Environmental Cancer Risk, Nutrition and Individual Susceptibility - EU Network of Excellence

Gesellschaft für Umwelt-Mutationsforschung e.V.

GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

ECNIS-sponsored Workshop on Biomarker of Exposure and Cancer Risk: DNA Damage and DNA Adduct Detection
&
6th GUM-32P-Postlabelling Workshop

September 29-30, 2006
German Cancer Research Center, Heidelberg, Germany

Workshop Organisation:

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Environmental Cancer Risk, Nutrition and Individual Susceptibility - EU Network of Excellence

GUM

Gesellschaft für Umwelt-Mutationsforschung e.V.

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We would like to thank our sponsors for supporting the workshop:

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We would like to thank the following companies for their donation to support the workshop:

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We would like to thank ECNIS for supporting young scientists with travel grants:

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Mr. Thomas Spormann, Technical University of Kaiserslautern, Germany

Ms. Nicole Verhofstad, University of Maastricht, The Netherlands

We would like to thank the GUM for supporting young scientists with travel grants:

Mrs. Chimgee Baasanjav, German Institute for Human Nutrition, Germany

Dr. Karen Brown, University of Leicester, UK

Mrs. Claudia Donath, German Institute for Human Nutrition, Germany

Dr. Roger Godschalk, University of Maastricht, The Netherlands

Ms. Berta Schulz, University of Würzburg, Germany

Mr. Markus Thiemann, University of Wuppertal, Germany

We would like to thank UKEMS for supporting young scientists with travel grants:

Dr. Hazel Greetham, University of Newcastle upon Tyne, UK

Dr. Gunter Kuhnle, MRC Dunn Human Nutrition Unit Cambridge, UK

Dr. Rachel Le Pla, University of Leicester, UK

Dr. Debbie Marsden, University of Leicester, UK

Mr. Jie Zuo, Institute of Cancer Research, UK

Programme

Friday, 29th September 2006

Venue: DKFZ Conference Center

Time	Session	Location
08.00 – 08.30 am	**Registration**	Foyer
08.30 – 08.35 am	Welcome/Introduction: **Volker Arlt**	Auditorium
08.35 – 08.45 am	**Welcome: Prof. Dr. Otmar Wiestler, Chairman and Scientific Director of the German Cancer Research Center**	Auditorium
	Session I Chair: Helmut Bartsch (German Cancer Research Center, Germany)	
08.45 – 09.30 am	***- Keynote Lecture -*** **Frederick Beland** (National Center for Toxicological Research, USA): *Acrylamide: adducts, mutations, and cancer* **(O#1)**	Auditorium
09.30 – 10.00 am	**Arthur Grollman** (State University of New York, USA): *Role of aristolochic acid in the etiology of endemic nephropathy* **(O#2)**	Auditorium
10.00 – 10.30 am	**Coffee Break**	Foyer
	Session II Chair: Peter Farmer (Biocentre, University of Leicester, UK)	
10.30 – 11.00 am	**David Phillips** (Institute of Cancer Research, UK): *Elucidating pathways of metabolic activation of carcinogens by quantifying and characterising their DNA adducts* **(O#3)**	Auditorium
11.00 – 11.25 am	**Bernadette Schoket** (National Institute of Environmental Health, Hungary): *Immunoassays for the determination of PAH-DNA adducts* **(O#4)**	Auditorium
11.25 – 11.50 am	**Karen Brown** (University Leicester, UK): *Accelerator mass spectrometry for DNA adduct detection* **(O#5)**	Auditorium
11.50 – 12.15 pm	**Lennart Möller** (Karolinska Institute, Sweden): *DNA-adduct analyses of human tissues by the ^{32}P-HPLC method* **(O#6)**	Auditorium
12.15 – 01.00 pm	**Lunch Break**	Canteen
01.00 – 01.45 pm	**Poster Presentation: *Session 1***	Foyer
01.45 – 02.30 pm	**Poster Presentation: *Session 2***	Foyer
	Session III Chair: David Phillips (Institute of Cancer Research, UK)	
02.30 – 02.55 pm	**Peter Farmer** (Biocentre Leicester, UK): *Alternative approaches to ^{32}P-postlabelling for detection of low levels of DNA adducts* **(O#7)**	Auditorium

02.55 – 03.20 pm	**Jagadeesan Nair** (German Cancer Research Center, Germany): *Novel ^{32}P-postlabelling method for the determination of adducted deoxynucleosides in human body fluids* **(O#8)**	Auditorium
03.20 – 03.45 pm	**Oliver Schmitz** (University Wuppertal, Germany): *Determination of the DNA methylation level with capillary electrophoresis and laser-induced fluorescence* **(O#9)**	Auditorium
03.45 – 04.15 pm	**Coffee Break**	Foyer
	Session IV Chair: Frederik-Jan van Schooten (University of Maastricht, The Netherlands)	
04.15 – 04.40 pm	**Matilde Marques** (Technical University of Lisbon, Portugal): *Tamoxifen and tamoxifen analogues: activation to DNA adducts in vitro and in vivo* **(O#10)**	Auditorium
04.40 – 05.05 pm	**Volker Manfred Arlt** (Institute of Cancer Research, UK): *The potential use of DNA adducts formed by the carcinogenic air pollutant 3-nitrobenzanthrone in human biomonitoring* **(O#11)**	Auditorium
05.05 – 05.30 pm	**Marie Stiborova** (Charles University Prague, Czech Republik): *Cytochrome P450- and peroxidase-mediated formation of covalent DNA adducts by an anticancer drug ellipticine - a novel mechanism of ellipticine action* **(O#12)**	Auditorium
05.30 – 05.55 pm	**Heinz Schmeiser** (German Cancer Research Center, Germany): *Molecular basis of Aristolochia carcinogenicity* **(O#13)**	Auditorium
05.55 – 06.15 pm	**Coffee Break**	Foyer
	Symposium Prof. Manfred Wiessler (65th birthday) Chair: Heinz Schmeiser & Eva Frei (German Cancer Research Center, Germany)	
06.15 – 07.15 pm	**Otmar Wiestler** (German Cancer Research Center, Germany): *Welcome* **Gerd Eisenbrand** (University Kaiserslautern, Germany): *Highlights of the scientific career of Prof. Manfred Wiessler* **Christopher Michejda** (National Cancer Institute at Frederick, USA): *Replication control in tumor cells as a target for DNA-interacting anti-tumor agents* **(O#14)**	Auditorium
07.30 – 11.00 pm	**Buffet Reception & Live Music**	Foyer

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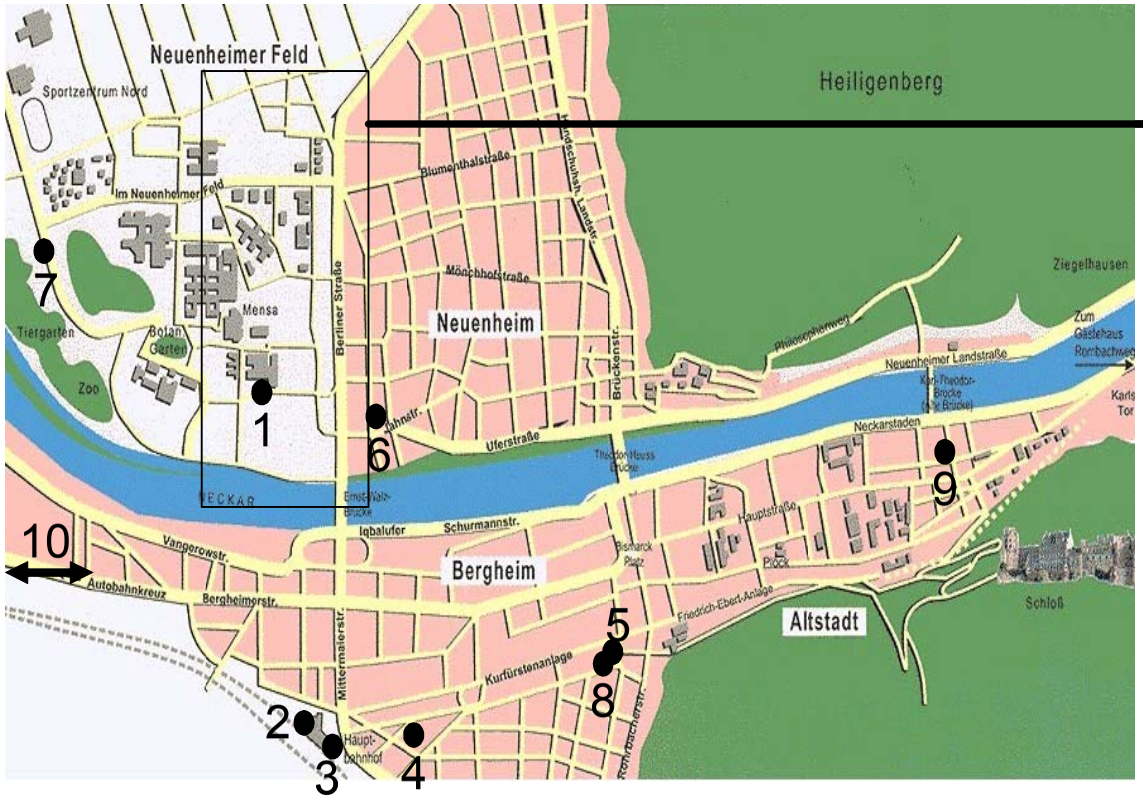
Saturday, 30th September 2006

