both the condition prevailling at the cell walls and measured by the thermic resistors, and the value deduced from the pressure decrease according to the perfect gas low. Determination of the absorption cross sections is made after several recording of the incident and absorbed light beams measured in the same temperature conditions. Condensation conditions of studied gases can restrict the use of fairly high gas pressure at low temperature and consequently limits the measurements of low absorption cross sections for the less-volatile compounds. In this case, values at 210 K can be extropolated from data obtained at higher temperature as it will be explained later.

RESULTS

The first measurements have been carried out on chloromethanes, chlorofluoromethanes and the methylchloroform. These compounds display continuous absorption in the wavelength interval 175-240 nm with absorption cross section ranging roughly from 10^{-21} to $2X10^{-18}$ cm^2 molecule $^{-1}$. In the case of chloromethanes and chlorofluoromethanes, the progressive substitution of the H atoms of the basic methane entity by chlorine atoms leads to increased absorption and extension of the absorption range towards higher wavelengths while fluorine atoms tend to stabilize the molecule whose absorption spectrum is depressed and shifted towards lower wavelength. With decreasing temperature, the absorption cross sections are lowered by a factor which depends on wavelength and on the chemical nature of the compounds (fig. 1 a and 1b). The analysis of the absorption cross section vs. temperature relationship at a given temperature shows an exponential decrease (fig. 2a) so that extrapolation down to 200 K for less-volatile compounds appears to be reasonable. The temperature effect is most important at low temperature, in the vicinity of the absorption threshold and in the case of highly chlorimated halocarbons. Fig. 2b displays our results obtained with the methylchloroform which, at room temperature are, in fair agreement with those reported by Robbins (1976), but significantly lower than the values proposed by Rowland (1977). Temperature dependance of absorption cross section measured down to 252 K are shown on the same figure.

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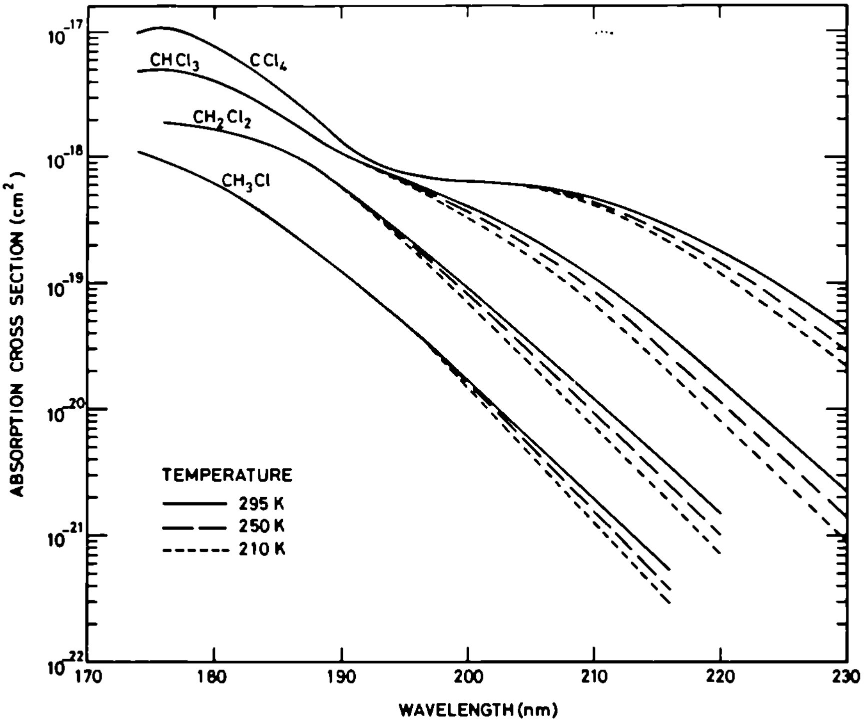

Fig 1a (top) : Absorption cross section of chloromethane vs. wavelength, as a function of temperature.

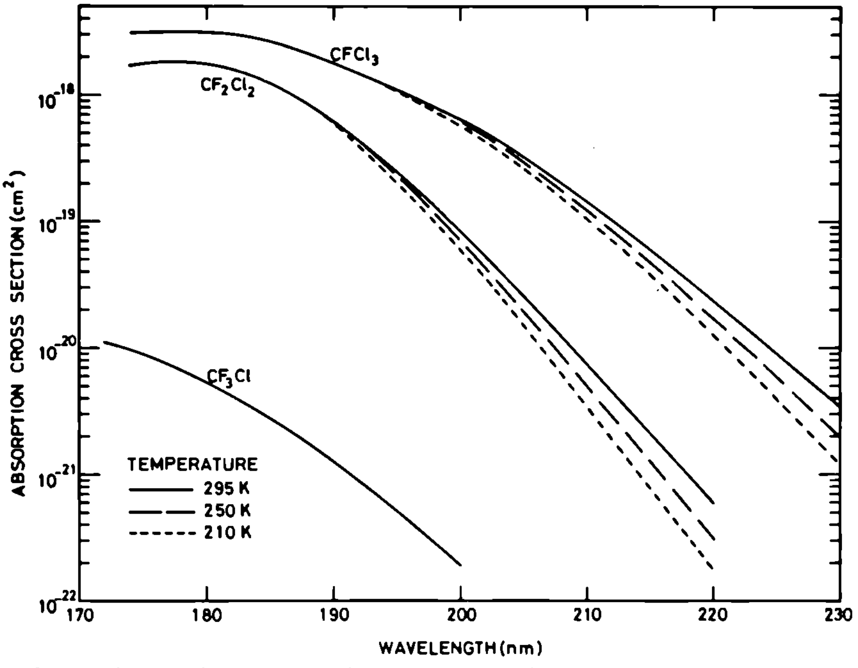

Fig 1b (bottom) : Absorption cross section of chlorofluoromethanes vs. wavelength as a function of temperatures.

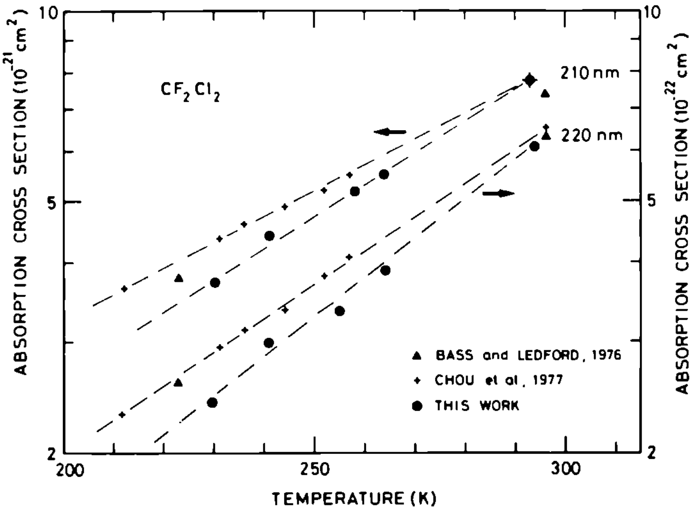

Fig 2a (top) : Temperature dependance of absorption cross section of CF$_2$ Cl$_2$ at wavelength.

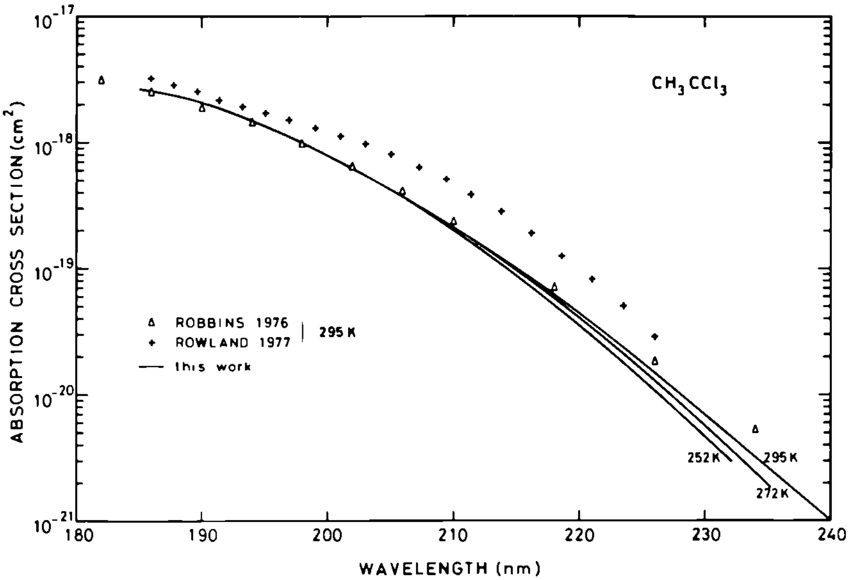

Fig 2b (bottom) : Absorption cross section of CH$_3$ Cl$_3$ vs. wavelength, as a function of temperature.

Contractor :	UKAEA-AERE, Harwell
Contract n° :	304-77-7 ENV UK
Project leader :	Dr. A.E.J. Eggleton
Title of project :	Kinetic studies using molecular modulation spectroscopy of active halogen species involved in the photochemistry of stratospheric ozone

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1. Objectives (for period 1.7.78 to 1.7.79)

(a) To obtain rate data on the reaction of ClO with NO_2 to form Chlorine Nitrate:

$$ClO + NO_2 + M \longrightarrow ClONO_2 + M \qquad (1)$$

Of particular interest is the experimental investigation of the fall-off behaviour of this reaction at pressures above ~10 Torr. Accordingly the pressure and temperature dependence of the rate constant k_1 over the range 25-615 Torr and 274-338 K has been studied.

(c) To obtain rate data for the reactions of Cl and ClO with the hydroperoxyl radical, HO_2.

$$Cl + HO_2 = HCl + O_2 \qquad (2)$$

$$ClO + HO_2 = HOCl + O_2 \qquad (3)$$

It is proposed to measure k_2 and k_3 at total pressures near 1 Atm, since the only other direct determinations of these rate constants have been made at low pressures. The temperature dependence of k_2 and k_3 will also be investigated if time is available.

2. Methods

The kinetics of the above reactions have been studied using molecular modulation ultraviolet spectroscopy, which enables direct observation and monitoring of ClO in the A-X band system near 279.5 nm and HO_2 near 220 nm. Cl atoms were generated photochemically by dissociation of Cl_2 using a square-wave modulated light source. For the study of the chlorine nitrate formation reaction (1), ClO radicals were produced by the reaction of Cl with chlorine monoxide, Cl_2O:

$$Cl + Cl_2O \longrightarrow Cl_2 + ClO \qquad (4)$$

In the presence of NO_2, ClO reacts by reaction (1) and observation of its kinetic behaviour allowed determination of k_1.

The reaction of Cl with HO_2 was studied in the modulated photolysis of Cl_2 in the presence of H_2 and excess O_2, when HO_2 is produced via:

$$Cl + H_2 \rightarrow HCl + H \qquad (5)$$

$$H + O_2 + M \rightarrow HO_2 + M \qquad (6)$$

HO_2 can either disproportionate to form H_2O_2, reaction (7), or react with Cl via reaction (2)

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \qquad (7)$$

k_2 was determined from measurements of the kinetic behaviour of HO_2 in this system.

When both Cl_2O and H_2 are present, the photochemically produced Cl atoms form ClO and HO_2 simultaneously, which enables the kinetics of reaction (3) to be investigated from observation of the two radicals in absorption.

3. Progress

During the reporting period, the experimental study of the reaction of ClO with NO_2 including the pressure and temperature dependence of k_1 has been completed and the results have been fully analysed. This work has been written up in the form of a paper which has been submitted for publication in J. Chem. Soc. Faraday Transactions. Additional experimental data on the reaction of Cl with HO_2 has been obtained and the results fully analysed. A paper on this topic is currently in preparation. Some preliminary experiments on the ClO + HO_2 reaction have been performed and a full experimental study designed to determine k_3 is currently underway.

4. Results

4.1 k_1 was determined at several pressures in the range 24-612 Torr. At the lowest pressures 25 and 50 Torr, k_1 was close to that calculated from the third order rate constant determined in previous low pressure studies. Over the temperature range 274-338 K the following Arrhenius expression for the second order rate constant was obtained:

$$k_1 = (4.3 \pm 0.6) \times 10^{-33} \exp((1085 \pm 43)/T) [N_2] \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$$

At pressures greater than 50 Torr the reaction enters the fall-off region. k_1 becomes increasingly lower than the extrapolated low pressure value and its temperature dependence decreases. At the highest pressure, 612 Torr, $k_1 = 1.5 \pm 0.2 \times 10^{-12}$ cm^3 molecule^{-1} s^{-1} at 298 K and the temperature coefficient had fallen to +200/T. There are some significant differences between the experimental values of k_1 and some recent theoretical calculations of the pressure dependence of this reaction. However over the relevant range of pressure and temperature for the stratosphere 1 to 50 Torr and 270-220 K, the values of k_1 can now be given with good reliability.

4.2 The experimental data for the reaction Cl + HO_2 had mainly been gathered previously. From the observed dependence of HO_2 absorption on H_2 concentration, the value of k_2 could be determined relative to k_5. A complete analysis of the data using computer simulation techniques gave the following expression for k_2 of the temperature range 273-338 K

$$k_2 = 1.38 \times 10^{-10} \exp(-219/T) \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$$

with an overall error of ± 30% on k_2. This gives a value a factor of two higher than the current consensus value for mid stratospheric conditions, implying an increased importance for reaction (2) in the conversion of active Cl species to HCl.

5. References

The following papers relating to the work carried out on this project have been prepared and accepted for publication in J. Chem. Soc.Faraday Transactions I.

(a) Kinetics of Chlorine Oxide Radical Reactions using Modulated Photolysis Part I - Disproportionation of ClO in the Cl_2 photosensitised decomposition of ozone.

(b) Part II - ClO and ClOO radical kinetics in the photolysis of Cl_2-O_2-N_2 mixtures

(c) Part III - Pressure and Temperature dependence of the reaction ClO + NO_2 (+M) $\longrightarrow$ $ClONO_2$(+M)

Additionally a paper is being prepared entitled 'Kinetics of the reaction Cl + HO_2 = HCl + O_2 using Molecular Modulation Spectrometry'.

A preliminary account of the experimental work on Chlorine Nitrate formation was presented at a Technical Meeting of the Study Group on the

Ozone Shield depletion problem in Brussels, October 1978.

6. Future Work

We are currently conducting experiments designed to determine the rate constant for the reaction of ClO with HO_2. The differential photometric arrangement for observation of ClO in the presence of strongly absorbing Cl_2O has been constructed and interfaced with the MMS. It is anticipated that this experimental work will be successfully completed by 1 July 1979.

A new cooler/thermostat device has been purchased to extend the temperature range of our experiments.

For future work relating to the ozone depletion problem we anticipate useful application of our techniques to obtain kinetic information on the reactions of active Bromine species.

Contractor: The University College Dublin

Contract No. : 305-77 ENV EIR

Project Leader: Dr. H. W. Sidebottom

Title of Project : The Determination of Possible Atmospheric Removal Pathways for Halocarbons

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RESEARCH OBJECTIVES

It is generally assumed that hydrogen containing halocarbons are removed from the troposphere by reaction with the hydroxyl radical. However, the fully halogenated chlorofluorocarbons are unreactive toward the hydroxyl radical and are believed to be inert in the troposphere and thus will eventually undergo vertical transport into the stratosphere. Molina and Rowland have pointed out that photolysis or reaction with excited oxygen atoms could provide a sink for these compounds and that the chlorine atoms produced might initiate a potentially destructive pathway for stratospheric ozone by means of the catalytic cycle,

$$\mathrm{Cl} + \mathrm{O_3} \longrightarrow \mathrm{ClO} + \mathrm{O_2}$$

$$\mathrm{ClO} + \mathrm{O} \longrightarrow \mathrm{Cl} + \mathrm{O_2}$$

There is comparatively little data on hydrogen or halogen atom abstraction from halocarbons by species other than the hydroxyl radical. The present study was initiated with the object of obtaining reliable kinetic data for hydrogen and chlorine atom abstraction from halocarbons by the methyl radical, It was hoped the results would provide a useful basis for predicting the general reactivity of halocarbons toward radical attack in the atmosphere, particularly with regard to any possible sinks for halocarbons in the troposphere.

A potential removal process for halocarbons in the troposphere is photosentized decomposition, in which a sensitizer molecule absorbs light of wavelengths longer than 280 nm and transfers sufficient energy in a collision to dissociate the halocarbon. In this work the importance of energy-transfer processes from the triplet state of a carbonyl compound (biacetyl) to a number of bromine and iodine containing halocarbons was investigated. The results should provide a useful basis for establishing the possible importance of photosensitized decomposition of halocarbons in the troposphere.

Photolysis of fully halogenated halocarbons in the stratosphere or hydroxyl radical attack on hydrogen containing halocarbons in the troposphere generates haloalkyl radicals. An understanding of the oxidation reaction of these radicals is of some importance since they may liberate further chlorine atoms via reaction in the atmosphere. The removal mechanism for these species has not so far been unequivocably established, though it has been generally assumed that the remaining chlorine atoms will eventually be released into the stratosphere. The aim of the present work is to study the oxidation of haloalkyl radicals in an attempt to determine the mechanism for their breakdown.