Venue: DKFZ Conference Center

Time	Session	Location
	Session V Chair: Bernadette Schoket (National Institute of Environmental Health,Hungary)	
08.30 – 08.55 am	**Panagiotis Georgiadis** (National Hellenic Research Foundation, Greece): *Progress towards the development of high-throughput immunochemical assays for DNA damage and repair* **(O#15)**	Auditorium
08.55 – 09.20 am	**Dan Segerbäck** (Karolinska Institute, Sweden): *^{32}P-Postlabelling analysis of UV-induced pyrimidine dimers from human skin and urine* **(O#16)**	Auditorium
09.20 – 09.45 am	**Hansruedi Glatt** (German Institute of Human Nutrition, Germany): *Formation of DNA adducts in humans and laboratory animals by phytochemicals from common vegetables and fruits* **(O#17)**	Auditorium
09.45 – 10.15 am	**Coffee Break**	Foyer
	Session VI Chair: Soterios Kyrtopoulos (National Hellenic Research Foundation, Greece)	
10.15 – 10.45 am	**Monica Hollstein** (German Cancer Research Center, Germany): *In vitro cell immortalization selects for p53 gene mutations found in human cancers* **(O#18)**	Auditorium
10.45 – 11.10 am	**Albrecht Seidel** (Biochemical Institute for Environmental Carcinogens, Germany): *Determination of urinary PAH metabolites as a non-invasive human biomonitoring method* **(O#19)**	Auditorium
11.10 – 11.35 am	**Jan Topinka** (Institute of Experimental Medicine AS, Czech Republic): *Use of DNA adduct analysis to study mechanism of drug genotoxicity - example cyproterone acetate (CPA)* **(O#20)**	Auditorium
11.35 – 12.00 pm	**Erwin Eder** (University of Würzburg, Germany): *Early markers in the development of colorectal tumours in the rat: DNA adducts compared with isoprostanes, histological and morphological alterations in the early stage* **(O#21)**	Auditorium
12.00 – 01.00 pm	**Coffee Break & Lunch Snacks**	Foyer
	Session VII Chair: Oliver Schmitz (University of Wuppertal, Germany)	
01.00 – 01.25 pm	**Roger Godschalk** (University of Maastricht, The Netherlands): *The use of DNA adducts in surrogate tissues: pitfalls and opportunities* **(O#22)**	Auditorium
01.25 – 01.50 pm	**Marco Peluso** (Tuscany Cancer Institute, Italy): *DNA adducts and lung cancer risk in a case-control study nested in the EPIC investigation* **(O#23)**	Auditorium
01.50 – 02.15 pm	**Elmar Richter** (University of Munich, Germany): *Sources of adducts releasing 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) from DNA and haemoglobin in humans* **(O#24)**	Auditorium
02.15 – 02.40 pm	**Wolfgang Pfau** (GAB-Consulting, Germany): *Dietary DNA adducts:*	Auditorium

	heterocyclic amines and pesticides **(O#25)**	
02.40 – 03.05 pm	**Werner Lutz** (University of Würzburg, Germany): *Correlation of DNA adducts with other endpoints of genotoxicity in mouse lymphoma cells treated with methyl methanesulfonate* **(O#26)**	Auditorium
03.05 – 03.10 pm	Closing Remarks: Heinz Schmeiser	Auditorium
03.15 pm	**End of Workshop**	



Conference Centre

1. German Cancer Research Center (DKFZ)
2. Heidelberg Train Station
3. Hotel Ibis Heidelberg
4. Hotel Central
5. Crown Plaza Heidelberg City Centre
6. Hotel Café Frisch
7. Youth Hostel Heidelberg
8. Arrival/Departure Lufthansa Airport Shuttle
9. Old Town
10. Direction to Highway
11. Tram stop [tram no. 4] “Jahnstr./Chirurgie”
12. Bus stop [bus no. 33] “Chirurgische Klinik”

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Programme Committee (tentative)

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Social Programme

Basel with its tri-national situation is located at the heart of Europe. The "Münster" in the medieval city centre and many museums are attractions on their own. Switzerland with its many world famous sites and views such as the UNESCO World cultural heritage city of Berne (www.berninfo.com), Lucerne (www.luzern.org) or the Eiger-Jungfrau mountain region (www.about.ch/cantons/bern/eiger_moench_jungfrau) are only less than two hours away by car or train. Also the french winery region of Alsace (www.tourisme-alsace.com) or the popular Black Forest (www.schwarzwald-sued.de) in Germany are within close vicinity and invite you for a tour before or after the conference. The social programme will reflect the cultural diversity of the tri-national region.

EEMS GUM

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Local Organizing Committee

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These colleagues are your primary contact partners for further information about the conference.

Pablo Tosin (pablo.tosin@bluewin.ch) *of TOSIN Productions*
Luftmattstrasse 17; CH-4052 Basel;
Tel: +41 (0)79 334 87 26 is the local conference organizer

Commercial Exhibitions

Space is available for commercial exihibions and companies active in the area of genetic toxicology; *in silico*, *in vitro* and *in vivo* testing services; cellular imaging; etc. are encouraged to present their business at this meeting.

For further information contact :
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Weblinks

WWW.EEMS-Basel-2007.ch (soon active)
EEMS: http://193.51.164.11/eems/eems1.htm
GUM: www.gum-net.de
www.basel.ch
www.unibas.ch
http://www.biozentrum.unibas.ch/
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www.mybasel.ch

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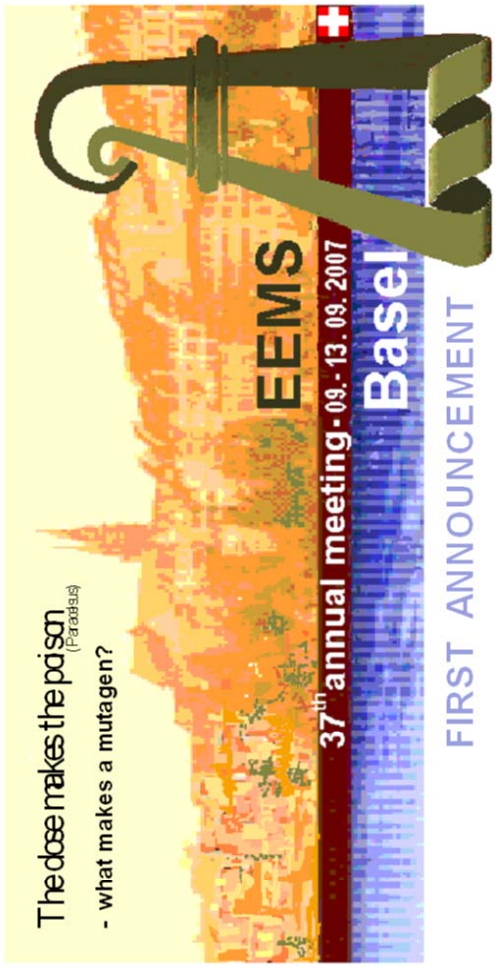
Basel EEMS 37th annual meeting 2007



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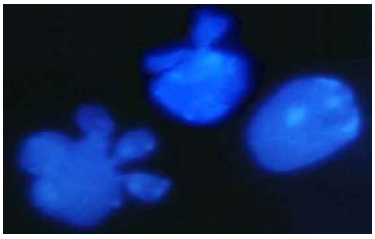
The Societies (EEMS and GUM)

The European Environmental Mutagen Society (EEMS) and the German speaking section GUM (Gesellschaft für Umweltmutationsforschung), home to scientists active in fundamental and applied research in genetic toxicology, mutagenesis and carcinogenesis, are announcing their joint meeting for **9-13 September 2007** in Basel.

EEMS GUM

The Subject: The dose makes the poison – what makes a mutagen?

Basel is one of the founding locations of the European chemical and pharmaceutical industry and its name figures prominently in the history of medical therapy and toxicology. Theophrastus Bombastus von Hohenheim (Paracelsus) taught in the city during the 16th century. The first part of the conference theme, which is assigned to him, laid the basis for toxicology as we know it today. Hence, it is only consistent that one topic of the EEMS 2007 meeting will be devoted to revisiting the dose-response concept and risk assessment in our science. In addition, Friedrich Miescher, the discoverer of nucleic acids has spent his scientific life in Basel, further connecting the venue with the object of our discipline.



„Blebbing chromosomes"

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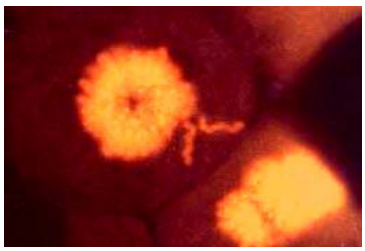
The Venue

Basel, the largest city of northwestern Switzerland, is located in the tri-national region of Switzerland, France and Germany. This unique location inspires a multilingual environment and multicultural flair. The international ambience is supported by the fact that Basel is home to the global headquarters of two of the worlds largest pharmaceutical companies, Novartis and Hoffmann-LaRoche.

The industrial background in Basel is matched by the basic research of the University and its various institutes. Founded in 1460, the University is the oldest in Switzerland and one of the most influential. The brand new Pharmazentrum at the Biozentrum of the Basel University right in the city centre is well known for its expertise and excellent scientific contributions in toxicology and will serve as the venue for the EEMS 2007, providing up-to-date conference facilities.



„Dislocated metaphase chromosomes"

Access to Basel

Basel/Mulhouse airport is located just outside the city (connecting European cities by various discount carriers), assuring a convenient journey to Basel. Zurich airport is one hour away by train/car. Hotels of various categories will allow you to find accommodation for any budget.

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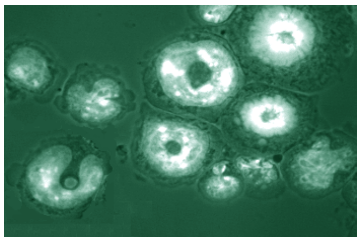
Scientific Programme

The main organizers of the EEMS-2007 will be the genotoxicity groups of Novartis and Hoffmann-La Roche. Aside of the fundamental research basis of the EEMS, an emphasis of the meeting will be on applied research and testing including risk assessment in a variety of workshops and lectures.



„Monopolar spindles"

Main Subjects (tentative)

- Dose response
- Risk assessment – risk perception – risk communication
- Stem cell toxicology / genotoxicity
- Nanomaterial toxicology / genotoxicity
- Image analysis – high content screening
- Structure-activity prediction
- DNA adducts and carcinogenesis
- Mutations in cancer genes
- Biologicals and genotoxicity/tumourigenicity
- Biomonitoring and risk assessment
- DNA repair and cancer risk
- Genotoxicity and non-cancer diseases
- Genetic engineering
- Industry – regulatory issues

Oral Presentations

Session I

O#1

Acrylamide: adducts, mutations, and cancer

F.A. Beland
National Center for Toxicological Research, Division of Biochemical Toxicology, Jefferson, AR, USA.

Acrylamide, a water-soluble α,β-unsaturated amide, is a high-volume industrial chemical, a component of cigarette smoke, and more recently, has been detected as a contaminant in baked and fried starchy foods, including French fries, potato chips, and bread. Acrylamide is carcinogenic in experimental animals; however, the mechanism of tumor induction is controversial, with both genotoxic and non-genotoxic pathways being proposed. Acrylamide is oxidized to the epoxide glycidamide, an electrophilic metabolite that could contribute to a genotoxic mechanism of tumorigenicity. Glycidamide reacts with DNA to form several DNA adducts including N7-(2-carbamoyl-2-hydroxyethyl)deoxyguanosine and N3-(2-carbamoyl-2-hydroxyethyl)deoxyadenosine, which readily depurinate to give N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua) and N3-(2-carbamoyl-2-hydroxyethyl)adenine (N3-GA-Ade). In order to investigate the contribution of glycidamide to the carcinogenicity of acrylamide, we have compared the extent of DNA adduct formation by acrylamide and glycidamide in experimental animals using HPLC coupled with electrospray ionization tandem mass spectrometry. The administration of a single dose of acrylamide or glycidamide to adult rats produced N7-GA-Gua and N3-GA-Ade in DNA from all tissues examined, with glycidamide giving adduct levels 160-560% higher than acrylamide. N7-GA-Gua and N3-GA-Ade were also detected in DNA from adult mice treated in a similar manner, but the adduct levels from glycidamide were only 16-150% higher than those observed with acrylamide, which reflects the more efficient conversion of acrylamide to glycidamide in adult mice compared to adult rats. In infant mice, glycidamide gave much higher adduct levels than acrylamide, and also induced micronuclei in reticulocytes and normochromatic erythrocytes and an increase in the *Hprt* mutant frequency in spleen lymphocytes. These data suggest that the carcinogenicity of acrylamide is mediated through its metabolism to glycidamide. Bioassays are currently being conducted to test this.

O#2

Role of aristolochic acid in the etiology of endemic nephropathy

A.P. Grollman[1], S. Shibutani[1], and B. Jelakovic[2]
1. State University of New York at Stony Brook, Department of Pharmacological Sciences, Laboratory of Chemical Biology, Stony Brook, New York; 2. University of Zagreb School of Medicine, Department of Medicine, Zagreb, Croatia.

Balkan endemic nephropathy (EN) affects men and women living in rural areas of Croatia, Bulgaria, Romania, Serbia and Bosnia. The disease, first recognized 50 years ago, is characterized by its invariable progression to renal failure and a strong association with upper urothelial cancer. Significant epidemiologic features of EN include its occurrence only in certain rural villages where only certain households are affected; a familial, but not inherited, pattern of disease; and overt manifestation in adults over 30 years of age. Based on clinical and pathologic findings in patients with aristolochic acid (AA) nephropathy, the results of a pilot epidemiologic study (Croat Med J 46: 116 (2005)), and detection of dA-AA and dG-AA adducts in the renal cortex of patients with EN, we have resurrected an earlier hypothesis that chronic dietary ingestion of aristolochic acid, in the form of home-baked bread prepared with contaminated wheat grain, is responsible for EN and its associated urothelial cancer. An alternative hypothesis invokes exposure of susceptible individuals to a mycotoxin, ochratoxin A (OTA). Data has been published in favor of (Chem Res Toxicol, 18:1091 (2005) and against (Chem Res Toxicol 18: 1082 (2005) the central proposal that OTA is genotoxic to humans and animals. In contrast, the genotoxicity and mutagenicity of aristolochic acid in humans and animals have been demonstrated clearly (Mutagenesis, 17: 265 (2002)). In this lecture, the strengths and pitfalls of using DNA adducts as biomarkers of disease will be illustrated by a critical evaluation of ochratoxin-induced DNA damage and presentation of new data suggesting the role of aristolochic acid in the etiology of EN. By resolving apparent discrepancies in these scientific reports, public health measures can be recommended for eradicating an environmental agent responsible for a devastating renal disease for which 100,000 people are currently at risk.

Session II

O#3

Elucidating pathways of metabolic activation of carcinogens by quantifying and characterising their DNA adducts

D.H. Phillips
Institute of Cancer Research, Section of Molecular Carcinogenesis, Sutton, Surrey SM2 5NG UK.

Detecting DNA adduct formation by carcinogens and deducing how they were formed has proved to be an effective strategy for elucidating their mechanisms of metabolic activation. Initially such studies were done using radiolabelled compounds and comparing the chromatographic properties of the radiolabelled adducts with those formed *in vitro* by synthesised reactive derivatives of the carcinogen. More recently ^{32}P-postlabelling analysis has largely replaced the need for radiolabelled test chemicals. The pathways of activation of carcinogens can now be readily deduced from the patterns and extent of adduct formation of putative metabolites and reactive derivatives of the parent compound. Examples from our lab include several PAHs, 3-nitrobenzanthrone and tamoxifen. Manipulating the enzyme functionality of the activating system and then observing the effect on DNA adduct formation is an effective way of determining which enzymes activate, or detoxify, carcinogens. Thus tamoxifen activation has been shown to be mediated by sulfotransferase(s) and not acetyltransferases. However, studies on benzo[a]pyrene-DNA adduct formation have revealed an apparent paradox, whereby CYP1A1 activity appears to be essential for metabolic activation *in vitro*, but in some *in vivo* situations, DNA adduct formation increases when this enzyme is inhibited or absent.

O#4

Immunoassays for the determination of PAH-DNA adducts

B. Schoket
Department of Molecular Environmental Epidemiology, National Institute of Environmental Health, József Fodor National Center for Public Health, Budapest, Hungary.

Molecular dosimetry of human genotoxic exposure and determination of carcinogen-DNA adducts have advanced substantially during the last two decades. After ^{32}P-postlabelling, immunoassay is the second most sensitive method for the detection of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in experimental samples and human tissues. (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7,8-diol 9,10-epoxide (BPDE)-DNA immunoassays, utilising monoclonal antibody or polyclonal antisera elicited against DNA modified with BPDE, recognise not only benzo[a]pyrene (BP)-derived but chemically related PAH-DNA adducts as well due to cross-reactivity of the antibody. Competitive enzyme-linked immunosorbent assay (ELISA), the first immunoassay used with human samples, is less sensitive than dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA), which has time-resolved measurement of the long-lived fluorescent signal and short-lived background fluorescence. Chemiluminescence immunoassay (CIA), the most sensitive and stable immunoassay, has the lowest percentage of samples below the detection limit, a detection limit of 1.5 BPdG adducts/10^9 nucleotides with 20 μg DNA/microtiter plate well and a high signal-to-noise ratio. The Automated Cellular Imaging System (ACIS) provides a novel approach to semi-quantification of PAH-DNA adducts in nuclei of tissues stained immunohistochemically with the BPDE-DNA antiserum. Approximation of the DNA adduct levels is obtained by comparison with a standard curve consisting of cultured human cells exposed to increasing concentrations of BPDE and assayed by both CIA and ACIS. DELFIA and CIA have been applied in the validation of a BPDE-DNA adduct standard, and PAH-DNA immunoassays have contributed to human biomonitoring of occupational, medicinal, environmental and tobacco-smoke PAH exposures.

Acknowledgements: Valuable discussions with Dr. Miriam C. Poirier, National Cancer Institute, Bethesda, MD, USA, are highly appreciated. The author of this abstract is partner of ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility), a network of excellence operating within the European Union 6th Framework Program, Priority 5: "Food Quality and Safety" (Contract No 513943).

O#5

Accelerator mass spectrometry for DNA adduct detection

K. Brown
Cancer Biomarkers and Prevention Group, Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, LE1 7RH, UK.

Accelerator mass spectrometry (AMS), a technique most commonly known for radiocarbon dating, is becoming increasingly popular in biomedical research and development where it can quantify the isotopes ^{14}C and ^{3}H down to attomole (10^{-18}) levels in biological samples. The exquisite sensitivity of AMS makes it one of the most sensitive methods for quantifying DNA and protein adduct formation by a ^{14}C-labelled chemical, capable of measuring as little as 1-10 adducts/10^{12} nucleotides. AMS has been used to illustrate DNA adduct formation *in vivo* by carcinogens such as PhIP, MeIQx and benzo[*a*]pyrene, following administration of dietary or environmentally relevant doses to humans. We have employed AMS to elucidate mechanisms of tamoxifen carcinogenesis and have identified CYP3A4 as the human CYP form that catalyses the metabolic activation and covalent binding of tamoxifen to DNA. We have also shown that tamoxifen is capable of binding at extremely low levels to DNA in the uterus of women administered a single ^{14}C-labelled therapeutic dose prior to hysterectomy. Importantly, AMS has been used by others to demonstrate a lack of DNA adduct formation by the chemicals TCDD and ochratoxin, supporting a non-genotoxic mechanism for these carcinogens. New ^{14}C-postlabelling approaches, which exploit the sensitivity of AMS for detecting DNA damage generated *in vivo*, without the need to administer a radioisotope labelled compound are currently under development. We have reported an assay for the detection of O^6-methyldeoxyguanosine by chemical postlabelling of HPLC-isolated adducts using ^{14}C-acetic anhydride, which can attain a theoretical limit of detection of 79amol. The availability of smaller AMS instruments dedicated to the analysis of biological samples, particularly those with on-line HPLC capability, together with advances in sample preparation methods should lead to a more widespread use of this technology in biomedical research, including the field of genetic toxicology.

O#6

DNA-adduct analyses of human tissues by the ^{32}P-HPLC method

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In the analysis of human tissues it is a need for a high-resolution chromatographic system to enable separation of individual DNA-adducts and clusters of DNA-adducts. The ^{32}P-post-labeling method is very sensitive in detecting DNA-adducts enabling analysis down to 1 DNA-adduct/10^9 normal nucleotides. When combining the post-labeling method with direct injection into the HPLC system with on-line detection of ^{32}P the high sensitivity can be combined with a high resolution. The ^{32}P-HPLC system can further be modified by changing flow and composition of solvents to optimize resolution and/or to optimize capacity. By changing the chromatographic properties a high resolution can be obtained between stereoisomers of a DNA-adduct forming metabolite. If a certain DNA-adduct or DNA-adduct cluster is the target, the analytical system can be modified to increase the number of samples to be analyzed with a factor of five. It is most common to analyze white blood cells for DNA-adducts due to accessibility. Such analyses can detect changes in the environment (air pollution) such as season, with high levels during the winter season and a recovery/repair during the summer period. White blood cells can also be treated in vitro (after monthly in vivo collections from the same individuals) by mutagens showing an induction of certain parts of the CYP 450 system with a consequence of a higher level of DNA-adducts, in this case during summer time. Another approach is to analyze DNA-adducts in solid tissues. One example was when human placenta was analyzed from different regions representing "control" vs. polluted areas. With blind samples it was possible to characterize the mothers as being from "control" or polluted areas. Biopsies of solid tissues were applied in a study of colon cancer. In all cases normal colonic mucosa was sampled, but the patients represented three groups, healthy, polyp- and cancer-patients. Most DNA-adducts were similar, but two declined from healthy, over polyp to cancer patients. One DNA-adduct was only observed in colon cancer patients. In autopsy samples the DNA-adduct pattern has a great variability. First there are great differences between people living in the same area. One example with liver tissue from people that lived in the same area showed a variation of 180 times in the total DNA-adduct level. Analyses of different tissues from one person can show a great variation, where lung is lower probably due to the high turnover of the lung cells. Other tissues like the brain and heart can on the other hand be high in DNA-adducts, suggesting a possible link to other diseases than cancer (i.e. ageing of tissues).

Session III

O#7

Alternative approaches to ^{32}P-postlabelling for detection of low levels of DNA adducts

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The methodology for the determination of DNA adducts is capable of determination of unlabelled adducts at levels down to 1 adduct per 10^{10} nucleotides. The main procedures used to date are based on immunoassay, HPLC with fluorescence or electrochemical detection, ^{32}P-postlabelling or mass spectrometry. Another method of detection that is currently being explored is laser induced fluorescence, which also yields very high sensitivity. If the adduct carries a ^{14}C or a ^{3}H label, accelerator mass spectrometry provides the highest sensitivity of all adduct determination techniques (1 adduct per 10^{12} nucleotides). All of these techniques hold particular advantages and disadvantages, but ^{32}P-postlabelling is at present the method which is applied to the largest range of adducts. Recently the sensitivity of mass spectrometric techniques, which have the ability to give enhanced structural information compared to ^{32}P-postlabelling, has improved to a level approaching that obtained by ^{32}P-postlabelling. Thus liquid chromatography-tandem mass spectrometry (LC-MS/MS) using electrospray ionisation and selected reaction monitoring has been used to determine a number of adducted deoxynucleosides in enzymic hydrolysates of adducted DNA. For example we have recently developed a method to determine the benzo[a]pyrene adduct with the N^2-position of 2'-deoxyguanosine, using LC-MS/MS and a stable isotope labelled internal standard, with a sensitivity of 3 adducts per 10^8 nucleotides (Singh et al, Chem. Res Toxicol., 2006, **19**, 868-878). Adducts with 2'-deoxyguanosine appear to have a common collision-induced MS/MS fragmentation, loss of m/z 116, corresponding to loss of the deoxyribose residue, which gives rise to the possibility of developing a screening system for these adducts. The first example of the use of such a technique (termed 'adductomics') has recently been published by Kanaly et al, Antiox. Redox Signal. **8**, 993-1001, 2006.