(A) THE REACTION OF METHYL RADICALS WITH HALOCARBONS

Methyl radicals were generated photolytically from acetone or by the pyrolysis of ditertiary butyl peroxide. Conventional static systems were used for all the reactions and products were analysed by gas chromatography.

The rate of chlorine atom abstraction by the methyl radical from a number of chlorofluorocarbons has been determined relative to the known rate of combination of methyl radicals

(1) $CH_3 + CCl_{4-n}F_n \longrightarrow CH_3Cl + CCl_{3-n}F_n$

(2) $CH_3 + CH_3 \longrightarrow C_2H_6$

Values for the derived rate parameters are given in Table I together with the carbon-chlorine bond dissociation energies.

Table I

Arrhenius Parameters for Chlorine Atom Abstraction from Chlorofluoromethanes by Methyl Radicals.

	D(C-Cl) kcal/mol	$Log_{10}A$ (l/mol-sec)	E kcal/mol
CCl_4	68	8.8	10.1
CCl_3F	71	8.4	8.9
CCl_2F_2	72	8.5	7.2
$CClF_3$	75	8.5	7.0

The Arrhenius parameters for hydrogen abstraction from chloromethanes by methyl radicals have been determined relative to bromine abstraction from bromotrichloromethane

(3) $CH_3 + CH_{4-n}Cl_n \longrightarrow CH_4 + CH_{3-n}Cl_n$

(4) $CH_3 + CCl_3Br \longrightarrow CH_3Br + CCl_3$

Combination of these results with the previously determined rate data for the reference reaction yields the Arrhenius parameters for hydrogen abstraction, Table II.

Table II

Arrhenius Parameters for Hydrogen Atom Abstraction from Chloromethanes by Methyl Radicals.

	D(C-H) kcal/mol	$\mathrm{Log}_{10}A$ (l/mol-sec)	E kcal/mol
CH_4	104	8.2	14.6
CH_3Cl	101	9.1	11.6
CH_2Cl_2	98	8.8	9.0
$CHCl_3$	96	8.5	6.7

The results for chlorine atom abstraction from the chlorofluorocarbons indicate a decrease in activation energy with increasing fluorination of the reactant. This result is contrary to that expected from consideration of the bond dissociation energies. It is proposed that this is due to reduced repulsion between the attacking CH_3 radical and the abstracted chlorine atom, arising from a decrease in electron density on the chlorine atom with increasing fluorination i.e.

$$CH_3 + \overset{\delta+}{Cl} — \overset{\delta-}{CF_3}$$

Similarly the marked increase in reactivity observed for the series of chloromethanes CH_4 to $CHCl_3$ can be ascribed to a combination of reduced bond dissociation energies and reduced repulsive polar forces.

The above results suggest that polar forces are important in radical reactions involving halocarbons and that halocarbons are likely to be more susceptible to attack by nucleophilic species.

(B) THE OXIDATION OF CHLOROALKYL RADICALS

The photo-oxidation of trichloromethyl radicals were carried out in a static system using either gas chromatography or infra-red analysis to determine product concentrations.

Photolysis of CCl_3Br or CCl_4 in the presence of O_2 leads to the production of CCl_2O with quantum yields of unity. Hence CCl_3 radicals in both systems are quantitatively converted to phosgene in reaction (5)

$$(5) \qquad CCl_3 + O_2 \longrightarrow CCl_2O$$

The overall rate of oxidation of CCl_3 radicals to yield CCl_2O has been determined using a competitive technique. Bromotrichloromethane was photolysed at 365 nm in the presence of oxygen-hydrogen bromide mixtures. The rate of the overall oxidation of CCl_3 radicals was determined relative to that for hydrogen abstraction from HBr reaction (6)

$$(6) \qquad CCl_3 + HBr \longrightarrow CHCl_3 + Br$$

hence $R_{CCl_2O}/R_{CHCl_3} = k_5[O_2]/k_6[HBr]$

Similarly the photolysis of CCl_4 at 253.7 nm in the presence of oxygen--bromine mixtures provides an estimate of the rate of the overall oxidation of CCl_3 radicals relative to that for bromine abstraction from Br_2, reaction (7)

(7) $$CCl_3 + Br_2 \longrightarrow CCl_3Br + Br$$

hence $R_{CCl_2O}/R_{CCl_3Br} = k_5[O_2]/k_6[Br_2]$

The ratios k_5/k_6 and k_5/k_7 were found to be independent of the relative concentrations of reactants and the total pressure (250 to 1000 torr). Combination of the values of k_5/k_6 and k_5/k_7 determined in this work with literature values for k_6 and k_7 yields the rate constant for the overall rate of phosgene formation in the oxidation of CCl_3 radicals,

$$k_5\ (298^0k) = (1.8 \pm 0.5) \times 10^{10}\ 1/mol\text{-}sec$$

A careful study of the chlorine atom sensitized oxidation of chloroform was made in an attempt to determine the detailed mechanism for oxidation of CCl_3 radicals. The results were consistent with the following reaction sequence,

(8) $$CCl_3 + O_2 + M \rightleftharpoons CCl_3O_2 + M$$

(9) $$2CCl_3O_2 \longrightarrow 2CCl_3O + O_2$$

(10) $$CCl_3O \longrightarrow CCl_2O + Cl$$

Hence the observed rate constant for the overall oxidation of CCl_3 radicals determined in the competitive studies described above is a measure of the high pressure limiting value of k_8.

It should be noted, however, that the combination of peroxyhaloalkyl radicals, reaction (9), is unlikely to be significant under atmospheric conditions and these radicals would be expected to be removed by some other atmospheric species,

e.g. $$CCl_3O_2 + NO \longrightarrow CCl_3O + NO_2$$

$$CCl_3O_2 + HO \longrightarrow CCl_3O + HO_2$$

(C) PHOTOSENSITIZED DECOMPOSITION OF HALOCARBONS

The triplet lifetime of biacetyl was investigated as a function of the concentration of various halocarbons using a time-resolved laser-excited phosphorescence technique.

It has been demonstrated that biacetyl can transfer its triplet energy to a large variety of acceptor molecules and that for reactions in the gas phase for which the process is exothermic the rate constant for quenching approaches the collision number. The continuous absorption spectra of CCl_3Br and CF_3I, corresponding to the $n \rightarrow \sigma^*$ transition, extend to over 400 nm. Although no evidence has been found for the direct population of the corresponding triplet state, this forbidden band would be expected to be found towards longer wavelengths than the observed allowed transition. The 0-0 band of the $'A_g \longrightarrow {}^3A_u$ transition of biacetyl occurs close to 500 nm and hence energy transfer to the triplet state of CCl_3Br and CF_3I might be expected to be relatively efficient. Since the carbon-bromine and carbon-iodine bonds in CCl_3Br and CF_3I respectively have bond dissociation energies less than the triplet energy of biacetyl, energy transfer to the triplet state of these halocarbons would presumably lead directly to dissociation products.

The results given below in Table III indicate that the quenching of the triplet state of biacetyl by CCl_3Br or CF_3I is relatively inefficient. The quenching rate constants are close to those found for CF_3Br which has a bond dissociation energy considerably higher than the triplet energy of biacetyl and whose triplet state energy is probably sufficiently high that the energy transfer process from biacetyl is endothermic. The similarity in the quenching efficiencies of CCl_3Br and CF_3Br suggests that quenching occurs not by energy transfer, but by a direct chemical reaction.

Table III

Quenching Rate Constant Data for Biacetyl Triplet Molecules with Various Halocarbons at 25°C.

Collision Partner	Quenching Rate Constant l/mol-sec
CF_3I	9.5×10^6
CF_3Br	5.0×10^5
CCl_3Br	8.9×10^5

In order for a sensitization removal pathway for halocarbons to be important in the atmosphere, the energy donor molecule must have a high absorption coefficient and also the energy transfer process must be extremely efficient compared to quenching by the atmospheric gases (particularly molecular oxygen). The above results indicate that triplet state sensitization of halocarbons appears not to be facile, at least for the model systems studied in this work.

PUBLICATIONS

1. The Reactions of Methyl Radicals with Chloromethanes, by K. V. Macken and H. W. Sidebottom. International Journal of Chemical Kinetics, in press.

2. The Oxidation of Trichloromethyl Radicals, by F. McKeown and H. W. Sidebottom, submitted to the International Journal of Chemical Kinetics.

3. The Quenching of the Triplet State of Biacetyl by Halocarbons, by M.P.A. Foley, C.O Concheanainn and H. W. Sidebottom, manuscript in preparation.

ORAL COMMUNICATIONS

1. Reactions of Methyl Radicals with Halogenated Methanes given at Fifth International Symposium on Gas Kinetics, Manchester, July 1977.

2. Some of the work described in this report was presented at the Meetings of the Coordination and Study Groups on the Ozone Depletion Problem, Brussels October 1977 and 1978.

Contractor: Danish Meteorological Institute
Lyngbyvej 100, 2100 Copenhagen Ø., Denmark

Contract no.: 306-77-1 ENV DK

Project leader: Lars P. Prahm, National Agency for Environmental
Protection, Air Pollution Laboratory, Risø,
4000 Roskilde, Denmark

Title of project: 2-D pseudospectral global dispersion modelling

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2-D Spectral Global Dispersion Modelling: Status report by

Ruwim Berkowicz and Lars P. Prahm

1.1 Objective of the research

Two-dimensional global models with both vertical and meridional resolution and the improved representation of transport, compared with one-dimensional models, are the ideal instrument for diagnostic and prognostic application for atmospheric experimental programs (WMO, 1977). Comparison between model simulations and measurements of different atmospheric traces, however, has shown that dispersion parameters which are adequate for simulation of some tracers are inadequate for others.

The present research focuses on the physical theory and numerical method for the dispersion computations. Improved procedures are tested and compared with conventional methods to give an estimate of the range of some errors originating from assumptions in the dispersion computations, and to asses the advantages of future application of the spectral diffusivity theory and numerical method for 2-D global dispersion simulations.

1.2 Introduction

A two-dimensional meridional global dispersion model is developed. The model is based on the new spectral diffusivity theory (Berkowicz and Prahm, 1979). The main feature of this theory is the introduction of diffusivities as a function of the wave number of the spectral components of the concentration distribution. This makes it possible to describe the turbulent diffusion as a scale-dependent process, but in an Eulerian framework.

In the case of dispersion on a global scale, eddies of a size of the order of 10.000 km play an important role. A dispersion model based on the gradient-transfer formulation of eddy fluxes cannot, in this case, be expected to yield satisfactory results because the characteristic extension of the considered tracers might be smaller than the size of the eddies. A properly designed scale-dependent dispersion model is thus of great importance.

Studies are presented of simulation of atmospheric dispersion of radioactive tracers resulting from nuclear explosions in the stratosphere. Preliminary results show a reasonable agreement between the model simulations and the existing measurements. Comparison is also made with models based on the gradient-transfer theory formulation. The model will also be tested on ozone dispersion with a simplified linearized chemistry.

2. Method

2.1 Theory

The gradient-transfer theory (K-theory) is widely used for the description of turbulent diffusion. However, it is known that, in order to obtain the K-theory approximation based on the hypothesis of a turbulent flux proportional to the gradient of the average concentration, it is necessary to assume that the characteristic length scale of the concentration field is much larger than the characteristic length scales of the turbulence. In studies of atmospheric dispersion, this is a very crude assumption. Horizontal dispersion of a continuous plume is a classical example where these assumptions are not satisfied. Since the pioneering works of Taylor (1921) and Richardson (1926) on the statistical theories of dispersion, it has been known that, in order to account for the scale-dependence of turbulent diffusion, the diffusivity K should in fact be made dependent on the time of travel or distance from a source. The price of such a reformulation is the loss of Eulerian properties of the gradient-transfer theory, just those properties that made the theory so applicable for many practical studies.

In two recent papers (Berkowicz and Prahm, 1979; Prahm et al., 1979), a new theory was formulated, the spectral turbulent diffusivity (STD) theory, which makes it possible to apply the diffusivity formulation for turbulent diffusion taking the scale-dependence into account, but without losing the Eulerian properties. The theory was formulated on the basis of a phenomenological understanding of the physics of turbulent diffusion. The basic concept of the STD-theory is that the diffusivity is a characteristic property of each Fourier-mode of the concentration distribution. A STD-coefficient K(k), a function of the wave-number k of the concentration distribution, was introduced. For a homogeneous one-dimensional diffusion, the diffusion equation for a Fourier-mode c(k,t) of the concentration distribution c(y,t) is

$$\frac{\partial}{\partial t}\tilde{c}(k,t) = -k^2K(k)\tilde{c}(k,t) \qquad (1)$$

and the solution is given by

$$\tilde{c}(k,t) = \tilde{c}(k,o)\exp(-k^2K(k)t) \qquad (2)$$

where $\tilde{c}(k,o)$ is the initial value. When (1) is converted to real space with K(k) = constant, the well-known gradient-transfer equation is obtained. As shown by Berkowicz and Prahm (1979), a k-dependent diffusivity results in an integro-differential equation. For more details, the reader is referred to the original paper where the case of non-homogeneous turbulence is also discussed.

It was shown by Berkowicz and Prahm (1979) that in order to account for the proper scale-dependence of the turbulent diffusion, the STD-coefficient must be a decreasing function of the wave number of the concentration distribution.

In this study, we use a simple form of the spectral diffusivity. The k-dependency of K(k) adapted by us is

$$K(k) = \frac{K_o}{1 + \left(\frac{k}{k_m}\right)^{4/3}} \qquad (3)$$

where K_o is the large-scale diffusivity, i.e. K(k) → K_o when k → o. $k_m = 2\pi/\ell_m$, where ℓ_m is the size of the most energetic turbulent eddies. The parameter ℓ_m is different for horizontal (meridional) and vertical motion. On the scale relevant for global dispersion, the turbulent energy in the meridional plane is mainly created by the temperature differences that exist between the polar and equatorial regions. It is, therefore, reasonable to assume that ℓ_m, in this case, is equal to the distance between the poles and the equator (about 10.000 km). In the vertical direction, the troposphere can be considered as the main source of the turbulent energy. This means that ℓ_m, in this case, is equal to the height of the tropopause ($\sim$8 km at the poles, and $\sim$16 km at the equator).

As the large-scale diffusivities (K_0), we used the values computed by Louis (1974). In the future, diffusivities will be estimated directly from measurements of wind fluctuations. The procedure to be used is treated by Prahm et al. (1979). The mean wind fields used in the model are computed from the stream function given by Louis (1974). Fourier expansion is used to evaluate gradients of the stream function.

2.2 Numerical method

The grid-point system extends from ground level up to 64 km with 2 km spacing and from north to south with spacing of (π/32) . The numerical scheme is based on the pseudospectral method. The spatial gradients are evaluated from expansion in Fourier series. This method is especially applicable for representation of advection with the mean circulation, because errors caused by numerical dispersion connected with the conventional finite difference methods are minimized. The main idea of the pseudospectral method is that the space derivative $\partial c/\partial x$ of the concentration field $c(x,t)$ is evaluated from finite Fourier series, while all other operations such as evaluation of local products and the time integration are performed on grid points in real space:

$$c(x,t) = \sum_k A(k_n,t)e^{ik_n x} \tag{4}$$

$$\partial c/\partial x = \sum_k ik_n A(k_n,t)e^{ik_n x} \tag{5}$$

where $A(k,t)$ are the Fourier coefficients and k_n are wave vectors defined by the discrete grid-point system. The procedure (4) and (5) is repeated for each dimension by use of one-dimensional Fast Fourier Transforms (FFT). For further details on the pseudospectral model used here, the reader is referred to previous presentations (Christensen and Prahm, 1926; Berkowicz and Prahm, 1978).

The pseudospectral numerical method is well-suited for treatment of the spectral diffusivity theory. When the spatial derivatives of the concentration field are computed for the diffusive form, the factor ik_n appearing in (5) in front of the Fourier coefficients is replaced by

$$ik_n f(k_n) = ik_n \frac{1}{1 + (\frac{k_n}{k_m})^{4/3}} \tag{6}$$

Note that only the diffusion term is treated in this way.

2.3 Initial and boundary conditions

In the study of dispersion of the radioactive tracer C^{14}, a seasonally and zonally averaged concentration distribution reported by Telegadas (1971) is used for initial conditions. We chose the period, March-April-May 1963, because the latest nuclear detonation took place about three months earlier and, therefore, we could expect that the zonal mixing would be well established. No new detonations were performed until 1967. We have also tried the period from 1959 to 1961, but because of the lack of the experimental data, no satisfactory comparison between the model predictions and measurements could be made.

A symmetrical Fourier transform is used in order to satisfy the boundary conditions of zero fluxes at the boundaries (Berkowicz and Prahm, 1978). At the bottom boundary (the earth's surface), a drain term is used in order to account for the deposition and removal of the tracer. The deposition velocity V_d = 0.01 cm/s is estimated from the decrease of the total burden of C^{14} and the measured surface concentrations.