O#8

Novel ^{32}P-postlabelling method for the determination of adducted deoxynucleosides in human body fluids

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Non-invasive detection methods such as urinalysis will expedite studies in humans aimed to elucidate etiopathological factors that cause DNA damage. Etheno-DNA adducts are generated from exogenous carcinogens such as vinyl chloride, urethane and also from, oxidative stress/ lipid peroxidation product such as trans-4-hydroxy-2-nonenal. We and others have established that 1,N^6-ethenodeoxyadenosine (εdA) and 3,N^4-ethenodeoxycytidine () are present in human urine which could be explored as biomarkers for monitoring whole body oxidative stress. Here, we report a new ultrasensitive ^{32}P-postlabeling/TLC method for the analysis of εdC as deoxynucleoside in human urine. The urine samples were purified and enriched on a solid-phase silica C-18 column followed by a semi-preparative reverse phase HPLC. The purified sample was labelled with a multisubstrate deoxyribonucleoside kinase from *Drosophila melanogaster* (Dm-dNK) in presence of 5'-bromo-2'-deoxyuridine (BrdU) as internal standard. The absolute sensitivity of the method was 0.1 fmol εdC detectable in 500 µl of human urine. The analysis of human urine samples from 15 healthy volunteers revealed a mean εdC level of 2.49 ± 1.76 (SD) fmol/µmol creatinine (range 0.66-6.42). By our non-invasive method, εdC in human urine could be explored as a biomarker for oxidative stress-related human diseases. Currently the method is being applied to the analysis of εdC in human pancreatic juice. [Ref: X. Sun, A. Karlsson, H. Bartsch, and J. Nair, Biomarkers **11**, 329-340, 2006]

O#9

Determination of the DNA methylation level with capillary electrophoresis-laser-induced fluorescence (CE-LIF)

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The epigenetic code, i.e. the methylation of cytosines, various histone modifications and DNA-binding proteins, is, unlike the genetic code, always being rewritten and erased. Epigenetic mistakes are involved not only in mutations in the genotype, but also in the development of abnormalities, cancer and other diseases. With a capillary electrophoretic

method we have described previously, the genomic methylation level can be determined very exactly. For coping with the increased quantity of samples in an adequate time, we present here a sample preparation that eliminates, after the derivatisation reaction, the surplus of fluorescence marker and the carbodiimide that is used for the coupling. With this method, sequences of up to 120 measurements can be carried out, which increased the sample throughput from 75 to 250 analyses per week. In addition the development of a new detector system is presented. The new detector unit is based on an Ar-ion laser with a spectrometer and a CCD camera, and the limit of detection of the fluorescence label BODIPY was determined as 5 pM. This allows us to determine DNA modification, after BODIPY labeling, at a concentration lower by a factor of approximately ten than with commercially available CE-LIF systems.

Session IV

O#10

Tamoxifen and tamoxifen analogues: activation to DNA adducts *in vitro* and *in vivo*

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The antiestrogen tamoxifen is used as an adjuvant chemotherapeutic agent for the treatment of breast cancer and as a chemopreventive agent in high risk women. Despite being beneficial in this context, tamoxifen increases the risk of endometrial cancer in women. The mechanisms for the induction of endometrial tumors by tamoxifen are presently uncertain, although both genotoxic and hormonal pathways could be involved. Tamoxifen is hepatocarcinogenic in rats, and this has been associated with DNA adduct formation by reactive derivatives of the metabolites α-hydroxytamoxifen and α-hydroxy-*N*-desmethyltamoxifen. However, the occurrence of tamoxifen-DNA adducts in the human endometrium remains controversial although a number of analytical methodologies, including ^{32}P-postlabeling and mass spectrometry, have been used to address the issue. Assuming a genotoxic pathway to tamoxifen-induced endometrial carcinogenesis, tamoxifen analogues less susceptible to metabolic activation to derivatives capable of reacting with DNA could be advantageous in the therapeutic setting. Two such analogues are toremifene, an effective chemotherapeutic agent in postmenopausal women with advanced breast cancer, and GW5638, which is being considered as an adjuvant chemotherapeutic agent. We have synthesized the α–hydroxy and α–sulfoxy derivatives of toremifene and GW5638, characterized the DNA adducts that are formed *in vitro*, and investigated whether or not these adducts are formed *in vivo*. α–Sulfoxytoremifene gave two major DNA adducts *in vitro*. ^{32}P-Postlabeling analyses of liver DNA from rats administered α-hydroxytoremifene indicated the same two DNA adducts; nonetheless, the ^{32}P-postlabeling data confirmed a much lower *in vivo* genotoxicity of toremifene and α-hydroxytoremifene, as compared to tamoxifen. Two DNA adducts were also obtained *in vitro* from reaction of α-sulfoxyGW5638 with DNA; however, hepatic DNA adducts were not detected by ^{32}P-postlabeling following administration of GW5638 to rats. Potential implications concerning the safety of both toremifene and GW5638 for long-term clinical use will be discussed in view of these results.

O#11

The potential use of DNA adducts formed by the carcinogenic air pollutant 3-nitrobenzanthrone in human biomonitoring

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Epidemiological studies have shown that exposure to diesel exhaust and urban air pollution is associated with an increased risk of lung cancer. 3-Nitrobenzanthrone (3-NBA) is an extremely potent mutagen and suspected human carcinogen identified in diesel exhaust and ambient air particulate matter. The main metabolite of 3-NBA, 3-aminobenzanthrone (3-ABA), was found in the urine of salt mine workers occupationally exposed to diesel emissions, indicating that human exposure to 3-NBA due to diesel emissions can be significant and is detectable. There is clear evidence that 3-NBA is a genotoxic mutagen forming DNA adducts after metabolic activation through simple reduction of the nitro group. Several human enzymes have been shown to activate 3-NBA and its metabolites in vitro and in cells leading to the formation of purine adducts at the C8 and N2 position of guanine and at the C8 and N^6 position of adenine. The predominant DNA adducts in vivo, 2-(2́-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (dG-N^2-ABA) and N-(2́-deoxyguanosin-8-yl)-3-aminobenzanthrone (dG-C8-N-ABA) are also the most persistent adducts in target tissue in rodents, and are most probably responsible for the induction of GC→TA transversion mutations observed in vivo. It is concluded that these adducts not only represent premutagenic lesions in DNA but are of primary importance for the initiation of the carcinogenic process and subsequent tumour formation in target tissue. Indeed, 3-NBA is carcinogenic

in rats after intratracheal instillation, inducing mainly squamous cell carcinoma in lung. Because of its widespread environmental presence, 3-NBA may represent not only an occupational health hazard but also a hazard for larger sections of the general population. For an accurate risk assessment more epidemiological studies on 3-NBA-exposed individuals and a broader monitoring of environmental levels of 3-NBA are required. [Arlt, Mutagenesis **20**, 399-410, 2005]

O#12

Cytochrome P450- and peroxidase-mediated formation of covalent DNA adducts by an anticancer drug ellipticine - a novel mechanism of ellipticine action

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Ellipticine is an antineoplastic agent exhibiting the multimodal mechanism of its action. While the prevalent mechanisms of ellipticine antitumor, mutagenic and cytotoxic activities were suggested to be intercalation into DNA and inhibition of DNA topoisomerase II activity, based on data found by us, ellipticine should be considered a drug, whose pharmacological efficiency and/or genotoxic side effects are dependent also on its cytochrome P450 (CYP)- and/or peroxidase-mediated activation in target tissues. We demonstrate a new mode of ellipticine action, formation of covalent DNA adducts mediated by its oxidation with CYPs and peroxidases. Using ^{32}P-postlabeling assay we found such DNA-adducts *in vitro*, in V79 cells transfected with human CYP1A1, 1A2 and 3A4, human breast adenocarcinoma MCF-7 cells, human leukemia HL-60 and CCRF-CEM and neuroblastoma cell lines and *in vivo* in rats exposed to ellipticine. We report the molecular mechanism of ellipticine oxidation by CYPs and identifies human and rat CYPs responsible for ellipticine metabolic activation and detoxication. We also present a role of peroxidases (i.e. myeloperoxidase, cyclooxygenases, lactoperoxidase) in ellipticine oxidation leading to ellipticine-DNA adducts. The 9-hydroxy- and 7-hydroxyellipticine metabolites formed by CYPs and the major product of ellipticine oxidation by peroxidases, the dimer, in which the two ellipticine residues are connected through nitrogen N^6 in the pyrrole ring of one of the ellipticine moieties and carbon C9 of the other ellipticine, are the detoxication metabolites. 13-Hydroxy- and 12-hydroxyellipticine, produced by ellipticine oxidation with CYPs, the latter one formed also spontaneously from another metabolite, ellipticine N^2-oxide, are metabolites responsible for formation of two ellipticine-derived deoxyguanosine adducts in DNA. The results shown here allow us to propose species, two carbenium ions, ellipticine-13-ylium and ellipticine-12-ylium, as reactive species generating two major DNA adducts seen *in vivo* in rats treated with ellipticine. The study forms the basis to further predict the susceptibility of human cancers to ellipticine. *Supported by GACR (grant 203/06/0329) and the Ministry of Education of the Czech Republic (grants 1M4635608802 and MSM0021620808).*

O#13

Molecular basis of *ARISTOLOCHIA* carcinogenicity

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The use of a herbal weight-loss product, containing *Aristolochia* species has been associated with the development of a novel nephropathy, and urothelial cancer. The observed nephropathy and urothelial carcinoma has been traced to the ingestion of aristolochic acid (AA) and is now called aristolochic acid nephropathy (AAN). The major components of the plant extract AA, aristolochic acid I (AAI) and aristolochic acid II (AAII), are genotoxic mutagens forming DNA adducts after metabolic activation catalysed by several human enzymes. The activating metabolism has been elucidated and is consistent with the formation of a cyclic nitreniumion with delocalised charge leading to the preferential formation of purine adducts bound to the exocyclic amino groups of deoxyadenosine and deoxyguanosine. The predominantly formed DNA adduct 7-(deoxyadenosin-N^6-yl)aristolactam I (dA-AAI) *in vivo* was detected in AAN patients unambiguously proofing the exposure to AA. dA-AAI the most persistent adduct in target tissue and therefore detectable in urothelial tissue from AAN patients 10 years after they stopped taking the weight-loss regimen is a mutagenic lesion leading to AT→TA transversions *in vitro*. This transversion mutation is found in high frequency in codon 61 of the H-*ras* oncogene in tumours of rodents induced by AAI, suggesting that dA-AAI might be the critical lesion in the carcinogenic process in rodents. Interestingly, in one AAN patient available for analysis the identical AT→TA transversion mutation was found in the p53 gene in urothelial tumour cells. AA is a powerful nephrotoxic and carcinogenic substance with an extremely short latency period not only in animals but also in humans. In particular the highly similar metabolic pathway of activation and resultant DNA adducts of AA which are detectable by the ^{32}P-postlabeling method, allows the extrapolation of carcinogenesis data from laboratory animals to the human situation. These findings draw one of the strongest links yet between use of a herbal product and cancer in humans. Therefore, all botanical-containing products known or suspected of containing AA should be banned from the market worldwide.