3. Results

The model results after 6 and 12 month integration are shown in Figures 2 and 3. Figures 2a and 3a show results obtained without use of the spectral turbulent diffusivity theory ($K = K_0$), while Figures 2b and 3b show the results obtained with the STD-theory applied. In the former case, one can observe that the model predicts results significantly smoothed compared with the measurements. Diffusion seems to be too strong. A significant improvement of the model simulation is obtained with the STD-theory introduced.

When the model results are compared with measurements, one should remember, however, that the isocontours given by Telegadas are quite subjective. There are no measurements above 30 km and only few for the southern hemisphere. The comparison should thus predominantly be made with the existing point-measurements and not isoplots.

4. Conclusions

A new diffusion model based on the Spectral Turbulent Diffusivity Theory is tested on dispersion of radioactive tracers in the atmosphere. A significant improvement is achieved compared with results obtained with the gradient-transfer formulation of diffusion. The STD-theory makes it possible to treat atmospheric diffusion with a scale-dependent diffusivity, but in an Eulerian framework. It is the authors' opinion that a model with a scale-dependent diffusivity will be able to describe dispersion of different atmospheric contaminants without any additional artificial parameterization. A future test of the STD-model on ozone dispersion will show the applicability of the model for simulation of tracers at different geographical scales and thus establish a general test of the STD-theory for global 2-D dispersion modelling.

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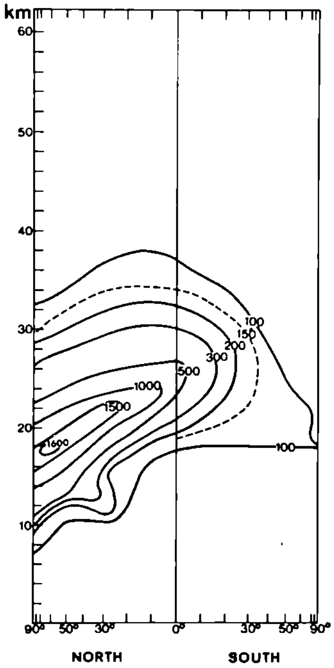


Fig. 1: Initial distribution.
March-May 1963 (Telegadas, 1971).
Units: 10^5 atoms/g air.

Fig. 2: Model simulation, 6 months after start of integration.

a) Gradient-transfer theory
b) STD-theory
c) Measurements (Telegadas, 1971)

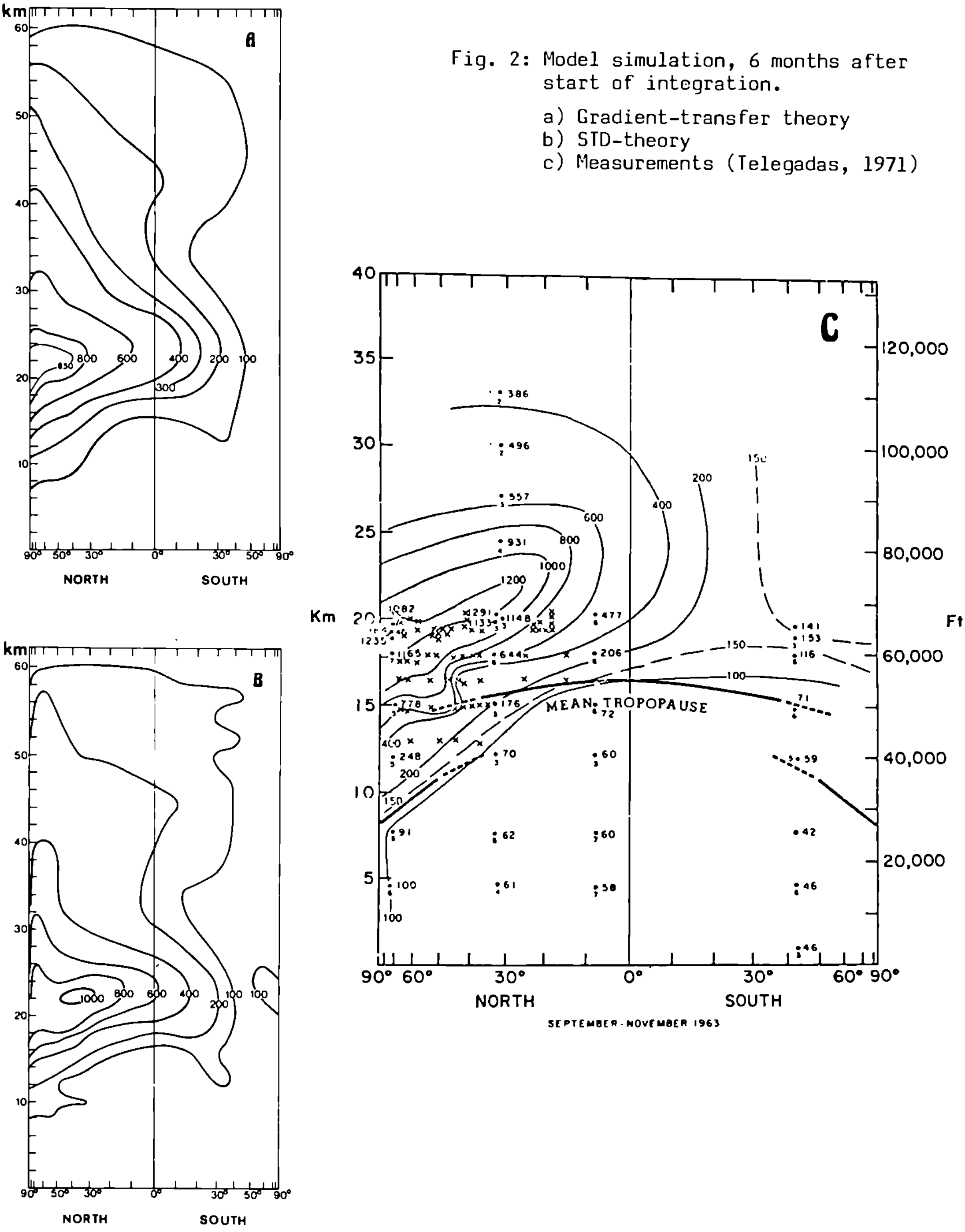
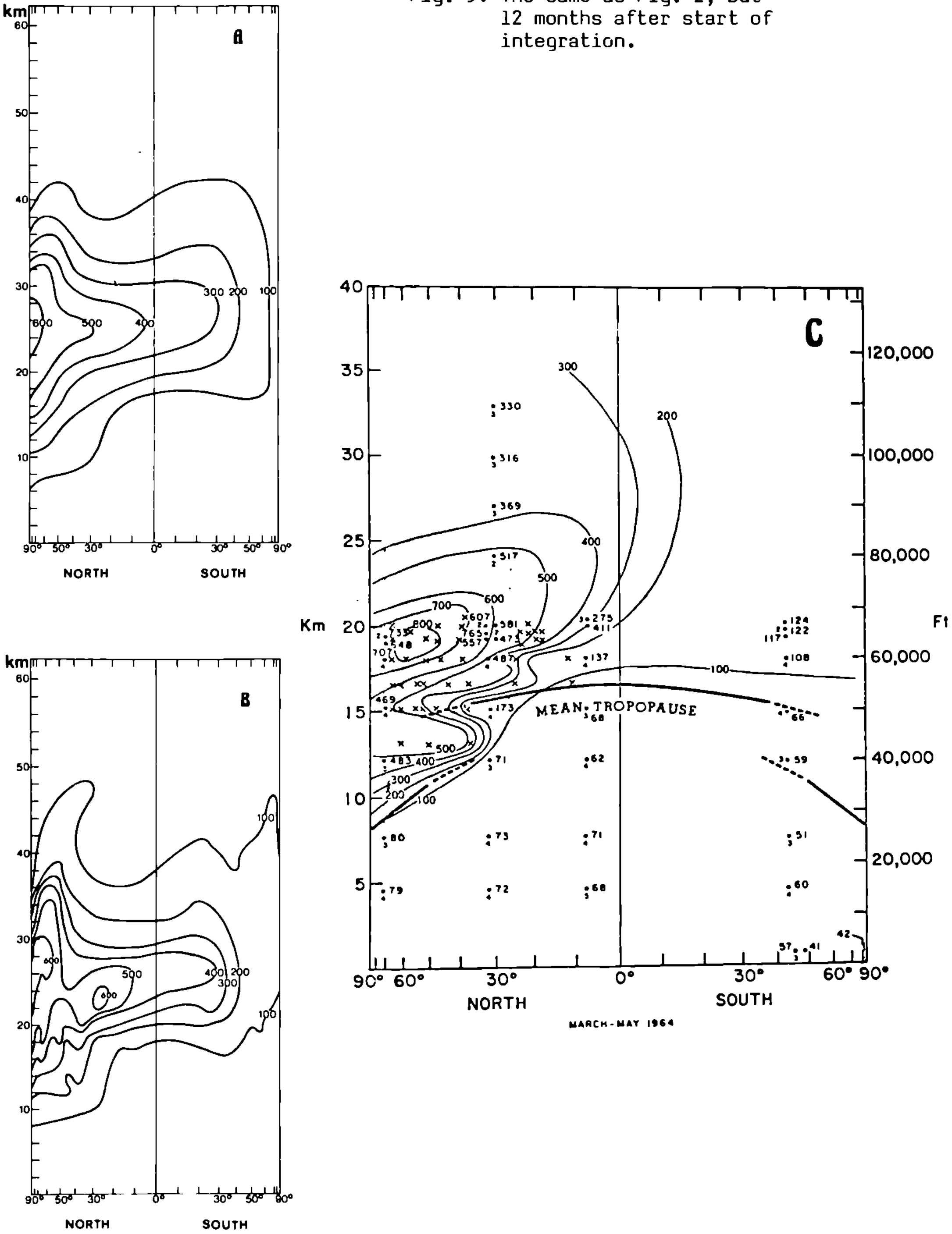


Fig. 3: The same as Fig. 2, but 12 months after start of integration.



Contractor: University of Copenhagen
Contract no. 307-77-ENV DK
Project leader: Prof. Ole Buchardt
Title of project: Halocarbons in the Environment

Objective of the research

The objective of the research was to carry out long- and short-term studies of the stability of halocarbons of the type supposed to threaten the ozone-shield, as well as model compounds. Eventual degradation pathway and mechanisms should be scrutinized.

Materials and methods

The halocarbons for these studies were chosen among those used industrially and in spray cans, especially CF_2Cl_2, $CFCl_3$, CH_3CCl_3 and $CHCl=CCl_2$.

The long-term studies are carried out by placing series of Pyrex-glass ampoules containing the halocarbon samples and air, and other agents like, e.g., acetone , outdoors and subjecting these to long term solar irradiation. Concessently laboratory studies using more intense light are carried out. After irradiation the samples are analyzed by adding water, and determining the halogenide content and pH by ion-selective electrochemical methods, as well as by GC-MS. Furthermore, energy transfer studies are in progress using various model compounds.

Results

Trichloroethylene, which due to its fast photodegradation is used as test substance, has given some quite unexpected results. We have observed that it photodegradates in a silica-surface catalyzed reaction to give first dichloroacetylchloride.

However, it turned out that the initial formation of this product is very slow, but after a certain period of irradiation, the degradation suddenly goes very fast. The further degradation to phosgene, *etc*. does not show this effect.

We have now been able to demonstrate a similar, although slower, behaviour for the environmentally more dangerous methylchloroform, and are still working on this problem, *i.e.*, the reason for the observed initiation period and the importance and mechanism of the silica-surface photobreakdown.

TOPIC 42 : RECLAMATION OF DERELICT LAND

Contractor :	Service Protection du Milieu Naturel Société du Canal de Provence et d'Aménagement de la Région Provençale (GERSAR), Le Tholonet, France
Contract n° :	202-77-1 ENV F
Project leader :	J. Portier
Title of project :	Reclamation of "red muds" by means of vegetal process

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Purpose of the study

"Red muds" is an industrial waste, from the extraction of alumina from bauxite, by an alkaline process, using mainly soda.

It is basically a mineral material which is almost sterile, with a compact structure for it is a composition of fine components (more than 50 % clay and loam). The ferric oxide and silico-aluminate of sodium (total Na = 5 %) are the essential components of that material, which can be found in nature as a thick solid outflow at the bases of hills, in the region of Aix-en-Provence, near Vitrolles (Bouches-du-Rhône - FRANCE) covering an approximate surface of fifteen hectares.

This study aims at reusing that muds by means of a vegetal process, viz, at re-creating "soils", capable of bearing some vegetation, in order to re-integrate that damaged site, as well as others, into the Provençal landscape.

Equipment and methods

The improvement of that basic material must be achieved through a process using organic matter providing fertilizing elements, microbial life and a necessary re-organization in order to obtain a good permeability and a better aeration of the soil.

These improvements are done by using urban and industrial wastes, available in an area surrounding the site which is to be revitalized.

On this basis, a series of experiments were tried in a laboratory, in two successive stages :

First stage : selection of the best "soils" prepared from mixtures of, on the one hand, organic elements (municipal sludge, domestic refuse compost and a biological fertilizer) and on the other hand of components improving the structure (sawdust, sand, polystyrene).

84 possibilities were tried (in containers of three litres) : seven of them were kept with which a cultivation of ray-grass (test plant) gave the best results over a period of three months.

Second stage : the best seven soils thus chosen are studied in vegetation containers, of fifty litres each, to check their physico-chemical and bacteriological evolution, in relation with the development of ray-grass cultivation.

Results :

a) a leaching of the sodium is absolutely necessary, after the various materials and supplementary components have been sorted out, and before the cultivation, which would correspond to the annual pluviometry of the region (about 700 millimeters).

b) it seems to be difficult to have a composition overrating the following proportions : 1/3 "red muds", the other 2/3 are distributed between the organic elements and the improving components of the structure, which approximately represents in a given case : 150 tons/per hectare (dry matter) sludge + 200 tons/hectare sawdust, with the possibility to reverse these proportions.

c) after 4 months of experiments, the evolution of these mixtures is satisfactory :

- decrease of pH rate reaching 8,2 (the usual pH rate of "red muds" is superior to 11)
- ratio C/N almost 10 (with very high organic matter and nitrogen proportions)
- high total P_2O_5 rate
- adsorbing complex saturated with Ca^{++} but still a high rate of Na^+ exchangeable.
- satisfactory physical proprieties (water reserve and stability of structure)
- intense microbiological life, with a tendency to ammonification and denitrification, and with an evolution of the basic components similar to the cycle of carbon .

Conclusion

1) the second stage of that series of experiments is carried on until June 1979 so as to get observation on an annual evolution.

2) the results will be applied to a study in lysimeters and experimental plots "in situ".

Contractor: UNIVERSITY OF YORK, UNITED KINGDOM

Contract no: 271-71-10 ENV UK

Project leader: DR M.J. CHADWICK

Title of project: Chemical characteristics of colliery spoil in relation to long term performance of grass and legume species under various fertilizer regimes

Objective of the research

The objective of the research was to test species performance in relation to spoil characteristics and different levels of fertilizer application. The range of grass species cultivars and spoils previously used (Chadwick et al. 1978) was to be extended using amenity species in extensive greenhouse trials. Research into the effect of applying different forms of nitrogen to swards deficient in nitrogen was to be extended with particular emphasis on the effect of this on yield and response to phosphorus. Studies were to be undertaken into the efficiency and long term effects of supplying phosphorus as a single application in solid form. The residual value of phosphorus fertilizer in relation to heavy liming rates and silicate additions was to be followed. In attaining these objectives spoils from Belgium, France and the Federal Republic of Germany, as well as spoils from the United Kingdom, were to be analysed.

These objectives have been achieved.

Materials and methods

The materials used and methods employed have been documented previously (Chadwick et al. 1978). Experimental details are provided under each section of work described.

Results

1. Species and cultivar screening on colliery spoil. Two large scale pot trials were conducted in the greenhouse during 1977 and 1978. The experimental details are given in Table 1.

Experiment	N	P	Blocks	Spoil	Species & cultivars
	kg ha^{-1}				
I	28 (N1)	18.8 (P1)	4	Cefn Pennar	Lolium perenne:
	56 (N2)	75 (P2)		Thorne	cv S23
	112 (N3)	300 (P3)			cv Sprinter
					Festuca rubra:
					cv S59
					cv Highlight
					Agrostis tenuis:
					cv Highland
					cv Tracenta
					Poa pratensis:
					cv Monopoly
					cv Nugget
					Poa compressa:
					cv Reubens

/continued

Experiment	N	P	Blocks	Spoil	Species & cultivar
	kg ha^{-1}				
II	28 (N1) 56 (N2) 112 (N3)	75 (P1) 300 (P2)	4	Cefn Pennar Thorne Ashington	*Dactylis glomerata*: cv S37 cv S143 *Festuca rubra*: cv S59 cv Merlin *Agrostis tenuis*: cv Highland cv Parys Mountain cv Goginan *Festuca pseudovina*: *Holcus lanatus*

Table 1. THE GREENHOUSE CULTIVAR SCREENING EXPERIMENTS

Spoil differences were considerable (Table 2) ranging from a high nitrogen, relatively low phosphorus spoil (Cefn Pennar) to a low nitrogen, relatively high phosphorus one (Ashington).

	SPOIL		
	Cefn Pennar	Thorne	Ashington
pH	3.4	7.5	4.2
Conductivity mS.cm^{-1}	2.6	1.8	0.5
N(NH_4^+ + NO_3^-) µg.g^{-1}	13.9	2.5	1.7
P µg.g^{-1}	2.2	1.4	16.8
K µg.g^{-1}	19.5	16.5	17.6
Ca µg.g^{-1}	237	30	36
Mg µg.g^{-1}	111	18.6	11.8
Na µg.g^{-1}	31	177	18.2
Fe µg.g^{-1}	78	0.09	0.05
Cu µg.g^{-1}	2.6	0.19	0.08
Zn µg.g^{-1}	2.6	0.04	0.30
Mn µg.g^{-1}	14.5	0	0.90
Al µg.g^{-1}	9.3	0.80	0.30

Table 2. CHEMICAL CHARACTERISTICS OF THE COLLIERY SPOIL USED IN THE EXPERIMENTS

The agricultural grass cultivars outyielded the amenity ones under fertile conditions but where fertility was lower the amenity cultivar of *Lolium perenne* (Sprinter) performed better than cv S23. However, cv S59 performed best of all the *Festuca rubra*-related cultivars, irrespective of the prevailing nitrogen and phosphorus levels. In *Agrostis tenuis*, Tracenta grew best at the highest nitrogen and phosphrous levels and Highland under the lower fertility conditions. Merlin and Goginan were, surprisingly similar to Tracenta in their response to nitrogen and phosphorus variation. No significant differences were found between the two cultivars of *Dactylis glomerata*, S37 and S143. The two cultivars of Poa pratensis (Monopoly and Nugget) grew well on colliery spoil and seem to be valuable alternatives to Lolium perenne where fertility levels cannot be maintained. Holcus lanatus and Poa compressa showed considerable promise for colliery spoil reclamation but require further testing under field conditions.

Under the relatively low nutrient regimes maintained in the field trials, Festuca rubra, Agrostis tenuis and Dactylis glomerata cultivars outyielded the Lolium perenne cultivars.

2. Long term nitrogen supply in colliery spoil. Field trials established in 1975 were supplied with various forms of nitrogen fertilizer on establishment and again in 1976. In 1976 sward yeilds were generally good (as high as 400 $g.m^{-2}$) but as the intention was to follow residual fertilizer effects these trials were not supplied with fertilizer in 1977. Consequently yields fell (on some plots to less than one-eighth of the 1976 value). The spoil nitrogen levels on these plots in early 1978 were found often to be lower than the pre-reclamation value in 1975 (Table 3).

	Thorne		Tibshelf		Abertysswg	
Plot N Treatment	1975	1978	1975	1978	1975	1978
Ammonium sulphate ($(NH_4)_2SO_4$)	9.5	4.3	4.9	8.9	3.2	8.6
Calcium nitrate ($Ca(NO_3)_2$)	10.5	3.2	2.9	7.0	3.8	5.3
Gold N (slow release)	8.0	6.8	3.9	8.1	3.0	8.2
IBDU (slow release)	9.0	8.5	4.1	7.1	2.4	17.5
Chiguano (organic manure)	9.0	11.0	-	-	-	-
Trifolium repens (S100)	7.0	10.0	4.1	7.0	2.8	9.7

Table 3. SPOIL NITROGEN LEVELS ($\mu g\ N.g^{-1}$) AT THREE COLLIERY SPOIL SITES BEFORE FERTILIZER ADDITION (1975) AND THREE YEARS AFTER TREATMENT (1978)

As spoil nitrogen levels had fallen so low, 62.5 kg N ha^{-1} was applied in Spring 1978. The form in which this was applied made little difference to the yields obtained on the less acid spoil sites although on an originally acid site (Cefn Pennar) one application of Gold N gave comparable yields to splitting the application of ammonium sulphate into two or four applications. A single application of Gold N gave superior yields to a single application of IBDU or ammonium sulphate on the originally acid site.

Nitrogen recovery by the sward in 1976 ranged from 9-27 per cent but only 1.5-4.2 per cent in 1978.

Legumes in the sward offer a potential alternative way of supplying nitrogen but their establishment on colliery spoil has given rise to problems (Chadwick et al. 1978). A greenhouse experiment investigated these problems by sowing Trifolium repens cv S100 into a Lolium perenne cv S23 sward established 12, 6 and 0 weeks previously, at various levels of nitrogen fertilization (125, 62.5, 31.25 and 15.63 kg N ha^{-1}).

The results showed that white clover (Trifolium repens) can be satisfactorily established in a sward, either by oversowing or when sown with grass seed, if spoil nitrogen levels and applied nitrogen fertilizer levels are low. Establishment of legumes in a sward which was growing on spoil with reasonable levels of residual nitrogen was poor. The ability to gauge the right level of spoil nitrogen - high enough to allow some grass growth but not too high to inhibit legume growth - is crucial.

3. Phosphorus studies. A pot experiment in the greenhouse investigated the interaction of lime additions to acid spoil (Cefn Pennar - Table 2) and superphosphate fertilization rate. The results of cumulative yield indicated that dressings of ground limestone are seldom efficacious above 35.5 tonnes.ha^{-1} and that high levels may be detrimental when low phosphate fertilizer additions are given (Table 4). Cumulative yield for three years (1976, 1977, 1978) in the field supports these findings.

Limestone (tonnes ha^{-1})	Phosphate applied (kg P_2O_5 ha^{-1}) 50	100	200	400	800	1600
15.8	5.76	9.32	13.44	14.00	16.57	17.87
23.7	4.99	11.21	14.87	17.97	20.32	20.99
35.5	3.85	10.14	16.55	19.71	20.70	20.87
53.3	3.81	9.67	14.72	18.14	20.09	20.88
80.0	3.48	9.88	16.58	18.44	20.72	21.56
120.0	3.91	9.05	16.04	18.41	21.18	20.31

l.s.d. ($p = 0.05$) = 1.994

Table 4. CUMULATIVE YIELD (SIX CUTS AT SIX WEEKLY INTERVALS) AS TONNES HA^{-1} ON AN ACID COLLIERY SPOIL

An experiment designed to investigate whether additions of sodium silicate to spoil (Cefn Pennar) would counteract low yields at low phosphate-high lime combinations suggested that 1377 ppm silicon to air-dry spoil (as sodium silicate) could do so but it would be more efficient to add greater amounts of phosphate fertilizer (Table 5). No significant yield reduction, due to overliming, occurs at 100 kg P_2O_5 ha^{-1}. A 300 percent increase in yield occurs when the P level is doubled (50 to 100 kg P_2O_5 ha^{-1}) but only a 200 percent increase for the sodium silicate addition.

From field trial data it appears that increased P status, due to phosphate fertilizer additions last little longer than one year on acid colliery spoil.

Si (ppm)	Low lime	High lime
0	7.0	3.2
3.8	6.1	4.2
23.0	5.7	4.2
137.7	6.9	5.6
l.s.d. ($p = 0.05$)	1.2	0.9

Table 5. DRY MATTER YIELD (TONNES HA^{-1}) OF A SWARD ON ACID COLLIERY SPOIL AT LOW PHOSPHATE FERTILIZER LEVELS (50 kg P_2O_5 HA^{-1})

4. Analysis of spoils from France, Belgium and the Federal Republic of Germany.

These are given in Table 6.

	pH	Ec mS.cm^{-1}	Al	Fe	Mn	Ca	Mg	Na	K	Cu	Zn	N
			µg.g^{-1} air dry spoil									
France:												
Auby 10 yr	4.2	1.16	1.35	0.46	6.01	177	44.7	10.3	11.5	0.06	3.27	260
Roost Warrendin 15 yr	4.9	0.27	0.85	0.45	0.64	19.2	10.4	7.7	6.8	0.04	1.42	7.4
Belgium:												
Borne 25 yr	5.0	0.33	0.77	2.23	0.60	30.8	9.5	12.6	6.4	0.06	0.18	11.0
Havré old	6.4	0.31	2.2	2.33	0.07	42	7.4	9.7	33.2	0.10	0.06	10.5
Roux 25 yr	6.1	0.23	3.5	2.68	0.41	21.2	8.4	7.9	11.8	0.14	0.11	8.5
FRG:												
A.Vict Fresh	7.6	6.83	0.45	0.10	0.06	110	22.8	1345	23.7	0.03	0.02	12.2
5 yr	3.6	1.26	4.10	1.27	4.53	93	53.4	17.4	7.9	0.33	0.93	15.6
Z. Hugo Fresh	7.8	2.89	3.70	0.32	0	4.5	1.19	620	8.2	0.02	0.02	17.4
5 yr	6.3	3.61	0	0.06	3.8	440	166	101	37.9	0.07	0.08	289

Table 6. CHEMICAL ANALYSIS OF SOME COLLIERY SPOIL SAMPLES FROM FRANCE, BELGIUM AND THE FEDERAL REPUBLIC OF GERMANY

Reference

Chadwick, M.J., Elias, C.O., Lloyd, A., Morgan, A.L., Palmer, J.P. & Williams, P.J. (1978). *Nutrient Problems in Relation to Vegetation Establishment and Maintenance on Colliery Spoil*. Department of the Environment, U.K.

Contractor :	UNIVERSITY OF NEWCASTLE UPON TYNE
Contract No. :	288-77-1 ENV. UK
Project Leader :	Professor P. W. Arnold
Title of Project :	Studies on the long term management of soils forming from colliery spoil

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- Objectives of the research

1. Continuation of field trials laid down in 1975 to look into a range of soil management techniques in the establishment and maintenance of a productive sward.
2. Continuation of lysimeter studies started in 1976, to study moisture movement and acid production in weathering colliery spoils.
3. Development of a standard, reliable method for measuring the concentrations of pyrite (FeS_2) in soil materials.
4. Greenhouse studies on shale compaction, root growth and the amelioration of acidity in colliery spoil.
5. The study of earthworm survival in shale.

- Methods

In general, methods used have been those described in a Special Report (Rimmer and Colbourn, March, 1978, see below).

Seven field trial sites were used (for locations see Additional comments). Four were renovation sites on land previously reclaimed, and 3 were establishment sites on newly reclaimed spoil. On the renovation sites there had been 5 cultivations, mould board plough, sub-soiling to 60 cm, chisel ploughing to 25 cm, minimum cultivation and addition of bulky organic manures and each of these had high and low seed and fertilizer rates applied. On the establishment sites there had been 3 levels of ripping at 60 cm, each one with treatments of organic matter addition, phosphate fertilizer incorporation, and 2 seed mixtures (one containing cocksfoot in place of some of the ryegrass). One site had included a treatment of 10 cm imported soil. The treatments were evaluated by comparing dry matter yields.

The 30 lysimeters had been packed with shale from various sites. Ion concentration in the effluents, particular SO_4, Fe, Ca, Mg, Al, had been monitored for 18 months. These observations had been combined with various other data into a two part numerical model describing pyrite oxidation within the lysimeter profiles.

A recommended method for measuring total pyrite using nitric acid oxidation was developed in co-operation with other research establishments (Feb. 1978 see below). A partial oxidation method for estimating potential oxidation rates of pyrite in the field, and a more rapid total pyrite procedure have been developed and tested on a range of samples.

In a pot experiment on the amelioration of acid shales, lime at 40 to 120 t/ha, and organic matter (peat and compost) were applied and their affect on germination and dry matter yield found.

In a second pot experiment phosphate fertilizer was either mixed in or applied to the surface of spoil and the effect on root growth measured approximately 1 and 2 months after germination.

Earthworms were introduced in a field trial and to pots buried to their rims in the open. Two treatments were applied to the pots, added horse manure and added water.

A necessary stage in the calculation of soil porosity from bulk density measurements is to find the coal content of the soil. The normal method does not distinguish between coal and soil organic matter, so laboratory tests were carried out to find a method of distinguishing between the two and, in the process, to check on the loss-on-ignition method used at present. This standard method ignites <2 mm soil material for 30 min. at 850^{o}C, and the weight loss is used to estimate coal content. In our tests the time and the temperature were varied, and 2 pretreatments with hydrogen peroxide (H_2O_2) used.

The pot experiment on compaction showed that root growth down the side of the pots might be a source of error. A method of lining the pot with hard setting resin that impregnated the colliery spoil was therefore investigated.

The field trial treatments might be better evaluated if the rate of water infiltration on the different plots was known. A field infiltrometer was built consisting of a cylinder that can be driven into the ground and a calibrated water container which gives a constant head of water. This has not yet been tested.

- Results

On renovation sites an analysis of results from 5 years showed that organic manure had given consistently higher yields than the other cultivations. The other cultivations gave improvements at individual harvests but showed no consistent trends. Increasing fertilizer rate by 33% gave a 10% increase in yield. There were no differences between yields from the 2 seed rates.

On establishment sites, the site in South Wales showed significant differences between ripping treatments in some years. The other two showed no benefit from ripping. Organic matter gave some improvements in yield, and the deep incorporation of phosphate fertilizer no improvement. In 1978 there was evidence that the cocksfoot containing swards were beginning to outyield the ryegrass dominated swards. The soil cover treatment gave improved yields.

In the numerical model based on lysimeter data, the first part (SOWAT) redistributes soil water in a profile on a week by week basis. The second part of the model (AUDITION) calculates total ion outputs and introduces the controlling mineral equilibria in four pH ranges into the calculation of total pyrite oxidized in the profile. The final stage will be to link up the estimate for total pyrite oxidation with soil moisture distribution and temperature within the profile. SOWAT and AUDITION are almost completely written but require compiling in FORTRAN. Part of SOWAT has already been successfully compiled and tested.

Comparisons of the rapid method of pyrite analysis with the standard procedure (Dacey & Colbourn, in preparation) have shown variation to be within acceptable limits. Increasing the temperature of oxidation by refluxing over a bunsen burner made no significant difference to the results. The partial oxidation method appears from tests made on shales used in lysimeter trials to give some indication of the potential acid production rate in weathering spoils.

The completion of a pot experiment to look at the effects of grass growth on compaction showed no significant difference between treatments.

The pot experiment on spoil amelioration showed a major effect of lime on germination and growth, and of peat on germination. There was no depression of yield with high lime application, and a trend towards higher yields with higher lime.

The first harvest of the phosphate fertilizer experiment showed surface application to give an increased weight of roots in the top half of pots. The second harvest showed no significant differences between treatments although in both harvests the mixed in phosphate gave higher mean root weights in the bottom halves of pots.

Weight of worms surviving under the manure plus water treatment was similar to the soil control, and the other treatments less than the control. There was no evidence of survival in the field trial.

In the tests for coal content a 30% H_2O_2 pretreatment was found to remove a fraction of material lost during ignition that was not part of the coal fraction. This was about 10% of spoil weight and 2% of a standard coal sample. It was found that samples only attained a steady weight after 90 minutes in the furnace at 850°C.

- Conclusions and additional comments

The application of both organic manure and soil improve the yield from soils forming on spoil. There is little evidence that deep cultivation affects yield. Swards containing cocksfoot may outyield ryegrass after a time.

When complete the pyrite oxidation model should enable more accurate calculation of pyrite oxidation rate in weathering spoils to be made from lysimeter data. These estimates may then be compared to laboratory estimates of potential oxidation rates to check on the usefulness of the laboratory method.

There is some evidence that high rates of lime application do not depress yield, and that worms can survive under good conditions in spoil. When measuring coal content the time of ignition should be extended to 90 mins.

Field trial locations are as follows:

1. Ashington, Northumberland
2. Tudhoe, County Durham
3. Abertysswg, Mid-Glamorgan

1. Big Waters, Tyne & Wear
2. West Sleekburn, Northumberland
3. Hunters Hill, Durham
4. Bryn Hall, Lancashire/ Greater Manchester

Publications and oral communications on contract research

1. Rimmer D. L. and Colbourn P. March 1978
"Problems in the management of soil forming on colliery spoils"
170 pp.
Special report produced for the United Kingdom Department of the Environment. Fully indexed. This report covers the period 1975-77 and includes an account of work carried out during 1977 which cannot be easily separated from the previous two years. It is intended that a condensed version will be produced in the near future for workers in the reclamation field.

2. Methods of pyrite analysis working party. February 1978
"The determination of pyrite in colliery spoil"
A 200 word press-release covering the recommendations of the working party prior to full publication. Dr. Colbourn of Newcastle Research group was a member of the working party which comprised of representatives of seven United Kingdom universities and research establishments.

3. Rimmer D. L April 1978
"The physical conditions of soils developing on reclaimed colliery shales"
A paper presented orally to a joint meeting of the British Society of Soil Science and the British Ecological Society at York.