Symposium Prof. Manfred Wiessler

O#14

Replication control in tumor cells as a target for DNA-interacting anti-tumor agents

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HKH40A, 2-(3-{4-[3-(8-methoxy-6-oxo-6H-2,10b-diaza-aceanthrylen-5-ylamino)-propyl]-piperazin-1-yl}-propyl)-5-nitro-benzo[*de*]isoquinoline-1,3-dione, and WMC79, the des-methoxy analog of HKH40A, are synthetic agents with potent activity against gastrointestinal and hematopoietic tumors. However, HKH40A has superior pharmacokinetic characteristics and significantly better in vivo activity. Cell-based assays have consistently shown that HKH40A is 3-5 times more active than WMC79, especially in tumors with mutated p53. Both compounds kill p53(+/+) tumors by up-regulation of the p53 apoptotic cascade, which appears to be induced by transcriptional down-regulation of ribonucleotide reductase (RNR) that controls dNTP pools and the cyclin-dependent kinases Cdc6 and Cdc7 that regulate replication. Up-regulation of RNR has recently been identified as a key factor in gemcitabine resistance. HKH40A causes dramatic down-regulation of these genes as well as those of the MCM family, which are important targets of Cdc7. Downregulation of the kinases in p53(-/-) tumor cells puts them into replicative arrest, and premature entry into S-phase that results in genomic instability, aberrant mitosis and cell death. Normal cells appear to be less affected by the drug because premature progression through the cell cycle is prevented by checkpoint controls, which are frequently disrupted in tumor cells. Consequently, HKH40A has a good therapeutic index. HKH40A is potently active (curative) in an orthotopic model of liver cancer (JM-1) in Fisher 344 rats when administered IP. However, HKH40A and WMC79 are most active in vivo against xenografted tumors when injected IV although WMC79 is significantly less stable in circulation, probably because of more rapid metabolic clearance in the liver. HKH40A showed good activity in HCT116 colon cancer xenografts in nu/nu mice (TGI 65%) and in BxPC-3 pancreatic cancer xenograft (TGI 71%). The combination of HKH40A and gemcitabine was tested in MIA PaCa-2 pancreatic cancer in nu/nu mice and produced regression or stasis in several groups. HKH40A is a candidate for Phase 1 clinical trials.

Session V

O#15

Progress towards the development of high-throughput immunochemical assays for DNA damage and repair

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The need for sensitive, high-throughput and economical methods to assay DNA damage and repair is widely recognized, especially in view of prospects of large-scale molecular epidemiology studies. Immunochemical methods hold a potential for satisfying these criteria, while at the same time providing the moderate-to-high specificity required for chemical-specific biomarkers of exposure. In this context we are making a systematic effort to develop such methodologies, and will present a short report of progress achieved.

a) The measurement of the activity of the repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) is important both for studies of chemical and environmental carcinogenesis by methylating carcinogens (eg nitrosamines) and for studies of the susceptibility of cancer patients to the therapeutic effects of methylating cytostatic drugs. The commonly used method for assaying MGMT activity in tissue or cell extracts is based on the use of a [3H]-methylated DNA substrate and the multi-step assay of the transfer of [3H]-methyl groups to the protein. Current experience indicates difficulties in the availability of the abovementioned substrate, while the throughput of the assay is limited by the need for multiple steps including centrifugation, precipitate dissolution and radiocounting. We have exploited the fact that O6-benzylguanine (BG) can serve as an efficient substrate for MGMT to develop a sensitive and rapid ELISA method for the assay of MGMT activity: Extracts are incubated with BG tagged with biotin, and the resulting MGMT-BG-biotin complex is immobilized on anti-MGMT-coated microtiter plates, followed by quantitation using streptavidine-conjugated horseradish peroxidase and a chemiluminescence-producing substrate. The method is highly sensitive (LOD: 0.5 fmol MGMT protein versus 3 fmol of the old method), has an extremely high dynamic range (0.5-500 fmol versus 3-20 fmol of the old method), is relatively inexpensive and can be easily automated.

b) Immunochemical assays of various types (RIA, competitive or non-competitive ELISA) have been reported for various types of DNA adducts. We are currently making an effort to develop a new approach to the immunochemical assay of DNA damage which, if successful, holds the potential not only of high sensitivity and throughput, but also of multi-adduct analysis on the same DNA sample. Our approach, still under development, is based on the use, in an ELISA set up, of immobilised anti-adduct antibodies to extract adduct-containing DNA fragments from bulk DNA, followed by their detection and quantitation using anti-DNA antibodies. The high ratio of normal nucleotides (providing epitopes for interaction with the latter) to adducts on each adduct-containing fragment provides a possibility for strong signal enhancement. Furthermore, the DNA not bound to the first anti-adduct antibody can be collected and used for the assay of additional types of adducts using a similar setup.

O#16

^{32}P-Postlabelling analysis of UV induced pyrimidine dimers from human skin and urine

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Ultraviolet irradiation (UV) is considered to be a major causative factor in human skin cancer. The major products induced by UV in DNA are dipyrimidinic lesions, which are likely to be important in the pathogenesis of skin cancer. In our studies of human volunteers exposed to solar simulated UV radiation we excised skin biopsies after exposure and analysed UV dimers in DNA by a sensitive ^{32}P-postlabelling assay. The obtained data showed that high levels of dimers are formed in human skin in situ after just a single dose of solar simulated UV light corresponding to what will be obtained during one hour in summer time Stockholm. Furthermore, those lesions were repaired, and with a mixed time kinetics, i.e. an initial fast removal followed by a slower phase. Large inter individual differences were observed, both in formation and repair of DNA damage. Use of sun screens was shown to be highly protective against formation of UV dimers. Case control studies with basal cell carcinoma patients indicated a reduced capacity to remove UV damage among the cancer cases. More recently we developed a postlabelling assay for detection of UV dimers present in urine as a result of DNA repair. This assay has the advantage that it is non-invasive and can thus be applied also to studies in children. So far carried out studies showed that the amount of dimer excreted into urine was correlated to the applied UV dose and that high levels of such lesions were also found in children following a couple of hours of sun exposure. In conclusion, our studies have shown that DNA damage by UV light can be analysed in humans following doses that everyone will receive just by spending a few hours outside and that there are inter individual differences that may have implications for who will develop cancer.

O#17

Formation of DNA adducts in humans and laboratory animals by phytochemicals from common vegetables and fruits

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Plants have to defend themselves against infecting microorganisms and herbivores animals, at least in certain stage of the vegetation cycle. We hypothesized that reactive chemicals could provide universal protection, in contrast to stable molecules interacting with specific structures of the enemy. These reactive species would have to be released directly after damage to the plant or after activation by common enzymes in enemies such as glycosidases. We observed that adducts are formed with the endogenous DNA in homogenates of various raw plants in a time-dependent manner. In general, the adducts were not formed when plants were cooked prior to homogenization. The patterns of adducts were similar within genetically closely related plants (e.g. *Brassicaceae*) but different between phylogenetically remote plants (e.g. broccoli, nutmeg, peer). Such homogenates or appropriate extracts were mutagenic in bacterial and mammalian target cells in culture, with formation of the same patterns of adducts as in the plant homogenates. Human volunteers have chewed such plants. Formation of plant-characteristics DNA adducts was detected in oral cells and, at lower levels, in blood cells. In one case, we also had the opportunity to analyse colon biopsies (with a negative result in this case). In rats accustomed to the consumption of raw broccoli, a treatment-associated DNA adduct was detected in liver that co-migrated with the major adduct in Broccoli homogenate. The study shows that plants can produce natural genotoxicants that may lead to damage in consumers. Damage is not seen when food is prepared in an appropriate way. It may be envisaged to searched for varieties that produce less genotoxicants.
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Session VI

O#18

In vitro cell immortalization selects for p53 gene mutations found in human cancers

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The ability to induce point mutations in the p53 tumor suppressor gene in its normal genomic context, and then select mammalian cells carrying the mutations would be useful for testing hypotheses on the origins of p53 mutations in human tumors. To design such an approach, we first introduced human p53 DNA binding domain sequences into the endogenous murine p53 locus by gene-targeting, and then generated mice harbouring the humanized p53 knock-in configuration in both alleles. This mouse (Hupki, for human p53 knock-in) is phenotypically p53 wild-type. Embryonic fibroblasts from this strain, like embryonic fibroblasts (MEFs) from mice with unperturbed endogenous mouse p53 wild-type alleles, senesce in vitro after a number of population doublings, but occasionally escape growth arrest, proliferate, and become established as immortalized cell lines. Bypass of senescence in mouse cells typically occurs as a consequence of a molecular event that disrupts p53/ARF function, such as a mutation in the p53 DNA binding domain (DBD). Overcoming senescence thus serves as a 'natural' method to select for p53 mutant mouse cells. Indeed, DNA from immortalized Hupki embryonic fibroblast cultures exposed during early passage to the mutagenic pro-carcinogens benzo(a)pyrene (BaP) and aristolochic acid I (AAI) reveal the presence of homozygous or heterozygous p53 DBD mutations. The predominant class of mutation in BaP-immortalized lines is G to T, whereas in AAI-treated cells, most mutations are A to T transversions, with strong evidence of strand-bias. BaP mutations are at codons frequently mutated in human cancers, and the base substitutions are characteristic for the major pre-mutagenic DNA adducts these chemicals form. To explore the effects on mutation and immortalization of the two common human p53 variants at codon 72 encoding arginine or proline we generated a second knock-in mouse encoding the proline variant of p53, but otherwise identical to the arginine p53 variant of the original Hupki strain.