4. In preparation:
A full and final report is being prepared as an amendment to Rimmer and Colbourn March 1978 (1). It will describe work completed in 1978.

TOPIC 44 : ECOLOGICAL CONSEQUENCES OF LAND USE PLANNING

Contractor:	Dornier System GmbH, Friedrichshafen
Contract No.	243-77-10 ENV D
Project Leader:	Dr. Peter Boese
Title of Project:	Study on the ecological consequences of traffic development in certain regions

1. Objective of the Research

The research contributes to the development of the environmental assessment methodology for regionally important planning. The ecological consequences and the environmental effects of traffic development (opening up for development by road network enlargement and road construction) in northern Schleswig-Holstein (FRG) and South-Jylland (Denmark) are being investigated to provide the empirical basis of the methodology to be introduced.

An area of about 4000 km^2 has to be evaluated in respect to its different suitability and conservation value. This assessment enables a pre-choice of areas which belong to different sensitivity classes according to the impact of the regionally significant measure (in particular land use changes).

The positive and negative effects on the environment shall be demonstrated in particular with the new built motorway E 3 between the cities of Rendsburg (FRG) and Haderslev (Denmark). The mediate environmental effects and their causes also have to be taken into account.

Finally the aggregation of effects should allow a system analytical balancing of economic and environmental factors.

2. Materials and Methods

The research comprehends three main levels of investigation:

- an analysis covering the whole investigation area,
- an investigation of the effects which directly come from the road line and road construction, including the mediate consequences,
- a more detailed analysis of problematic areas where land use conflicts are to be expected.

The levels demand different procedures while the general methodological approach remains more or less the same.

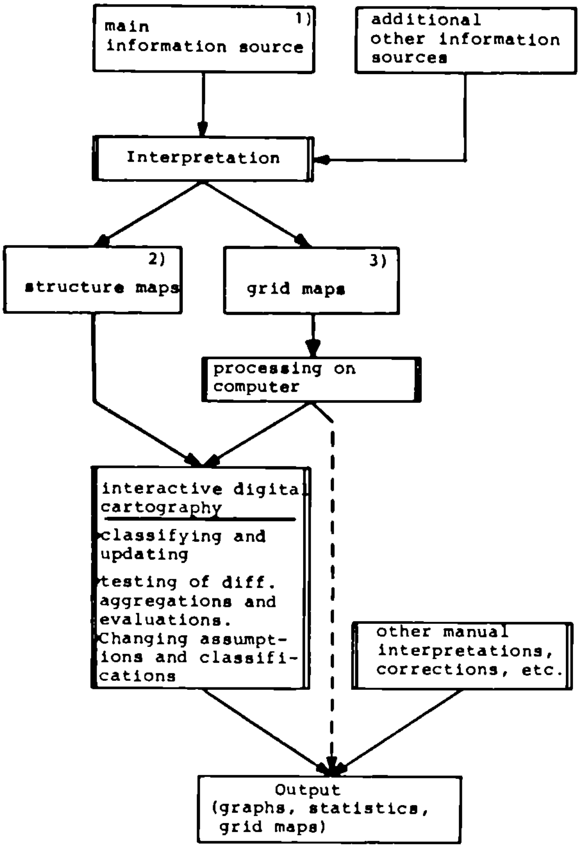
The research is based on the data acquisition by a geographic inventory of the area where mainly the material which is available without further inquiry shall give the input for the further analyses. However, because of the fact that the available material had been very diverse, fragmentary,of different quality and different scales, additional survey had been necessary. This means that the results show different reliability rates for the planning statements (e.g. about the risk of a proposed land use change). It is useful to define a "minimum reliability rate or minimum error rate" for each planning statement which should indicate the limits of data sufficiency or the level where additional information is needed.

According to its different research fields and investigation methods the project can be divided into a methodological and an empirical part.

The methodological research, as the main objective of this study, aims at the development of means for the environmental assessment of regionally siginificant measures.It concentrates on substantiations of aggregation procedures
and on the improvement of their applicability in the planning practice.The empiricalpart of the project covers the acquisition of socio-economic and geographical data, and the tests of the mentioned methods for environmental planning.

The processing of the basic data is manually done by direct interpretation and by means of an interactive digital cartographic device in connection with a standard computer.

The working steps are shown on the following scheme (tab. 1).



e.g.: 1) topogr. map 1:50 000
2) delineation of wooded areas
3) length of hedge rows

Tab. 1: Steps of information processing

The methodology is particularly based on the procedures as developed for the "Handbook for Ecological Planning" (Umweltbundesamt (Hrsg.): Abschlußbericht zum FE-Vorhaben "Handbuch zur ökologischen Planung", 1978).

The main methodológical task lies in aggregating area-related informations. The most important principles can be described as:

- indicator concept: The environmental situation and processes are characterized by means of informations which stand for more complex facts. E.g. the degree of air pollution of an area can be derived from the energy consumption, the different energy sources and the topographic and climatic factors.
- assessment and aggregation: The informations to be aggregated are transferred into a uniform scale according to instructions which had been developed for different applications and potential land uses. This include an assessment of the many basic data. E.g. the recreational suitability becomes assessed by the diversity of land uses.
- grid-transformation: The basic regional unit is a grid of a certain size. The grid allows the spatial combination of different geographical factors.
- EDP-assisted procedure: The informations are processed by means of a computer and a device for interactive digital cartography. This facilitates updating and changing of assessments and planning or scientific assumptions (as shown in the aggregation instructions).

3. Results and Conclusions

As the project is not yet finished, only some aspects of the general regional analysis should be shown here.

a) Data sources

The reality of planning on the level of regional, state-wide planning demands a relatively fast and reliable statement on the environmental impact of a certain regionally siginificant measure.

The demand of "fast results" entails that comprehensive e.g. geo-ecological research with long-range analyses is not practicable. It means rather to use the available material and data sources on the basis of which an ecologically assessable regionalisation can be established, which allows to differentiate the possibility and risk of conflicts between different land uses. In such a case the data requirements have to be defined in accordance to the accessable data sources. Tab. 2 shows the combination between the environmentally significant fields on which information is needed, and the data sources, which were available for the Schleswig-Holstein part of the mentioned project.

SUBJECT FIELD OF RESEARCH	DATA ON	MAIN SOURCES (supporting sources not mentioned)
0. general		
0.1 administrative boundaries	o communities, districts, sphere of influence	outline maps
	o population	statistical returns
0.2 land use	o settlement areas o traffic areas o forests	TK 50[1]
	o areas of special land use	regional planning cadastre[2]
0.3 hydrography	o rivers, brooks o lakes o coastline o catchment areas	orohydr. maps or TK 50
0.4 industrial sites	o emitting industry	reg. planning cadastre, statistical returns
0.5 traffic on roads	o road network	TK 50
	o density	traffic density maps or returns
0.6 railways	o railway network	TK 50
	o density	records
0.7 waterways	o ship canals	TK 50
	o density	records
1. air pollution		
1.1 emission of settlement areas	o distribution of settlement	TK 50 (see above)
	o density	
	o population	statist. returns
	o energy consumption	" "
1.2 emission of industries and power plants	o sites and type	TK 50 and statist. returns
	o capacity	reports
1.3 emission of traffic ways	o network	TK 50 and statist. returns
	o density	statist. returns
1.4 climate	o distribution factors (topographic and climatic)	TK 50 meteorological records
2. noise		partly based on TK 50. Thematic and applied maps, supported by data from local and regional agencies.
3. surface waters		
4. recreation		
5. ground water		
6. agriculture		
7. areas for the regeneration of water resources		
8. areas for the regeneration of air		
9. areas for the regeneration of fauna and flora/biotopes		

1): TK 50: Topographische Karte (topographic map) 1:50 000

2): regional policy register on a state-wide level

tab. 2: research fields and main data sources (abridged)

Most of the material had been very diverse, fragmentary, of different quality and different scales. The topographic map 1:50 000 (TK 50 of the FRG) proved to be one of the most important data sources[1], because it covered the whole area

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[1] additionally the regional planning register (1:25 000) could be used where up-dating of topographic features were necessary and the status of areas and land use were important.

and was relatively actual what the topographic content concerned. Aerial photographies were only additionally used because they covered only small areas of the region to be investigated.

b) Fidelity requirements

In order to assess the research results it is important to know how reliable, accurate and actual the informations are.

The actuality of the map material plays a great role in particular with environmental planning. Artificial changes as e.g. the change of road networks and the spreading of settlement areas are sufficiently up-dated or can relatively easily be corrected. The natural factors however, which are very important for an ecological regionalisation are insufficiently up-dated and principally show a state of land use which leads to an environmentally more positive assessment than reality is. Small groupings of trees, wetlands, bogs, lakes and hedges can be mentioned here. Changes by land clearing for example, are not represented on maps. An analysis of different land uses showed e.g. that only 34 % of the wetland-areas as figured on topographic maps could be refound on aerial photos or by site survey. Other land uses, however, had a much better map/reality ratio. In order to keep the interpretation effort in the scope of this research only areas of special interest had been closerly investigated by means of direct survey while sample areas gave an idea of the general actual geographical situation.

The regional analysis does not yet allow to give a preliminary statement on the environmental impact of the enlarged road network. This is due to the fact that the area under investigation in general has a rather low data level and prognoses on the traffic acceptance can by now only be done within a larger probability range.

List of References:

a) Oral communications with representatives of:

- Miljøministeriet, Copenhagen
- Fredningstyrelsen, Copenhagen
- Sozialministerium, Abt. Umwelt, Kiel
- Straßenneubaubehörde-Mitte, Neumünster
- Planlegningsafdeling, Aabenraa
- Ministerium für Ernährung, Landwirtschaft und Forsten, Kiel
- Innenministerium, Kiel
- Landesamt für Naturschutz und Landespflege, Kiel
- Kreisverwaltung Rendsburg
- Kreisverwaltung Schleswig-Flensburg
- Sønderjylland Amtskommune, Tønder und Aabenraa
- Landbrugsministeriet, Vejle

b) Publications or papers referring to project:

- paper, read in Sankelmark (Schleswig-Holstein): "Instrumentarium zur Beurteilung ökologischer Folgen der verkehrlichen Erschließung"; Symposium "Umweltschutz und Straße", June 1978.

Contractor:	ECOSYSTEM Ges. f. Umweltsysteme mbH
Contract n°:	295-77-2 ENV D
Project Leader:	Prof. Dr. P. Müller/Dr. E. Keller
Title of Project:	Ecological criteria for assessing transnational space

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Transnational environmental impacts are increasingly becoming a focus of interstate conflict. Differing legal norms, varying economic policy goals, and sometimes basically different natural resources make the definition of generally acceptable guidelines a difficult task. This is why the research carried out under this contract is aimed at establishing generally applicable ecological criteria to be employed for the assessment of transnational space by a systems analytical approach to information contained in living systems. In a second step the ecological data matrix thus established was tied to other space-relevant factors by a systems analytical model study. The state of Saarland, the urban agglomeration of Saarbruecken and a point source of emission (power plant at Ensdorf), were employed as testing areas.

1. Ecological Investigation

For the ecological assessment of these testing areas beyond an ecological data base (inter-alia epidemiological studies, recording of important faunal and floral species on a UTM grid of 1 x 1 km resp., 500 x 500 m, plant sociological charts, recording of radiological parameters) the establishment and validation of both a passive and an active monitoring program were central to the research accomplished in this phase. The passive monitoring program consisted of effects monitoring and trend monitoring of wild living organisms.

Trend monitoring was initially limited to heavy metals (Cd, Pb, Zn, Be, Se, As) in wild living animals (Lumbricus terrestris, Carabus sp., various vertebrates and plants (Picea sp., Plantago, Taraxacum, Acer, Platanus). Trend monitoring was later extended to include organic substances (PCB).

Effects monitoring was applied by charting visible emission damage (i.e., forest damage zones; lichen zones) and synecological changes to be tied to anthropological factors.

An active monitoring program was also initiated, using exposed organisms (Müller and Erhardt, 1978). Here again, both effects and trends records (accumulation) were used.

For the "effects register", (Wirkungskataster), the species Hyposymnia physodes, tobacco Bel W3 and a number of lupines and lucernes were tested. Using multiple analyses of air composition at various sites, it was possible to demonstrate that after only 45 days of exposure of Hyposymnia physodes, excellent data on the air quality class of these sites could be obtained.

Moreover, it became clear that, by using exposure methods, a validation of distribution models was possible in the immediate vicinity of the tested power plant.

The exposed lichens were less suitable for trend monitoring as their thallus size was not sufficiently large for multiple analyses. Plant species of larger biomass were therefore exposed (Picea sp., Plantago, Taraxacum, Lolium multiflorum, Brassica oleracea). Picea omorica, for example, was found to be far more appropriate for trend monitoring than Picea abies (cf. Müller and Wagner, 1979) because the former is less sensitive to emissions and is genetically more uniform. In order to ensure genetic comparability of the test organisms, disc electrophoresis was employed to measure allelic polymorphism (Müller and Steiniger, 1978, 1979, Steiniger, 1978). Their results demonstrated that emissions have a selective effect on allelotype variety. Thus, some heavily stressed sites are populated only by certain allelotypes (shown for 74 carabidae populations at both urban and non-urban sites) and individual allelotypes have developed different adaptive strategies (i.e., different heavy metals accumulation, different protective mechanisms of enzyme systems). Our cultural landscapes are thus presently experiencing evolutionary processes, analysis of which has so far been neglected.

The area and point source (power plant) data obtained in this first phase were subjected to a systems analytical assessment and were thereby interrelated with a great number of space relevant factors.

2. Systems Analysis and Assessment

As a preliminary step in this phase of the study, a value (cost-benefit) assessment method was developed. Initially, a number of subsystems (and dimensions to measure them) were defined that could be influenced if the use of the space under consideration were changed: ecological factors, ecological effects, resources, economic and settlement structure, social structure, material infrastructure, and indirect data pertaining to the individual human environment. In a first assessment step, the relative importance of the above subsystems is to be defined. Each subsystem dimension will then be weighted (indicator weighting on a 1-10 scale). In a third step, the goal achievement rate of each space-relevant indicator will be established. Thus the "value" of the space-relevant project under consideration may be assessed using the following formula:

$$V = \sum_{i} Di \sum_{l} \sum_{m} \bullet I_{l,m} \bullet Z_{l,m}$$

where by

Di = weight of subsystem dimension

$I_{l,m}$ = weight of indicators

$Z_{l,m}$ = goal-achievement rate of indicators

Among the critical indicators (criteria for assessment) employed for the various subsystems, the following is cited as an indication.

- ecological factors
  - emissions of particles
  - emissions of noise
  - emissions of waste water
  - emissions of waste heat
  - solid waste disposal/treatment
  - environmental health
- ecological effects
  - effects on lichens
  - effects on grass
  - effects on pines
- resources
  - availability of space and surface aquatic bodies
  - availability of minimum surface in agglomerated areas
  - availability of labor force
  - availability of water
  - availability of energy

To validate the results obtained, sensitivity analyses of relevant parameters were recommended and commented on.

Contractor :	EDEN (Ecologie-Développement-Energie) University of Paris VII
Contract n° :	203-77-1 ENV F
Project leaders :	Prof. J. Viera da Silva and Ph. Mirenowicz
Title of project :	Integrated analysis of eco-sociosystems; application to regional environmental management policies (case of the Seine and Marne department)

I. SETTING OF THE PROBLEM

The environment, which can be defined as the center of interdependances between social, cultural, and economic phenomena and ecological processes, appears to-day as the specific field where the expression of the movment of integration/desintegration takes place. In other words :

- the interdependances between phenomena and various levels of space, time and organization scales are increasing in number, getting more complexe and generating new forms of causalities.

- at the level of perception and analysis as well as at the level of management, sectoral approach is always predominent and more synthetical approach is still lacking.

This paradox of simultaneous complexification and sectorization results in the field of the environment by the need of a natural environment of high quality, which assumes all its functions in the medium and the long term, and also by a quick downgrading of this natural environment and the feeling of an incapacity to manage it.

In fact, when the regional ecological data banks become avaliable and when the region (in its geographical sense) appears as a pertinent level for the management of the man-made and natural environment, it is clear we do not really have a good methodology nor practical tools allowing the definition of evaluation and implementation of management policies which would be coherent in the short and longer term, in relation to economic development and environmental politic objectives.

II. OBJECTIVES AND CHARACTERISTICS OF THE RESEARCH

Our research has started from the above-mentioned framework and has tried to bring some answers to these problems. Two main objectives are therefore aimed at :

- the building of the conceptual model for the analysis of "eco-sociosystems" based on three basic methodological tools : PROSPECTIVE ANALYSIS, SYSTEMS THEORY and SYSTEMIC ECOLOGY.

- the practical experimentation of this conceptual model in the department of Seine et Marne (France), which has led us to define alternative management propositions.

Those objectives determine some basic characteristics of our research :

- include man not only through its activities but also by its social and cultural aspects : values systems, conflicts, and finally its actions must be placed at the center of the model.

- take into account the various forms of interactions between the "sociosystem" and the "eco-system".

- finally, include the problem of the integrated development and the reproduction of natural and human systems in the evaluation process.

III. BASIC PRINCIPLES AND CONCEPTS

Three principles make the link between the general above mentioned analysis and the practical development of the research.

1°/ The basic unit of the study : the eco-sociosystems

The center of attention has been directly put on the interactions and interdependances between the social system and the ecosystem, which has led us to the definition of the eco-sociosystem.

2°/ A key-element for the evaluation : the patrimony value

The research field being defined and structured in units of analysis, we have set evaluation criteria and indicators so that we can assess the state of the eco-sociosystem from the point of vue of its patrimony value : this means to be able to understand the conditions of its reproduction and long term evolution. Such questions as the reproductive capacity of natural systems, the differencial time length of the ecological and economic cycles, the measure of the diversity, complexity, stability of these systems and the ecological thresholds and constraints have been addressed.

3°/ The institutionnal approach

We have discussed the traditionnal sectorial planning and the need of a more integrated approach which would be embodied in the appropriate institutions.

IV. THE METHODOLOGICAL TOOLS

1°/ Systems theory

Having divided up the area (the department of Seine et Marne)in homogeneous sub-areas, each of which being an eco-socio-system, we have studied, for each one, the linkages and interdependances between the natural and social environments ; we have then described the interactions among those sub-areas ; we have studied the structure and evolution of these systems through the tools and concepts of systems analysis.

2°/ Systemic ecology

The importance of transfers and flows among eco-sociosystems show that they are open-systems for which the exchanges with the exterior are key-factors

Furthermore, energy and more generally ressources are transmetted and utilized with various efficiency levels within systems. All these characteristics are those of natural ecosystems about which a scientific corpus, systemic ecology exists. Therefore, it has seemed interesting to use, by analogy, some of the concepts of the systemic ecology for the analysis of eco-sociosystems.

3°/ The prospective approach

The explicit need to consider medium and long term time horizons, the need to be conscious to-day of the future environmental problems and the need to examine the strategies of the actors of the system and their conflicts, has led us to the use of the prospective approach ; in particular, the scenario method has been applied to the case of Seine et Marne department.

V. THE DEVELOPMENT OF THE STUDY

It has taken place through five successive phases :

1°/ Definition of contrasted scenarios

Three scenarios of regional development at horizon 2000 have been defined and built in order to explore various futures for the Seine et Marne department.

2°/ Identification and description of the eco-sociosystem

In a first step, we have identified the ecological systems and described them by the classical ecologic indicators but also through elements taken from the concepts of the systemic ecology ; in a second step, we have specified and delineated the social and economic systems which take into account the specificities of the populations of the region. Finally, in a third step, being aware of the interaction between the eco and socio systems, we have defined the eco-sociosystems which are both structural and functionnal entities including at the same time the ecological and socio-economical speficicited of a sub-area.

In this process, an important data-bank has been built which has been used by computer ; also an ecological synthetic map of the department of Seine et Marne has been drawn. Furthermore, various statistical treatments have been performed on all these data.

3°/ The functionning and the dynamic of the eco-sociosystems

The dynamic of the eco-sociosystem has been studied for itself and in relation to the other eco-sociosystems so that the behaviour of their endogenous variables can be assessed in relation to the environmental constraints and to the scenario variables (exogenous variables).

4°/ Diagnostic and evaluations of the present and the future state of the Seine et Marne department

In this phase, we have not analysed the images of the future given by the scenarios in terms of impact, aptitudes, or sectorial diagnostic, but in terms of evaluation of the degree of coherence of the simultaneous evolution of the natural and socio-econimical systems ; we haved shed light the potential contradictions and conflicts in the evolution of these systems (in time and space) in order to make clear the thresholds and conflicts in the future use of space and natural ressources.

5°/ Regional environmental management policies

From these various evaluations (one per scenario) recommandations for alternative regional environmental management have been made, based on the following ideas :

- conceive systems of complementary activities within a given eco-sociosystem, in the use of ressources and space.
- take into account the uncertainty about preferencies and future use about the environment through an increased flexibility of the options and the saving of margin of manoeuvre for future options ; this applies particularly for irreversible orientations.
- look for a dynamic harmonization between sectorial policies (transportation, urbanisation, energy, ...) and the specific environmental policies (air, vater, solid wastes, ...) rather than a static compromise.

- propose a real patrimonial management which leads to a broader conception of environmental policies (i.e. not only in terms of protection).
- take into account the heterogeneity of the social, economic and ecological time units in order to define the horizons which are pertinent for planning.
- keep together environmental planning and the management of daily activities.
- organize environmental and patrimonial management at the level of and by the populations which are directly concerned (the eco-sociosystem brings a first answer to that question since it expresses some kind of possible harmony between a population and an ecosystem on a given sub-area).
- keep or reconstitute the diversity of the human systems.
- develop the environmental management by steps applying permanently the trial and error process.

Remark : the "global" model which is presented here, is only a conceptual model, not completely formalized.
The next coming research for the CEE (1978-79) should be based on the conception and the perfection of a quantified and computerized model of an eco-sociosystem, allowing simulations in time.

This research has been presented as a communication to the GERMES Seminar at Arc et Senans (France).

A summary of the research must be published soon in the journal : Aménagement et Nature and also in the publication of the GERMES Association.

Contractor : Centre d'Analyse spatiale quantitative et appliquée (Laboratoire
R. Blanchard) University of Nice

Contract n° 206-77-1 ENV F

Project leader : A. Dauphiné

Title of project : Previsional models of the dynamics of periurban natural spaces in mediterranean France and Italy·

Objective of the research

Natural spaces in the Mediterranean area suffer from an important lack of balance ; minor changes result in crise, particularly the urban growth which causes damage to he landscape without possible reversal. The building of models about the evolution of periurban natural spaces is what we aim at in the scheme. It has a double prospect :

- to devise empirical and cartographical models
- to devise mathematical diffusion models.

Material and methods

In the first part, we shall try to define, from an epistemological point of view, the concept of environment (the ecologist's, the idealist's or the geographer's environment).

The geographer thinks that periurban environment is a morphology, an assemblage of forms, a stream of flows with complex temporal evolutions. These forms and flows are organised into a hierarchy. Therefore the environment is something which is given, built and perceived.

To explain and forecast its evolution, we don't have any global theory but a lot of parttheories. However, we do have rudimentary though formal global theories at our disposal particulary of Thom (1974) about morphogenesis, that of I. Prigogine (1977) about dissipative structures and finally the general systems theory. The objectives of our research are situated within this epistemological and theoretical framework.

Two test cities have been chosen : Nice and Genoa ; a large seaside resort and a large harbour. Field work and the analysis of air-views have allowed us to select four types of suburbs. Eastern, residential suburbs grow dense, exotic spaces are destroyed.

Narrow vales follow a second type of evolution with the disappearance of their physical characteristics in the talweg, the uspsetting of the crests and the gradual building up of the slopes which gives them a very particular "moth-eaten" design or dash and dot aspect. In wide valleys, agriculture resists much better and the decay of the environment obeys different rules. Finally western suburbs, overrun by industrial and transportation structures present a fourth aspect of developement.

In Nice, each suburb has been cut out into regular squares, where as in Genoa it was in homogeneous although irregular spaces, so as to test the influence of the partition.

The data have been chosen according to the given aims, the theories and their own quality. To reach our aim and estimate the dynamics of environment, we have retained variables which evolve more or less quickely. Besides, if some transformations are reversible, others are not. These variables essentially represent forms because flows have not been analysed because of the lack of machines.

Most of the variables have been studied before, particularly by the phyto-ecological school of L. Emberger. Each variable was taken at two different dates (two series of air view, one in 1964 and one in 1974), sometimes three, and they were coded in nominal mode.

Three techniques have been retained : field work, cartographical and mathematical formalisation. The cartographical treatment lead us to establish three maps : one for each year, 1964 and 1974, and one about evolution comparing both states. Another group of map, more synthetical is under way. These maps allow us to draw two conclusions. On the theoretical level, they put in view the type of diffusion processes of evolution. It is then possible to forecast a land use, minimizing the decay thanks to planning (land use planning in France, Pianno regulatore in Italy). An Atlas of about 150 maps has been elaborated this way.

To get a satisfying mathematical formalisation we have taken empirical and cartographical models into account. Those models demonstrate that :

- Simple, neighbourhood, hierarchical and random diffusion forms are nearly always overlapping ; neighbourhood and hierarchical diffusion works simultaneously and successively,
- The random diffusion process is really effective only inside limits,
- Barrier effects appear clearly, even in relatively flat irregular in narrow vales.
- The diffusion process complexity is increased by the interaction between variables which don't always work in the same direction. Taking these results as a starting point, we have tested the models perfected by Hagerstrand and Berry. In a second pha-

se, we have used more realistic models perfected at the geographical institute of Karlsruhe. The models (programs hayer 1 and hayer 2) integrate barrier effects more particulary.

Finally we have transformed and applied a diffusion model resting on entropy, perfected by RK. Semple and MT. Wasilenko (1978).

Results

Cartographical analysis allox us first to classify the types of evolution of environment components. We have distinguished stable parameters that don't vary at a human scale, for instance the nature of rocks and the topographical situation. These components are very few.

Andthen, the second type is constituted by slowly evolution componenents, slowness that we can measures thanks to the square mapping system. Some variables have a slow and continuous evolution, for instance the artificialisation of vegetation (we notice changes in 4 to 10% of the land parcels). That evolution, which is always oriented in the same direction, to a greater artificialisation, is relatively continuous except in weastern suburbs where important infrastructures grow.

On the other hand some components follow a slow but discontinuous evolution, such as the humidity of soils ; then the urban effect is a desertification by fits and starts, according to the process of housing zones. In the same way, erosion develop slowly, but with sometimes unexpected jumps over limits, particularly in narrow vales. Thoughtless urbanisation results in the upsetting of erosion schedules and entails heavy additional works to put a limit to erosive crises.

Other components have fast continuous or discontinuous transformations. The albedo is one of those variables which is quickely modified by urbanisation. So is it whith the structure of vegetation which changes unexpectedly with a lot of discontinuity.

Spatial forms of this evolution are numerous. We have counted four elementary types. First, diffusion can spring up out of neighbourhood or contiguity. That's what happens with the percentage of rock outcrop, we can draw a real front of decay. Sometimes the process is blocked by a barrier effect particularly for the vegetation forms lining the rivers Var and Polccevera.

This type of contiguity diffusion with barrier effect can be observed mainly in narrow vales where the steepness of the slopes annihilates diffusion processes. Irrigations systems follow the same rules.

For these components, T. Hagerstrand's simulation models, based on the "Method" of Monte Carlo, are satisfactory, mainly in wide valleys (program haeger 2).

Other components undergo a transformation in clusters. This evolution is akin to a hierarchical diffusion. The characteristics of the vegetation sub system have a tendancy to follow this law, as well as physical and chemical pollution. But these cells which develop ahead of the urban front, don't build a real hierarchical system.

A great number of variables have an evolution which creates a hallow spatial structure, mainly in narrow vales eastern residential suburbs.

The processes are complex when the whole of natural environment is concerned; the different types of evolution appear simultaneously and can even overlap. To apprehend these transformations K. Semple and M.T. Wasilenko's mathematical model (1978) is quite satisfactory at the level of analysis. Yet, it does not allow us to devise quite enduring spatial forecast. It would be useful to make some research to fill up this blank.

The natural spaces of the suburb follow different path of evolution. In the west and in wide plains, the natural environment is hit by waves (contiguity effect) or by patches. In the eastern residential suburbs and in narrow vales diffusions in "dots and dashes" is predominant.

Conclusions

Cartographical and informatic results express the complexity of the diffusion processes to which periurban natural environment is submitted. If some forms resist well, such as the vegetation which is fundamentally transformed only by urban implantation, other characteristics are deeply adulterated even before the intrusion of buildings, mainly the water cycle and may be the energy cycle.

Simplified maps elaborated out of evolution maps should permit to devise actions to fight against decay. Then according to the contemplated actions, it is possible to simulate the results we get from informatic models, at least for the simplest characteristics.

Anyway this research includes blanks that are left ot be filled. First, on the level of methods, two progress were left undone : to make a global analysis of forms and not only a sectorial study ; but it requires technical progresses and then to improve the interrogation of spatial reality in mathematical models.

Another important need to be met is the study of the main flows of water, energy and material. This requires the establishment of material. This requires the establishment of heavy stations like in URSS, or Bomes in USA, and automatic stations.

Publications and oral communications

- Le concept d'environnement péri-urbain et ses applications.
  Analyse spatiale quantitative et appliquée 1978 n° 6, 20 p.

- Analyse dynamique de l'environnement dans la banlieue de Nice.
  Recherches urbaines, n° 40 pages.

- Les Modèles de diffusion appliqués à l'environnement péri urbain.
  L'espace géographique (à paraitre)

- Les types d'environnement péri urbains à Gènes
  Cahiers de l'ILRES, 1978, n° (en Italien)

- Vers une planification de l'environnement péri urbain
  Oral communications, journées nationales de géographie, Montpellier 1978, Commission géographie appliquée.

- Le potentiel naturel de la basse plaine du Var
  Oral communications, journées des chambres de Commerce de la Côte d'Azur.

- Les formes touristiques et les transformations des espaces naturels péri urbains.
  Journées franco italienne sur le tourisme, Association des cadres du tourisme, Menton, novembre 1978.

- Les modifications des micro climats péri urbains en Méditerranée
  Journées nationales de climatologie, Paris, décembre 1978.

- D'autres publications sont prévues, notamment dans les revues Méditérranée (Aix en Provence), et (Paris).

TOPIC 45 : ECOLOGICAL CONSEQUENCES OF MODERN AGRICULTURAL PRACTICES

Contractor:	Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Abt. Pflanzenschutz, München
Contract n°	209-77-1 ENV D
Project Leader:	Prof. Dr. P. Wallnöfer
Title of project:	Degradation of xenobiotics and their metabolites by soil microorganisms

Objective of the research

Degradation of xenobioties in the environment is often incomplete leaving metabolites, frequently the aromatic moiety of the parent molecule, which are more recalcitrant to microbial attack. There is a need to investigate the further degradation.
The purpose of this investigation is to elucidate the metabolism of anilines and phenolic compounds, which are liberated during the microbial degradation of aniline-based and phenol-based pesticides in soil.

1) Halogen-substituted anilines

Aniline based pesticides, particularly phenylurea, phenylcarbamate, and acylanilide herbicides and fungicides are being degraded in the environment with the ultimate liberation of halogenated anilines. Four major modes of transformation of halogenated anilines in soil and by microorganisms isolated from soil have already been described: (i) condensation of two molecules which leads to the formation of both symmetrical and asymmetrical azobenzenes (Bartha and Pramer, 1967), and the condensation of three molecules with the formation of halogen-substituted anilinoazobenzene (Plimmer et al., 1970); (ii) condensation of two molecules of a primarily hydroxylated 4-chloroaniline with the formation of 7-chloro-2-amino-3H-phenoxazin-3-one (Briggs and Walker, 1973); (iii) acylation resulting in a formylated (Kearney and Plimmer, 1972), acetylated (Wallnöfer et al., 1977), or malonic acid conjugated (Kaufman et al., 1973), derivative; (iiii) oxidation of the amino to a nitro group (Kaufman et al., 1973). Anaerobic metabolism of halogenated anilines results in the release of a not yet identified volatile compound (Bollag and Russel, 1976).

2) Phenolic compounds

Chlorinated phenols are intermediates in the microbial degradation of the widely used chlorophenoxyacetic acid herbicides (Bollag et al., 1968; Loos et al., 1967). Methyl-, methylthio-, and nitrosubstituted phenols are released in soil from a number of organophosphorus and methylcarbamate insecticides and nematicides by chemical and enzymatic hydrolysis in water, soil, plants and animals (Fest and Schmidt, 1973).

Materials and methods

Chemicals. All substituted phenols were purchased from EGA Chemie, Steinheim, Germany, the chemicals are commercially available compounds.

^{14}C-Phenyl-labeled 4-chloroaniline (spec. activity 57 mCi/mg) was purchased from Buchler, Amersham, Braunschweig, Germany.

Culture conditions. Degradation studies were performed using Hegeman's mineral base (Hegeman, 1966) modified by addition of 0.02% yeast extract or 0.1 M phosphate buffer pH 7.5. Cultures were incubated on a rotary shaker (New Brunswick, G 10) at 28 °C and 220 rpm. Liquid cultures were grown in 100 ml-Erlenmeyer flasks containing 25 ml mineral base with the addition of 0.01-0.02% of a substituted phenol and 0.4% of fumarate or ethanol, respectively, as carbon source. For large scale preparation of metabolites, cultivation was performed in 2 l-Erlenmeyer flasks with 1 l of medium.

Degradation of substituted anilines and phenols was assayed by extraction of residual substrate and metabolites followed by quantitative UV-analysis as described (Engelhardt et al., 1977).