Oral Presentations

O#19

Determination of urinary excreted PAH metabolites as a non-invasive human biomonitoring method

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Polycyclic aromatic hydrocarbons (PAH) are widespread occurring environmental contaminants some of which are well known human carcinogens. Apart from personal smoking habits, the general population is exposed to low level of PAH in ambient air particulate matter and contaminated food. Biological monitoring of PAH metabolites in human urine is the method of choice to assess the internal dose of an individual as it reflects all different uptake routes (inhalation, ingestion, and dermal resorption). Urinary excreted phenols of naphthalene, pyrene and phenanthrene are selected to be used as biomarkers of an exposure to PAH because metabolites of higher moleculat weight PAH, such as benzo[*a*]pyrene, are urinary excreted only in very small amounts. Among the phenols 1-hydroxypyrene (1-OHP) is the most frequently determined PAH metabolite and in Germany and USA references values for this biomarker have been calculated for the general population from environmental surveys performed in these countries. During the last years several analytical procedures have been developed based on HPLC and GC/MS methodologies allowing to determine 1-OHP and an additional battery of phenols from naphthalene, phenanthrene and other PAH. Recently, analytical procedures have been developed to determine more polar PAH metabolites excreted in urine. The levels of the new biomarkers 1,6- and 1,8-dihydroxypyrene, formed from 1-OHP by further oxidation, have been determined in the general population. Notably, the ratio of 1-OHP and dihydroxypyrenes varies greatly depending on the exposure level. Other examples are phenanthrene diols and the highly polar phenanthrene tetrol. The latter biomarker is excreted in similar amounts as the phenanthrols and reflects the metabolic activation pathway of carcinogenic PAH to bay region diol epoxides. The various types of phenanthrene metabolites have been proposed to be used in future studies for phenotyping of individuals to allow to determine their possible susceptibility to develop cancer upon exposure to PAH mixtures.

O#20

Use of DNA adduct analysis to study mechanisms of drug genotoxicity - example cyproterone acetate (CPA)

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The mechanistic study was performed in rat hepatocytes on the synthetic gestagen and antiandrogen cyproterone acetate (CPA), an active component of several drugs widely used in human therapy for many years as an active component of oral contraceptives (Diane 35) or treatment of hypersexual behavior and several other indications (Androcur). CPA was originally regarded as a typical tumor promoter causing in high doses liver tumors in rats. In the basic battery of tests of genotoxicity, CPA exhibited no indication of any mutagenic potential. However, by using of rat hepatocytes we have clearly demonstrated that CPA, as a first synthetic steroid, induced formation of high levels of DNA adducts [Topinka et al., Carcinogenesis 14 (1993) 423-427]. These DNA adduct levels were much higher than those induced by structural analogues of CPA [Topinka et al., Carcinogenesis 16 (1995) 1483-1487]. The CPA induced DNA adducts were persistent and accumulating [Werner et al., Carcinogenesis 16 (1995) 2369-2372]. We have also shown that CPA is an integral part of DNA adducts detected in hepatocytes treated with CPA [Topinka et al., Carcinogenesis 17 (1996) 167-169] and the activation pathway of CPA to DNA reactive metabolites via the action of keto-reductases and hydroxy-steroid sulfo-transferases has been demonstrated [Schwarz et al., Advances in Experimental Biology, 387 (1996) 243-251]. Hepatic CPA-DNA adducts are fixed in the liver as point mutations [Topinka et al., Mutation Res. 550 (2004) 89-99] and we have shown that DNA adduct formation is not the only prerequisite of mutagenic effect of CPA [Topinka et al., Mutation Res. 550 (2004) 101-108]. Non-linearity of dose response curve of mutagenic effect *in vivo* in target tissue of carcinogenesis and the existence of no-effect level of CPA proved in agreement with epidemiologic studies that contraceptive dose of CPA does not represent any significant risk.

O#21

Early markers in the development of colorectal tumours in the rat: DNA adducts compared with isoprostanes, histological and morphological alterations in the early stage

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Dextrane sulfate induced colitis leads to increased formation of polyps and colon cancer via inflammation, oxidative stress and lipid peroxidation. Anti-inflammatory substances, anti-oxidative compounds, other secondary plant ingredients or selenium substitution in case of selenium lack in nutrition can act as chemopreventives in colorectal cancer. Neither the detailed mechanisms of such chemopreventives are entirely known, nor are the single states of colon carcinogenesis by oxidative stress and the course from inflammation to precarcinogenic lesions and final colo-rectal carcinoma investigated in detail. In the study the impacts of chemopreventives were investigated on each single step in the development of colon cancer on a morphological and molecular biological base. Early markers are prostaglandin $F_{2\alpha}$ for inflammation, 8-iso prostaglandin $F_{2\alpha}$ for oxidative stress, E-cadherin, beta-catenin, Ki67, rate of apoptosis and 1,N^2-propanodeoxyguanosine adducts of the lipid peroxidation product 4-hydroxy-2-nonenal (HNE-dGp) as early markers for DNA damage, mutation and cancer initiation. These parameters are compared with morphological changes like crypt architecture, preneoplastic aberrations and late markers like colon polyps and carcinoma. We established a special ^{32}P-postlabelling technique for HNE-dGp with a detection sensitivity of 2-3 adducts / 10^9 nucleotides and found background adducts levels in human and animal tissues. Adduct levels were increased after induction of lipid peroxidation. The HNE-dGp adducts are promutagenic DNA lesions leading to mutations in tumour suppressor genes and proto-oncogenes, are not repaired by base excision repair and are highly specific for 4-hydroxynonenal because they still contain the structural moiety of 4-hydroxy-2-nonenal. After 16, 28 days and 6 month we found increased levels of the early markers in rats induced with dextrane sulfate and fed with vitamin E and selenium deficient diet. We expect polyps 12 months after start of the application of dextrane sulfate and of carcinoma after 24 months in the animal groups which had increased early markers.

Session VII

O#22

The use of DNA adducts in surrogate tissues: pitfalls and opportunities

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Carcinogen-DNA adducts can be applied to assess the biologically effective dose and to identify individuals at high cancer risk. However, practical and ethical considerations limit the types of tissues available for DNA adduct analysis. Therefore, DNA adducts have predominantly been studied in easily available white blood cells (WBC) or in cells obtained by broncho-alveolar lavages (BAL). WBC consist of 3 subpopulations: monocytes, lymphocytes and granulocytes, which differ in their relative abundance in blood (ca. 5-10%, 20-40% and 50-75%, respectively). DNA adduct levels after *in vitro* and *in vivo* exposure to polycyclic aromatic hydrocarbons were higher in monocytes and lymphocytes than in granulocytes. Furthermore, DNA adduct levels were more persistent in lymphocytes than in short-lived monocytes or granulocytes, which makes the number of adducts that are detectable in total WBC after a given amount of time uncertain. On the other hand, these differences in adduct persistency give the opportunity to get information about the time since exposure has ended by comparing DNA adduct levels in lymphocytes with those in monocytes. Overall, assessment of DNA adducts in total WBC instead of isolated subpopulations could attenuate the relationship between DNA adducts and carcinogen exposure. Although granulocytes seem to have a minor contribution to DNA adduct levels in peripheral blood, they play an important role in the formation of DNA adducts in BAL-cells; activated granulocytes release myeloperoxidase, which was found to increase DNA adduct levels in target cells by increased metabolic activation of pre-carcinogens and by the inhibition of DNA repair. Information on the influx of granulocytes into the lung and their subsequent activation is thus required to establish a more reliable dose-response relationship. The analysis of DNA adducts in separated cell-types present in BAL has not yet been performed. It should be recommended, if feasible in molecular epidemiological studies, to separate WBC-subpopulations and to characterize cells in BAL before the analysis of DNA adducts.

O#23

^{32}P-postlabelling DNA adducts and lung cancer risk in Gen-Air, a case-control study nested in the EPIC investigation

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Air pollution has been reported to increase lung cancer risk. The risk of lung cancer death has been suggested to increase by 8% for every 10 micrograms of fine particles in a cubic meter of inhaled air. In the present case-control study nested in the EPIC investigation, we investigated prospectively the ability of DNA adducts to predict cancer in non-smokers in 10 European countries (France, Denmark, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden, United Kingdom). Cases included newly diagnosed lung cancer (N=115), upper respiratory cancers (pharynx, larynx) (N=82), bladder cancer (N=124), leukemia (N=166) and COPD or emphysema deaths (N=77), accrued after a median follow-up of 7 years among the EPIC former smokers and never smokers. Three controls per case were matched for questionnaire analyses, and two controls per case for laboratory analyses. Matching criteria were gender, age, smoking status, country of recruitment, and follow-up time. Individual exposure to air pollution was assessed using concentration data from monitoring stations in routine air quality monitoring networks. Leukocyte DNA adducts were blindly analysed using the ^{32}P-postlabelling technique. DNA adducts were associated with the subsequent risk of lung cancer, with an odds ratio of 1.86 (95% CI 0.88-3.93) when comparing detectable versus non-detectable adducts. The association with lung cancer was stronger in never smokers (OR=4.04; 1.06-15.42) and among the younger age groups. After exclusion of the cancers occurring in the first 36 months of follow-up, the OR was 4.16 (1.24-13.88). A positive association was found between DNA adducts and O_3 concentration, indicating that the average levels of O_3 play a role in the modulation of DNA adducts of non-smokers. Gen-Air study suggests that leukocyte DNA adducts may predict lung cancer risk of never smokers. Besides, the association of DNA adduct levels with O_3 indicates a possible role for photochemical smog in determining DNA damage.

O#24

Sources of adducts releasing 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) from DNA and haemoglobin in humans

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Recently, the tobacco-specific nitrosamines (TSNA), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN), have been classified by IARC as carcinogenic for humans. From determination of urinary NNK metabolites it can be concluded that smokers take up at least 100-fold higher amounts of TSNA than nonsmokers. However, HPB-releasing haemoglobin adducts resulting from metabolic activation of NNK and NNN did not show the expected specificity for smoking. Using an improved analytical method we found about sixfold higher levels of HPB-releasing adducts in lung DNA from smoking (N=22; Mean±SE: 385±56 fmol HPB/mg DNA) compared to nonsmoking (N=12; 67±18 fmol/mg; $p<0.0001$) lung cancer patients undergoing lung surgery. In a subset of these patients haemoglobin adducts were only marginally higher in smokers (N=14; 60±13 fmol/g haemoglobin) compared to nonsmokers (N=8; 41±11 fmol/g; n.s.) and did not correlate with lung DNA adducts. In tumour-free sudden death victims current smoking status was verified by determination of cotinine in urine or blood. The differences in lung DNA adducts between 49 active smokers and 34 nonsmokers (91±19 versus 50±7 fmol/mg DNA; n.s.) were much lower in sudden death victims than in lung cancer patients. Even higher adduct levels were found in the mucosa close to the oesophageal-gastric junction. These mucosal adducts were completely independent of the smoking status and did not correlate with adducts in the lung. The tobacco alkaloid myosmine could be an important tobacco-independent source of HPB-releasing adducts for several reasons. Myosmine has been found in a large variety of edible plants. Independent from smoking status, myosmine is present in human plasma and saliva in the lower ng/ml range indicating daily uptake of mg amounts of myosmine from the diet. Finally, due to its imine structure myosmine is not only nitrosated rapidly to NNN but gives rise to reactive precursors of HPB by nitrosative as well as peroxidative mechanisms.