Identification of metabolites

Melting points were determined using a Kofler hot stage (Reichert, Austria). Absorption spectra were recorded on a Zeiss spectrophotometer model DM 4 and mass spectra were obtained from an AEI mass spectrometer, type DB MS 1073 at an ionization potential of 70 eV using the direct insertion probe.

Preparation of crude extract and enzyme assay. Crude extracts were prepared by the alumina grinding technique as described (Engelhardt et al., 1977).
Catechol 1,2-dioxygenase was assayed by measuring O_2-consumption with a WTW-oxygen electrode (Wissenschaftlich Technische Werkstätten, Weilheim, Germany).

Results

Transformation of 4-Chloroaniline

From sixty bacterial strains isolated from a soil, previously treated with 4-chloroaniline (4-CA), 33 organisms were active in transforming 4-CA to at least two metabolites. One of the bacterial strains could be identified as Bacillus firmus, which transformed 80 % of 4-CA applied. Since the organism did not grow with 4-CA as sole source of carbon ethanol was used as carbon source. Under this conditions 4-CA was converted to 4-chloroacetanilide and 4-chloropropionanilide due to a cometabolic reaction. When the 4-CA concentration in the culture medium was increased up to 3.20 mg/ml, in addition to the formation of the two anilides the two phenoxazinone derivatives, 7-chloro-2-amino-3H-phenoxazine-3-one and 7-chloro-2-amino-3-H-3-hydroxyphenoxazine, respectively, appeared. These phenoxazinones are most probably formed by condensation of two molecules of 4-CA, hydroxylated ortho to the amino-group.

Degradation of substituted phenols

Nocardia sp. DSM 43251 grown on sucrose in the presence of substituted phenols metabolized these compounds with the intermediate formation of the corresponding substituted catechols (table). The phenols were added to the media giving final concentrations slightly below the concentrations toxic for the organism.

Phenols	Oxidation products
4-Chlorophenol	4 chlorocatechol
2-Chlorophenol	3-chlorocatechol
2,4-Dichlorophenol	3,5-dichlorocatechol
3,4-Dichlorophenol	4,5-dichlorocatechol
3-Methyl-4-(methylthio)-phenol	4-methyl-5-(methylthio)-catechol 3-methyl-4-(methylthio)-catechol

Table: Oxidation of various substituted phenols by *Nocardia sp.*

Isolation and identification of further degradation products.

Metabolites arising from further degradation of substituted catechol intermediates were isolated from cultures of *N. spec.* DSM 43251 grown in the presence of 4-chlorophenol, 4-chlorophenol-2-methylphenol, and 3-methyl-4-(methylthio)-phenol, respectively. They were identified by means of their UV-absorption spectra, melting points, and mass spectra as 5-carboxymethylene-2-oxo-2,5-dihydrofuran, 5-carboxymethylene-2-oxo-3-methyl-2,5-dihydrofuran, 2,5-dihydro-3-(methylthio)-4-methyl-5-oxo-furan-2-acetic acid, and 2,5-dihydro-2-methyl-3-(methylthio)-5-oxo-furan-2-acetic acid, respectively. These compounds are produced from the corresponding substituted muconic acids which arise from the catechols by 1,2-dioxygenative ring cleavage. Therefore 4-chlorophenol, 4-chloro-2-methylphenol, and 3-methyl-4-methylthiophenol are metabolized in *N. spec.* DSM 43251 by the common 1,2-cleavage.

Catechol 1,2-dioxygenase activity with substituted catechols.

Cells grown with substituted phenols in the presence of a carbon source to support growth contained only very low catechol 1,2-dioxygenase activity, which is presumably due to the very low substrate concentrations in the media. However, during growth on 0.1% phenol or 0.4% benzoate, respectively, as carbon sources a high activity of catechol 1,2-dioxygenase is induced.

Conclusion

Substituted anilines, e.g. 4-chloroaniline are transformed by soil microorganisms to several metabolites due to cometabolic reactions. However, a complete mineralization of these compounds has not been reported until know, although very small amounts of the less chlorinated derivatives can be degraded to CO_2.

Since up to 90% of halogenated anilines released during the biodegradation in soils become unextractable by solvents, it seems most likely that they are reacting with humic substances, forming non hydrolyzable bindings between aniline and monomeric constituents of humic substances. Microbial degradation tests with model substances consisting of known lignin monomers and substituted anilines are indicated to elucidate the fate of bound residues in soils.

Phenols substituted by one or two halogen-substituents are readily oxidized by several soil bacteria by 1,2-dioxygenative ring cleavage accompanied by chlorine elimination from the molecule. The further metabolism leads to the ultimate liberation of CO_2 and chlorine. However, in soils even these phenols are often withdrawn from microbial attack for a while due to adsorption reactions to the soil organic matter.

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Contractor : Universität Hohenheim, Postf. 106, D-7000 Stuttgart-70

Contract n° 210 - 77 - 1 ENV D

Project Leader : Prof.Dr. F. Großmann

Title of project : Differences between conventional and biological fertilization relevant to the environment

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Differences between „conventional" and „biological" fertilization relevant to environment

by E. Schlichting and G. Kahnt

The following questions were investigated :

1. Under which site conditions is N from organic fertilizers mineralized synchroneously with plant demands and thus leached or volatilized less than mineral N (E. Schlichting)?
2. Which plants use best the N from liquid manure or high doses of P from such impoverished in N by aeration and
3. Which legumes fix under which conditions the most N and when is how much of this N available for subsequent crops (G. Kahnt)?

Since the experiments are still pending, only a preliminary report can be given.

1. Nitrogen turnover under different management at various sites

At the following sites

Locality	Mean annual temp. °C	leaching mm	Soil
1. Reutsachsen	7-8	200-300	Pseudogley from loess/k_u-clay
2. Tiefenbach	"	"	" " " "
3. Gochsheim	9-10	"	Brownearth " "
4. Kirchheim/N.	"	"	eroded Para " " " "
5. Möttlingen	7-8	"	Pseudogley-Pelosol from m_u-marl
6. Ehningen	"	"	" " brownearth from loess
7. Wilsingen	6-7	300-400	Terra fusca from w_δ-limestone

one field was supplied with conventional (organic + mineral) and the other with biological (no mineral) fertilizers; additional plots at sites 4 and 6 were fertilized with 0, 60 or 120, at site 7 with 0, 50 or 100 kg N/ha either as lime-ammoniumnitrate (conv.)or as liquid cattle manure (biol.). The nitrogen turnover in soils, plants and soil solutions was investigated as is shown for site 1 in figure 1.

In the soil of 1. biol. the N_{min}(= NO_3+ NH_4) contents were highest in the autumn, decreased very much during the winter and less during plant growth. In the soil of 1. conv. the N_{min} contents were much lower in the autumn, decreased less during the winter, reached a maximum after spring fertilization and dropped sharply under the growing plants. During uptake by plants N_{min} in the soil was restored by mineralization (dashed lines in fig. 1).

Since the uptake (fig. 2, section a) exceeded the mineralization (section b), the difference (c) decreased the N_{min}-contents. But the decrease between N_{min}-maximum (d) and minimum at harvest time (e) f(=d-e) exceeds c by the amount of g (= f-c), which was lost. In the soil of 1. conv. the loss of N_{min} before it reached its maximum (h) has to be added. Then g+h = i is the total loss of N_{min} between autumn and harvest.

Evaluation of the measurements in this way lead to the following results: At all sites cropped with winter wheat the course of N_{min} was similar to that at site 1. Without additional N, plants took up less from „conv." than from „biol." soils. At a given site the differences in yields and N uptakes corresponded with those in the N_{min}-maxima. Uptakes (a) exceeded the mineralization during the growth period (b) at all sites, except some plots of the cool humid site 7.

– At site 5, which was cropped with legumes + oats, the N_{min}-contents decreased only slightly during the winter, corresponded with the uptakes and decreased less than the uptakes increased (f-c = g $\simeq$ -100 kg N/ha due to symbiontic N fixation) in „conv." and „biol."-

A general relation between kind or level of fertilization and N losses could not be found.But from the temporal and spatial N_{min}-distribution it can be concluded that N losses occur from „conv." soils more by volatilization and from „biol." soils more by leaching (which is proved by analyses of seepage water).

2. Use of N and P from liquid manure at different sites and by various plants

2.1 Field experiments: At the sites 4, 6 and 7 (cf 1.) the yields of winter wheat depended more on the preceeding crops (g = grain crops, p = potatoes, l = legumes) than on either the site or the fertilizing system (cf 1.), as can be derived from the following data (dt/ha):

Site	4 conv.	4 biol.	6 conv.	6 biol.	7 conv.	7 biol.
Rotation	g-g-p	l-l-p	g-g-p	g-g-p	l-g-l	g-g-l
N 0	33	64	39	50	46	37
60	42	70	52	45	50	42
120	54	66	55	44	56	49

In an unsuitable rotation low yields of unfertilized plots apparently can be increased better by mineral than by organic fertilizers.

2.2 Pot experiment: 0.47 g N, 0.38 g P and 0.66 g K / pot given either as liquid cattle (Iho, G) or pig manure (Z) or as mineral fertilizer were used by Trifolium alexandrinum (Trif.al.) or Lolium perenne (Lol.per.) in the first and -after fallowing- by summer wheat (Trit.L.) in the second year at the following rates (%):

Plant	Trif.al.			Lol.per.			Trit.L.		
Fertilizer	N	P	K	N	P	K	N	P	K
Iho	-	21	94	34	11	76	21	10	44
G	-	21	79	46	14	89	37	10	52
Z	-	30	65	37	13	75	20	13	36
Mineral.	-	8	29	58	13	91	50	18	79

From the data it can be concluded, that the clover uses much more of the manure-P than the grass. But its N-fixation allows only medium yields of the following wheat (for high yields either a more efficient legume or an additional fertilization would be needed).

3. Nitrogen fixation by legumes

In a pot experiment the increase of soil rhizobia as well as the effect of repeated legume cropping on yields of dry matter and protein were tested. More than 20 legumes were also investigated in the field with respect to growth during the season, yield of dry matter and protein as well as to N-accumulation in the shoots (x 1.4 ≈ N-fixation). The following groups can be made:

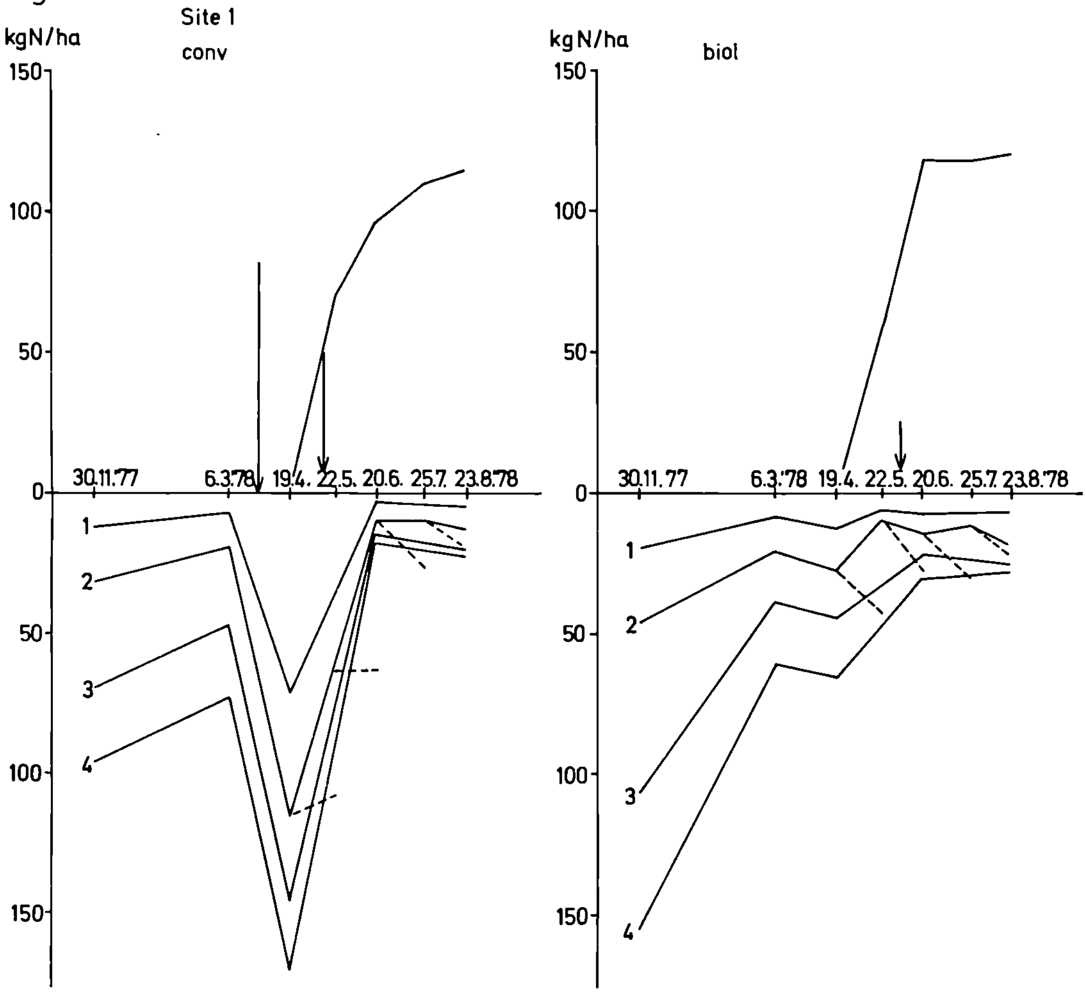
30-90 (= Lolium per.):	Lupinus lutens, Ornithopus sativus, Vicia sativa, Lathyrus sativus, Anthyllis vulneraria
90-180:	Onoboychis viciifolia, Lupinus augustifolius, L.albus, Trif.pratense, Trif.resupinatum, Trif. repens, Trif.hybridum, Medicago sativa, Melilotus albus

180-360: Trif.inkarnatum, Medicago lupulina, Pisum sativum

>360 kg N/ha: Vicia faba

The availability of this N for subsequent crops is tested in pot and field experiments.

Fig. 1:



Legend to Fig.1:

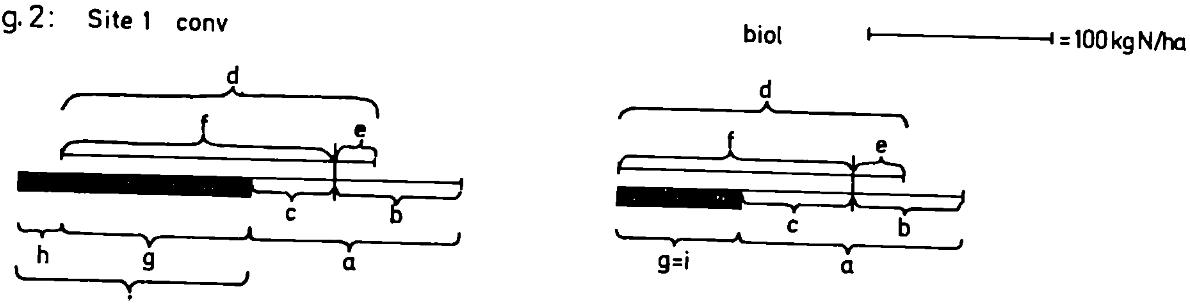
above abscissa: ↓ N fertilization (amount = arrow length)

—— N uptake

below " : —— N_{min} until 1)10,2)25,3)50,4)100 cm

· accumulated N_{min} in plough layer

Fig. 2:



Contractor : Bundesforschungsanstalt für Getreide-u.Kartoffelverarbeitung

Contract n° 252-77-4 ENV D

Project Leader : Dir. und Prof. Dr. H.D. OCKER

Title of project : Toxic heavy metals in cereals and cereal products

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Summary report of results to date.

The objective of the research was first to receive knowledge to which constituents of cereals and cereal products heavy metals (mercury, lead and cadmium) are bound and secondly why the heavy metals are distributed in a specific and different manner within the cereal kernel, as shown in earlier publications.

Samples of commercial wheat and rye were milled on a laboratory scale mill; the corresponding milling products were examinated for their content of heavy metals. It was tried to isolate organic-bound fractions of the heavy metals by extraction with organic solvents (petrol, ether, acetone). The results of these experiments showed clearly, that the fraction of organic-bound, lipophile heavy metal compounds can only be very small, if at all. Most of the heavy metals seem to be bound to fractions of the cereal proteins. Rather surprising was the fact, that cereal and cereal products can absorb mercury from contaminated atmosphere in large quantities. After a storage period of three month the mercury content of wheat grains and wheat flour was found at a level of more than 3 ppm, while the average mercury content of untreated wheat is below 0,01 ppm. Wheat bran absorbs the volatile mercury to an even higher degree (up to 15 ppm). The absorbed mercury was found to be rather strongly bound, because it cannot be removed by aeration with a mercury-free stream of air or nitrogen. About 20% of the absorbed mercury vanishes, by heating the contaminated flour to 100°C.

It is assumed that mercury reacts irreversible with certain protein fractions. Isoelectric focussing experiments have shown an influence on the activity of some isoenzymes of esterases and other enzymes, however,these indications of biochemical changes have to be checked by future work. Further experiments with other cereal products of oat, maize, rye and hard wheat are still in progress.

Contractor : Landwirtschaftskammer Weser-Ems
Landwirtschaftliche Untersuchungs- und Forschungsanstalt

Contract n° 253-77-1 ENV D

Project leader : Prof. Dr H. Vetter

Project title : Methods of decreasing the heavy-metal content of plants in areas affected by pollution, and means of reducing its harmful effects

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Objective of the research

The objective of this research is the development of methods to decrease the contents in plants of the heavy metals lead, zinc and cadmium, and the reduction of their harmful effects, not only on the plants themselves, but also in the ensuing links of the food chain. To this end, it is necessary to solve the following problems :

(1) How do the lead, zinc and cadmium contents of various forage and food plants, growing in an area affected by pollution, differ ?

(2) How great are the amounts of heavy metal taken up from the soil in the polluted area, and how great are the amounts of heavy metal taken up from the air via the foliage ?

(3) To what extent can the heavy-metal content of the plants be decreased by means of improved soil cultivation and fertilization ?

(4) What sorts of pollution cause illness in cattle, particularly a rise in the rate of cattle mortality during spring grazing ?

Materials and Methods

Three plant experiments and one cattle experiment were carried out in an area which over a period of 60 years has been affected by lead, zinc and cadmium pollution in the vicinity of metallurgical plant . Three locations, at distances of one, two and seven kilometres, were selected for the field experiments. The area involved is fenland, which was previously used as grassland. The pollutant contents in the soil and the air are shown in summarized form in Table 1 for the three localities.

Table 1 : Pollutant contents in the soil and the air (25 April-5 November) at distances of 7, 2 and 1 km from the emitters.

Distance (km)	Pb		Cd		Zn		F	
	Soil ppm	Air $\mu g/m^3$	Soil ppm	Air $\mu g/m^3$	Soil ppm	Air $\mu g/m^3$	Soil ppm	Air $\mu g/m^3$
7	15	0.35	0.4	0.004	36	0.05	300	0.3
2	70	0.56	1.9	0.010	190	0.11	350	0.4
1	510	1.5	10	0.03	1250	0.60	560	0.7

In the experiment to determine the separate heavy-metal uptakes from the soil and from the air, two sets of plots were laid out at a locality 7 km from the metallurgical plant, one set with local soils and the other with polluted soils from areas 2 and 1 km distant; conversely, plots with low-pollution soils from an area 7km away were laid out in localities with a high level of air pollution at distances of 2 and 1 km. When the exchange of soils was carried out, displacements were performed separately for the layers from depths of 0-15, 15-30 and 30-45 cm.

In the fertilization experiments, the effect of a phosphate and chalk fertilizer on the heavy-metal uptake of various plants was examined. This experiment was set up at a distance of 1 km with increments of 0-160-320-640 kg P_2O_5/ha for the calcined phosphate fertilizer and 0-10-20-40 t CaO/ha for the quicklime.

In both experiments, grass, beet, cereals, spinach, green cabbage and Chinese cabbage were used as the experimental plants. The heavy-metal contents were investigated in : grass : three plant cuttings ; beet : leaves and root ; Chinese cabbage : head and outer leaves ; spinach and green cabbage : leaves ; cereal : grains and straw.

In a further tillage and fertilization experiment with grass, the effects of two recultivation measures, one-way rotary hoeing and deep ploughing, were investigated with a succession of different fertilization intensities.

The heavy-metal concentration in the plants was measured in an atomic absorption spectrometer (AAS) after wet digestion with a mixture of HNO_3 and $HClO_4$, in some cases after enrichment by means of extraction with ammonium pyrilidindithiocarbonate (APDC).

For the cattle-feeding experiment, 88 one to one- and-a-half year old cattle from unpolluted areas were purchased, divided into three groups and given different doses during the 1977 grazing period and the 1977-78 winter-feeding period. Animals in Group I were given fodder only(grass and/or hay and silage) from a distance of 0.9-1.4 km (Zone I), animals in Group II fodder from a distance of 1.8-2.3 km (Zone II), and animals in Group III, fodder from a distance of 10 km from the metallurgical plant works (Zone III). When the cattle were driven out to graze in spring 1978, each of the previously dosed groups was again subdivided into three groups, which were then distributed between the meadows of Zones I, II and III. After six weeks of grazing, the animals were slaughtered. Liver, kidneys, spleen, muscle, long bones and caudal vertebrae were investigated, for their heavy-metal content.

Results

The lead, cadmium and zinc contents in various types of plant and various parts of the same plant differed to an unusually marked extent (Table II).

Table 2 : Lead, cadmium and zinc contents (mg/kg dry weight) in various forage plants and vegetables at distances of 7 and 1 km from the emitter.

	7 km			1 km		
	Pb	Cd	Zn	Pb	Cd	Zn
Summer barley grain	0.3	0.14	53	5.2	0.8	145
Fodder beet	0.3	0.3	43	2.7	1.8	215
Fodder-beet leaves	8.2	0.7	80	168	7.9	785
Grass, 2 cuttings each	5.7	0.4	48	73	4.5	496
Chinese cabbage head	2.7	1.1	70	21	2.3	393
Spinach	3.5	1.6	173	70	14	1150
Green cabbage	8.4	0.2	44	189	4.6	565

Plant enrichment via the soil and via the air varies widely in respect of the three polluting elements. As regards lead, the plant uptake in the vicinity of the emitter is eight times as great via the air as via the soil, while with cadmium and zinc, the uptake via the soil is 1.5 times as great as via the air.

The fertilization and tillage experiments show that all measures that promote plant growth contribute to decreasing the pollutant content in plants through a dilution effect. An optimum supply of lime also reduced the availability of Cd and Zn considerably. The grass reacted the most perceptibly, with a decrease in content of up to 30% in respect of both elements.

The structure of the cattle experiment, the animals'lead uptake via respiratory air and fodder and the number of dead and sick animals are presented in Table 3.

Table 3 : Lead uptake via air and fodder and number of animals seriously affected in the cattle experiment.

	Zone I (1.2 km)			Zone II (2.2 km)			Zone III (10 km)		
Pb content of the air ($\mu g/m^3$)	1.5			0.6			0.3		
Pb content of the fodder									
Summer 1977 (mg/kg dry weight)	135) Ø 238			72.6) Ø 120			6.5) Ø 13.1		
Winter 1977-78 (mg/kg dry weight)	367)			180.0)			21.4)		
Experimental animals (number)	35			33			13		
Dead (number)	1			-			-		
Sick (number)	6			1			-		
	Zone			Zone			Zone		
	I	II	III	I	II	III	I	II	III
Pb content of the fodder									
Spring 1978 (mg/kg dry weight)	123	97	5	123	97	5	123	97	1
Experimental animals	17	9	8	17	8	8	5	5	3
Dead	2								
Sick	-	1							

After nine months'exposure via fodder from the vicinity of the metallurgical plant works (Zone I), six animals became ill and one animal died, all with typical symptoms of lead poisoning. After the cattle had been driven out to graze in the spring of 1978, there were dead animals only in the group that had been intensively exposed to lead during the winter and after being driven out to graze. One animal became sick in the group subjected to a high preliminary exposure in winter and medium grazing exposure in spring. In animals that had undergone medium and low-level preliminary exposure, even high levels of exposure to lead during the grazing period caused no symptoms. The varying exposures of the cattle to lead could not be seen from the contents in the muscle, but were clearly recognizable in the liver (0.8-5.9 mg Pb/kg dry weight), the spleen (0.3-6.2) and the kidneys (2.9-29.1. The highest values were obtained from the long bones and the caudal vertebrae, namely, 77 and 128 mg Pb/kg dry weight respectively. The lead contents of the organs show that all organs get rid of lead during the grazing period. In the animals subjected to a high level of exposure, there were indications of a slightly lower weight increase in the preliminary-exposure and grazing-exposure phases.

Not only the level but also the duration of exposure is of decisive significance as regards the harmful effects on the animals.

Conclusions

All measures which promote plant growth bring about a decrease in the pollutant contents. Lead is taken up by the plants almost exclysively via the air, while cadmium and zinc are mainly taken up via the soil. The enrichment of the plants in these two elements can be reduced by means of liming.

Animals are harmed by pollutants in their fodder. Cases of death occur mainly during the grazing period, even if the lead uptake at that time is lower than during the winter. The preliminary exposure during the preceding nine months was decisive.

Publications and oral communications

Vetter, H., W. Schulte im Walde und H.H. Meyer : Schadstoffe in der Nahrungskette in der Nachbarschaft einer Blei- und Zinkhütte
Vortrag : Tagung Umweltforschung, Universität Hohenheim, 19.1.1979

Vetter, H. :Belastung der landwirtschaftlichen Produktion mit Schadstoffen - Möglichkeiten zur Belastungs- und Schadensminderung
Vortrag : Kammerversammlung der Landwirtschaftskammer Weser-Ems, Oldenburg, 9.2.1979

Vetter, H. und W. Schulte im Walde : Aufnahme von Blei, Cadmium und Zink durch verschiedene Pflanzenarten aus der Luft und dem Boden mit unterschiedlicher Düngung in einem Immissionsgebiet
Vortrag : VDLUFA-Tagung Augsburg, 22.9.1978

Contractor : **SNPN-CNRS, Centre d'écologie de Camargue, Arles**

Contract n°: 204-77-1 ENV F

Project Leader : **P. Heurteaux**

Title of project : Aquatic pollution in the Camargue. Ecology of rice-fields and the role of rice cultivation in the contamination of the aquatic environment

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OBJECTIVE OF THE RESEARCH

The SNPN and the Centre d' écologie de Camargue were brought to study the structure and functioning of the rice-paddy agro-ecosystem for two reasons:

1. The presence of pollution originating from the Rhone river as well as from agriculture in the lakes of the Réserve Nationale de Camargue and the danger of the bioaccumulation of these pollutants in trophic chains.
2. Evidence that rice cultivation is the principal polluting agent in the Camargue, due to the large value of water that it drains (200 million m^3), the chemical treatments that it requires (herbicides, insecticides and fertilizers), and the ecological role it plays as a temporary summer aquatic habitat.

This study involves both fundamental and applied research and its goal is to better understand:

1. The organisation of an aquatic ecosystem that is shallow, temporary estival and has a dense vegetation cover.
2. The processes of water contamination
3. The immediate and chronic effects of pesticides on the flora and fauna of both rice fields and natural bodies of water
4. Propositions for reducing this pollution.

MATERIALS AND METHODS

On the three hectares of experimental rice fields and with the scientific facilities available at the Centre d' écologie de Camargue for the various chemical (notably for pesticides) and biological analyses both in the laboratory and in the field, an interdisciplinary team of eight researchers (six full time) and three technicians study:

1. The parameters of the water balance evapotranspiration (evaporation pans),precipitations (pluviometers), irrigation, drainage (recorder weirs), percolation,
2. The dynamics of pesticides and fertilizers employed in rice cultivation and the role of drainage water in the contamination of the natural aquatic environment,
3. The soil bacteria, particularly to study the fixation of atmospheric nitrogen in the rhizosphere of rice,

4. The adventitious phanerogams, especially Echinochloa,
5. The ecology of the principal populations of algae and aquatic invertebrates in terms of the evolution of the physical and chemical factors of the aquatic environment and the trophic relations existing between them,
6. The action of pesticides on the flora and fauna of rice fields.

RESULTS

The results presented here concern particularly the influence of rice cultivation on the environment of the Camargue.

1. Evidence of poor management of irrigation water

The basic water requirement of rice fields in the Camargue is between 15 000 and 18 000 m^3 ha year, representing evapotranspiration (9 to 10 000 m^3) and percolation into the soil (6 to 8 000 m^3). The real consumation is approximately 40 000 m^3 ha year, with the difference, some 20 000 m^3 representing superficial drainage. Without waste, this drainage could be limited to 7 000 m^3/ha/year. The surplus is the result of a poor irrigation practice ("arrosage à la surverse"), which, without any advantages for the crop (the desalinisation of the soil is ensured by the deep percolation), severely disturbs the natural balance (desalinisation and increased pollution of natural water bodies) and is as well harmful for agriculture (hazard of flooding and poor winter drainage of cultivable land).

2. Dynamics of pesticides applied to rice paddies in the Camargue and the role of drainage water in the contamination of the aquatic environment

2.1. Nature and quantity of pesticides (g/ha/year)

insecticides, gamma HCH : 80 parathion ethyl : 60
herbicides, molinate : 5 000 propanil : 4 000

(no algicide treatments - CuSO4 or nabasan - no phyto hormones on the experimental fields).

2.2. Longevity of pesticides in water (days)

	half-life	disappearance or residual dose
gamma HCH	1.8	10
parathion ethyl	0.8	9
molinate	2.8	15
propanil	0.5	15
3,4 DCA	1.1	10

These time periods are dependent upon the physical and chemical conditions of the environment (temperature, anaerobiosis).

2.3. Ways of disappearing

2 3 .1. Transfer in drainage water

	g ha	% of input
gamma HCH	3	3.1
parathion ethyl	1	1.3
molinate	800	16
propanil	34	0.9

	g/ha	% of input
3,4 DCA	54	1.1(expressed as propanil)

23.2 Adsorption-metabolisation on the surface soil

-maximum values after treatment (after 1 to 3 days)

	ppb	g/ha	% of input
gamma HCH	250	19	24
parathion ethyl	200	15	25
molinate	9 400	750	13

-values 1 and 10 months after treatment		g/ha	ppb (10 months after)
gamma HCH	30	2	11
parathion ethyl		undetected	
molinate	700	34	15

NB - propanil and its metabolite, 3,4 dichloroaniline, were not dosed (analytical difficulties)

23.3 Percolation

It is negligible by interstitial porosity because adsorption on the soil is very important and the percolation is slow. It is possible through cracks in the plough sole, but represents at the most 1/20 of that emptied by drainage.

23.4 Volatilization

gamma HCH and parathion ethyl	negligible
molinate	50% of input

23.5 Harvest of grain

negligible

23.6 Degradation-metabolisation in water, soil and living organisms

2.4. Effluent quantities received by the drainage system in 1978 (kg)

rice paddies	entire Camargue : (ca 10 000 ha)	water drained into Vaccarè : (ca 1 500 ha)
gamma HCH	150	23
parathion ethyl	100	15
molinate	10 '000	1 500
propanil) 3,4 DCA)	1 000	150

Degradation-metabolisation ensures the disappearance of part of the pollutants before their dilution in the lakes of the Réserve. The residual values in the Vaccarès for gamma HCH are 0.1 ppb,at the most, and for molinate from 2 to 4 ppb. Molinate is toxic at a low dose for certain aquatic invertebrates, and is a definite danger for the environment (chronic intoxication). It can remain in drainage canals for a long time at doses lethal for numerous invertebrate species (its concentration can reach 1.5 ppm). Parathion ethyl and propanil are also present in canals at lethal doses for certain phytoplankton and zooplankton.

3. Transfer of nutrients (N and P) by water drained from rice fields

For the "Grande Camargue" as a whole this transfer is estimated at 100 tons of nitrogen and 20 tons of phosphorus per year.

The Vaccarès receives respectively about 15 and 3 tons, which considering the capacity of this lake (120 million m^3), are not negligible quantities, even if they dc not present a middle term danger of eutrophication.

4. Action of pesticides on the biocoenosis of rice fields

-insecticides

The association of gamma HCH and parathion ethyl drastically reduces the invertebrate fauna and fulfills its function (destruction of chironomidae larva and *Triops cancriformis*). Zooplankton react differently, certain rotifera and nauplii resist whereas cladocera are the most affected followed by ostracoda and cyclopidae. This difference in reaction to insecticides favors the most resistant when the populations are reconstituted and results in a decrease in their diversity. Insecticides do not have a direct effect on algae, but the disappearance of phytophaga entails an increase in algal biomasses. *Egretta garzetta* and their young, which feed on invertebrates inhabiting rice fields during the period of insecticide application, do not show any sign of chronic intoxication by gamma HCH.

-herbicides

Molinate: employed preventively, its action on *Echinochloa* is uncontrollable. It is generally accepted that its efficiency is diminishing. It has no direct effect on algae but is toxic for moinidae and seems to have sublethal effects on other invertebrates for which it slows down the feeding activity, thus indirectly aiding algae.

Propanil: very toxic for algae. Lethal and sublethal effects on invertebrates.

CONCLUSIONS

How to make rice cultivation in the Camargue less polluting

1. Improve water management by avoiding waste.

2. Establish an efficient program of chemical treatment.

   The need for insecticide treatments is very questionable. In any case they shoul not be systematically employed as is presently done, and the simultaneous use of lindane and parathion ethyl is not justified since the dose of parathion ethyl applied is sufficient. Propanil treatments are inevitable, but the efficiency and thus the utility of molinate are doubtful. Overdosing, frequently practised, is use less and costly.

3. Stop the emptying of rice field drainage water into the Vaccarès.

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Dr. Pierre HEURTEAUX
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