O#25

Dietary DNA adducts: heterocyclic amines and pesticides

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Diet is influencing human cancer risk and, among others, dietary residues of plant protection products (PPP) and heterocyclic aromatic amines (HCA) derived from frying or grilling meat have been suspected as contributing factors. PPP are a heterogeneous group comprising diverse chemical structures and various toxicological profiles. Experimental studies on the formation of DNA adducts by PPP are lacking confirmation and structural characterisation of adducts. Using the ^{32}P-postlabelling assay isolated studies have reported on elevated DNA modification levels in human peripheral lymphocytes upon occupational exposure towards PPP but DNA adducts were not characterised as PPP-derived. No association of DNA modifications in humans with dietary intake of residual plant protection products have been reported, and, considering also the low residue levels, are not to be expected. The current re-evaluation of all existing PPP according to Directive 91/414/EC reduces health risks from dietary residues of PPP or occupational exposure to PPP in the EU. This process is, in fact, eliminating genotoxic active substances including those that have been shown experimentally to induce DNA adducts. There are about twenty different but structurally similar HCA. These have been demonstrated to be genotoxic, to form DNA adducts and to induce tumours in rodents. HCA-derived DNA adducts in human tissues have been identified only in a few studies using ^{32}P-postlabelling, immunochemical or mass spectrometric methods. Elevated levels of DNA modification in humans with a high intake of fried meat have been demonstrated in isolated studies. Several case control studies confirmed the association of high dietary intake of HCA and increased human cancer risk of the colon, breast and pancreas while no epidemiological association of dietary PPP-residues and cancer risk has been demonstrated.

O#26

Correlation of DNA adducts with other endpoints of genotoxicity in mouse lymphoma cells treated with methyl methanesulfonate

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The biological significance of DNA adducts as a risk factor for mutation and cancer is under continuous debate. In view of the background rate of DNA damage this question could be addressed quantitatively by relating increments of DNA adducts from an exogenous source to the increments measured for other endpoints of genotoxicity. In this study, background levels of four adducts, O^6-methyl-2'-deoxyguanosine (O^6-mdGuo) and 7-methylguanine (7mG), 8-oxo-7,8-

dihydro-2'-deoxyguanosine (8-oxodGuo) and 1,N^6-etheno-2'-deoxyadenosine (εdAdo) were measured by LC-MS/MS in DNA isolated from large batches of L5178Y mouse lymphoma cells treated with methyl methanesulfonate (MMS) at 0, 50, 100, 200 μM concentration. Aliquots of the cell suspension were used to perform simultaneously (i) the alkaline version of the comet assay, (ii) the micronucleus test, and (iii) the thymidine kinase $tk^{+/-}$ gene mutation assay. Means and standard deviations from at least four independent replicate studies were determined for the four adducts and the three biological endpoints, and increments above control were expressed in relative terms. Fifty μM MMS resulted in a 1.6 to 2.2-fold increase above background for the biological endpoints, whereas both O^6-mdGuo and 7mG increased about 8-fold. At 100 μM MMS, the biological endpoints showed 2.6 to 4.4-fold increases, while the factors for both O^6-mdGuo and 7mG were 19-fold. At 200 μM, the respective factors ranged from 4.4 to 7.5 for general genotoxicity and were 28 and 189, respectively, for the guanine methylations. No dose-related increase was observed for either 8-oxodGuo or □dAdo. Except for the sublinear dose response observed for 7mG at high dose, linearity could not be rejected for the other endpoints. Our data indicate that DNA-adduct formation as a first interaction of a genotoxic agent with its target may lead to a many-fold overestimation of its biological relevance for subsequent, more complex endpoints.

Poster Presentations

Posters mounting:
Friday, September 29, 2006, 08.00 am – 01.00 pm.

Posters display:
Posters will be displayed continuously throughout whole workshop September 29-30, 2006.

Posters removal:
Saturday, September 30, 2006, not later than 03.00 pm.

Poster viewing:
Friday, September 29, 2006, 01.00 – 02.30 pm.

Session 1:
01.00 – 01.45 pm, all presenters with odd numbered posters should be present by their poster.
Session 2:
01.45 – 02.30 pm, all presenters with even numbered posters should be present by their poster.

Pins for mounting posters will be available at the poster area and/or registration desk.

Poster dimensions:
Height: max. 130 cm
Width: max. 100 cm

Poster Titles

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^{32}P-POSTLABELLING ANALYSIS OF THYMINE DIMER IN URINE FROM CHILDREN OF DIFFERENT SKIN TYPES

T. Sandberg, N. Kotova, P.C. Turner, A. Sylla, M.S. Diallo, C.P. Wild, and D. Segerbäck

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OCHRATOXIN A (OTA)-DNA ADDUCT DETECTION AS A USEFUL BIOMARKER OF OTA EXPOSURE AND FOR STUDIES ON MOLECULAR MECHANISM OF OTA CARCINOGENICITY

A. Pfohl-Leszkowicz, V. Faucet-Marquis, M. Tozlovanu, F. Pont, P. Mantle, M. Castegnaro, and R. Manderville

P#3

EVALUATION OF ELISA AND HPLC-MS/MS METHODOLOGY FOR THE ANALYSIS OF 8-OXO-7,8-DIHYDRO-2'-DEOXYGUANOSINE IN SALIVA AND URINE

M.D. Evans, R. Singh, G.K. Hall, V. Mistry, T. Duarte, P.B. Farmer, and M.S. Cooke

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A. Dyrcz and J. Konopa

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J. Lewandowska, A. Bartoszek, and J. Konopa

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J. Zuo, C.S. Cooper, V.M. Arlt, and D.H. Phillips

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ENZYMES AND CONDITIONS USED FOR DNA HYDROLYSIS AFFECT THE RELEASE OF PROPANO ADDUCTS: COMPARISON OF LC-MS/MS USING COLUMN SWITCHING WITH ^{32}P-POSTLABELING METHODS
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EUROPEAN STANDARDS COMMITTEE ON URINARY (DNA) LESION ANALYSIS (ESCULA): TOWARDS CONSENSUS FOR THE MEASUREMENT OF URINARY 8-OXO-7,8-DIHYDRO-2'-DEOXYGUANOSINE
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DNA ADDUCT FORMATION CAPACITY AND INDUCTION OF OXIDATIVE STRESS BY THE CARCINOGENIC URBAN AIR POLLUTANT 3-NITROBENZANTHRONE AND ITS ABUNDANT ISOMER 2-NITROBENZANTHRONE, MEASURED *IN VITRO* AND *IN VIVO*
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PATERNAL EXPOSURES TO BENZO[A]PYRENE AND GENETIC RISK IN THEIR OFFSPRING

N. Verhofstad, R. Godschalk, J. van Benthem, H. van Steeg, and F.J. van Schooten

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ANALYTICAL CHARACTERIZATION OF AZANUCLEOSIDES AS NOVEL CHEMOTHERAPEUTIC AGENTS

D. Stach, F. Schmitges, and F. Lyko

Poster Abstracts

P#1

^{32}P-Postlabelling analysis of thymine dimer in urine from children of different skin types

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Formation of UV induced DNA lesions is associated with the development of different forms of skin cancer. The predominant lesion induced is a thymine-thymine (T=T) which following DNA repair can be released intact into the urine. A highly sensitive ^{32}P-postlabelling assay has been developed, and it was shown that the amount of excreted T=T in urine was directly correlated to the UV dose received. Epidemiological studies show that dark skin is highly protective against skin cancer. In this study we compared urinary T=T levels in children of different skin types after exposure to natural sunlight. Urine samples were collected from Swedish children of Caucasian origin and in black children from Guinea, West Africa. The T=T levels in the Guinean children did not differ dramatically from that of Swedish children in spite of the higher UV exposure in Africa. A preliminary assessment of the difference after correction for the different UV doses received by the two populations indicate that the amount of lesions in the African children were 1-2 orders of magnitude less than in the Swedish children. This protection factor of black skin is in line with what has been found when analysing DNA damage in skin earlier, and shows that other factors than just DNA damage are important to determine the risk of skin cancer.

P#2

Ochratoxin A (OTA)-DNA adduct detection as a useful biomarker of OTA exposure and for studies on molecular mechanism of OTA carcinogenicity

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Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin, suspected of being the etiological agent of Balkan endemic nephropathy (BEN) and associated urinary tract cancers. Conflicting results have been obtained regarding the genotoxicity of OTA and its ability to react directly with DNA upon oxidative bioactivation to yield covalent DNA adducts. This presentation gives an overview of data studying the metabolic pathways involved in OTA genotoxicity and the nature of DNA adduction observed in cells or tissues. By ^{32}P-Post-labelling technique, a dose- and time-dependent DNA adduct formation is observed *in vivo* (mice, rat, pig) and *in vitro* (human or opposum cells cultures; microsomes incubations). Use of several inducers or inhibitors of biotransforming enzymes (including cytochrome P450, cyclooxygenase, lipoxygenase, glutathione-S-transferase), demonstrated that OTA is biotransformed into genotoxic derivatives damaging for DNA. Authentic C8dG-OTA standards have been synthesised by photo-oxidation. Both of them (C-C8 & O-C8) comigrate on TLC with two adducts formed by *in vitro* incubation of OTA in the presence of kidney microsomes, and *in vivo* in kidney of pig or rodent fed OTA as well as in kidney and bladder tumour of humans naturally exposed to OTA. DNA-binding of OTA is related to formation of OTA-quinone derivatives, some of them being identified by mass spectrometry.

P#3

Evaluation of ELISA and HPLC-MS/MS methodology for the analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine in saliva and urine

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Background: 8-oxo-7,8-dihydro-2'deoxyguanosine (8-oxodG) has often been measured in urine as a marker of DNA oxidation, by immunochemical or chromatographic methods. Whilst ELISA is a frequently used means of assessing 8-oxodG in biological fluids, differences in baseline urinary 8-oxodG levels, compared to chromatographic techniques, has raised questions regarding the specificity of immunoassays. Recently, ELISA of salivary 8-oxodG has been used to report on periodontal disease.
Methods: Urine and saliva samples were collected from 30 healthy subjects (12 males, 18 females, age range 20-48) for analysis. Comparison of salivary and urinary 8-oxodG levels, using two commercially available ELISA kits, to high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), with prior solid phase extraction (SPE), was performed. The SPE/HPLC-MS/MS methodology was developed based on methodology reported by Lin *et al.* (H.S. Lin *et al.* (2004) *Biochem. J.* 380:541-548). Synthesis, purification and characterisation of [$^{15}N_5$]-8oxodG for HPLC-MS/MS was done in house. Urinary creatinine values were also determined by The Department of Clinical Biochemistry at Leicester Royal Infirmary.
Results: Salivary 8-oxodG values were obtained with both ELISA kits, although HPLC-MS/MS results for 8-oxodG in saliva approached the limit of detection for this assay. As the limit of detection for the HPLC-MS/MS procedure is much lower than ELISA, we concluded that the assessment of salivary 8-oxodG by ELISA is not accurate. In contrast to previous studies, ELISA levels of urinary 8-oxodG (1.67 ± 0.53 pmol/μmol creatinine) were within the range reported previously only for chromatographic assays, although still significantly different to HPLC-MS/MS (0.41 ± 0.39 pmol/μmol creatinine; p = 0.002). Furthermore, no correlation with HPLC-MS/MS was seen.
Conclusions: These results question the ability of ELISA approaches to specifically determine absolute levels of 8-oxodG in saliva and urine. On-going investigation in our laboratories aims to identify the basis of the discrepancy between ELISA and HPLC-MS/MS.

P#4

Urinary biomarkers of aflatoxin exposure in young children in Egypt and Guinea

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Chronic exposure to the fungal toxins aflatoxins (AFs) is considered to be a major risk factor in the development of primary liver cancer. In parts of Africa AF exposure is common and early life exposure could be a contributing factor towards the early onset of liver cancer in adulthood. This project assessed the level of exposure of children to aflatoxin in Egypt (predicted moderate aflatoxin exposure) and Guinea (predicted high aflatoxin exposure) by measurement of AF metabolites in urine. Aflatoxins were extracted from urine samples of 50 Egyptian (aged 1-1.5 years old) and 50 Guinean children (aged 2-4 years old) by C18 cartridges and aflatoxin immunoaffinity columns and analyzed by HPLC-fluorescence. AFB1, AFB2, AFG1, AFG2 and AFM1 were detected in children's urine and the identities of the aflatoxins were confirmed by spiking with aflatoxin standards and co-chromatography using different HPLC conditions. AF were less frequently present in Egyptian than Guinean children (38% Vs 86%) with statistically significant differences in prevalence for most of the detected toxins (AFB1 (2% Vs 16%, p= 0.016), AFB2 (10% Vs 58%, p= 0.000), AFG1 (4% Vs 2%), AFG2 (24% Vs 36%, p= 0.190) and AFM1 (8% Vs 64%, p= 0.000). For AFM1 the mean levels in Guinea were 18-fold higher than in Egypt. Worldwide there is a scarcity of urinary biomarker data for AF exposure in children. Overall AF exposure in Egypt is modest in comparison to Guinea though it should be noted that more of the Egyptian children in this study were still at least partially breast feeding, and thus may be protected against exposure due to the limited passage of AF into breast milk. These data would suggest that measures to reduce AF exposure in both regions are important although the situation is particularly pressing in Guinea where exposure is more prevalent and at higher levels.

P#5

Assessment of the relative contribution of exogenous and endogenously derived *N*7-(2-hydroxyethyl)guanine adducts in ethylene oxide treated rats

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Ethylene oxide (EO) is a widely used intermediate in the chemical industry and is also formed endogenously from the metabolism of ethylene, which is generated during normal physiological processes. EO reacts with DNA, primarily forming *N*7-(2-hydroxyethyl)guanine adducts (*N*7-HEG), which can be used as a biomarker of exposure and potential cancer risk. In order to accurately determine the increase in DNA damage caused by low exposures to EO we have established background and induced levels of *N*7-HEG adducts in 6-week old male F-344 rats (3/group) using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Endogenous *N*7-HEG was present in all tissues examined, ranging from 1.1-3.5 adducts/10^8 nucleotides. Following administration (i.p.) of a single or three daily doses of EO (0.01-1.0 mg/kg) *N*7-HEG adducts increased in a dose dependent manner, with the highest levels achieved in the liver. In the 3-day study, hepatic adduct levels were elevated 2h after the final dose, subsequently decreasing over time, with a second increase observed at 10h. Since a similar trend was observed in other tissues in both treated and control animals, this suggests a natural variation throughout the day in the production of endogenous adducts is responsible for the later increase. To examine the effects of lower EO concentrations, including doses that should attain blood levels several orders of magnitude lower than that produced after occupationally relevant exposures, ^{14}C-labeled EO was administered to rats (4/group) at (0.1-0.0001 mg/kg), for three consecutive days. This resulted in a dose dependent increase in tissue concentrations of ^{14}C-EO (~2-5000 fmols EO/mg liver). DNA samples extracted from tissues are currently being analysed for the presence of background and exogenous *N*7-HEG adducts by LC-MS/MS and accelerator mass spectrometry respectively, which will also allow determination of whether exogenous EO exposure modulates background levels of *N*7-HEG adducts. Funded by the American Chemistry Council (MTH0311-02).

P#6

DNA modification by 1-nitroacridine derivatives. Interstrand crosslinking in nuclear and mitochondrial DNA of tumor cells by 1-nitroacridine C-1748 and Nitracrine

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The significant role for biological activity of several antitumor compounds is their ability to induce DNA adducts and DNA crosslinking. Earlier studies proved that mitochondrial DNA (mtDNA) was much more susceptible to covalent modification than nuclear DNA (nDNA) by different xenobiotics, particularly DNA alkylating agents. The induction of interstrand mtDNA crosslinking was demonstrated only for few antitumor drugs. Our aim was to investigate the ability of 4-methyl-1-nitroacridine derivative C-1748, developed in our department, selected to phase I clinical trial, to form crosslinks in mtDNA of tumor cells and compare it with similar ability to bind nDNA. Compared to its parent analogue Ledakrin (Nitracrine), C-1748 is definitely less toxic to patients. Both compounds crosslink DNA after metabolic activation.All experiments were carried out on tumor cells of human colon cancer HCT-8. The mtDNA crosslinking were investigated with the electrophoretic Hartley's method and detected with the aid of specific molecular mtDNA probe in Southern hybridization. Whereas nDNA crosslinking were conducted with the usage of modified, radioactive Parsons method. Both 1-nitroacridines were able to induce mtDNA and nDNA crosslinks in a dose-dependent manner after 6 and 14 hours of cells' treatment. There was no significant difference between first crosslinking bonds which appeared in mtDNA and nDNA. We detected them for 0,5 μM of C-1748 concentration and 0,05 μM of Nitracrine. The level of crosslinking modification was much higher for mtDNA and gained 100 % for 5 μM of C-1748. We did not detect this for Nitracrine as it induced mtDNA fragmentation above 1 μM concentration. We observed also that Nitracrine was stronger and faster crosslinking agent than C-1748 derivative. To conclude, mtDNA can be the molecular target for both 1-nitroacridines.

P#7

Genotoxic effect of PAHs and their mixtures in THP-1 cells

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To find an appropriate in vitro model for studies on genotoxic potential of polycyclic aromatic hydrocarbons (PAHs) and their real environmental mixtures, human acute monocytic leukaemia cells THP-1 were incubated with individual

PAHs, their synthetic mixtures and the organic extract from particulate matter of the ambient air. We determined bulky DNA adducts by ^{32}P-postlabelling and the expression of p53 and p21^{WAF1} proteins by Western blotting. Out of 4 carcinogenic PAHs tested (benzo[a]pyrene, B[a]P; dibenzo[a,l]pyrene, DB[a,l]P; benz[a]anthracene, B[a]A; benzo[b]fluoranthene, B[b]F) the highest DNA adduct forming activity was observed for DB[a,l]P inducing more than 70 adducts/10^8 nucleotides at very low concentration of 10 nmol/l. The incubation of THP-1 cells with a synthetic mixture of 8 carcinogenic PAHs (c-PAHs) prepared proportionally to the content of individual c-PAHs in real environmental mixtures (B[a]P in concentrations of 0.1, 0.5 and 1 μmol/l was taken as a basis) resulted in significantly lower DNA adduct levels as compared with DNA adduct levels resulting from single components. For example, at B[a]P concentration of 0.1 μmol/l, 26-fold higher DNA adduct levels were observed than for c-PAH mixture containing equal B[a]P concentration. Even lower DNA adduct levels were induced by extractable organic matter (EOM) from ambient air indicating inhibition effect of various extract components. The results suggest a competition for the metabolic activation enzymes, their saturation and/or inactivation by PAH metabolites. Compared to other cell cultures used in genetic toxicology, THP-1 cells exhibited much lower DNA adduct levels induced by PAHs. We did not observed induced levels of both p53 and p21^{WAF1} proteins by any PAHs and mixtures. This finding is in agreement with our previous studies suggesting that certain minimum of DNA adduct levels should be reached to observe induction of these proteins. We concluded that due to their limited metabolic capacity THP-1 cells are not sensitive enough and therefore they are not suitable model system for in vitro testing of genotoxicity of PAHs and complex mixtures of organic air pollutants. *The work was supported by Czech ministry of Environment, grant No. VaV/740/05/03.*

P#8

DNA modification by 1-nitroacridine derivatives. Comparison of ^{32}P-Postlabeling and restriction enzyme analysis method for the detection of DNA adducts formed by 1-nitroacridines C-1748 and C-857

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4-Substituted 1-nitroacridines represent a new group of acridine derivatives synthesized at Gdansk University of Technology. Compared to parent 1-nitroacridines, these compounds exhibit lower toxicity and enhanced antitumor efficacy. The leading derivative 4-methyl-1-nitroacridine denoted C-1748 is being prepared for the I phase clinical evaluation towards prostate cancer. In the present study, we compare DNA binding properties of C-1748 and the parent 4-unsubstituted analogue C-857. Two methods were used in this study. ^{32}P-Postlabelling technique was employed for the detection of DNA adduct formation in cellular and cell-free systems. The DNA adducts were studied in HT29 (colon cancer) and LNCaP (prostate cancer) cells as well as in cell-free system employing DTT or microsomes for the activation of the compounds. C-1748 and C-857 gave rise to different patterns of 4-6 adducts spots. However, for a given compound the same DNA patterns were observed in all studied systems. Also, in cell-free system, the same DNA adduct patterns were found with using the different systems activation (DTT or microsomes). DNA modification level was 1 adduct per 10^4 nucleotides in the cells, and 1 adduct per 10^5 nucleotides in cell-free system. The second technique, developed by us, is a method which exploits restriction enzymes to the detection of covalent modification of specifically designed PCR-amplified DNA fragment. This amplicon includes two restriction sites recognized by enzymes: MspI (GC specific) and Tru1I (AT specific). The inhibition of cleavage was a measure of covalent modification of the restriction site(s) simultaneously pointing at the kind of base pairs involved in DNA binding. By this approach we were able to follow the kinetics of covalent DNA modification by C-1748 and C-857 and to determine their base pair specificity.

P#9

Seasonal variability in genotoxic potential of urban air particulate matter

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The main aim of this study was to compare genotoxic potential of organic extracts from urban air particles collected in various seasons in the center of Prague (Czech Republic). For this purpose we analyzed DNA adduct forming activity of organic extracts from urban air particles (PM10) in human hepatoma cell line HepG2. DNA adducts were analyzed by ^{32}P-postlabelling with nuclease P1 enrichment. Concentrations of PM10 were 36.9 μg/m^3, 39.0 μg/m^3, 62.6 μg/m^3 in summer, autumn and winter, respectively. Corresponding EOM contents were 5.0 μg/m^3 (13.9% of PM10), 6.7 μg/m^3 (17.2%) and 14.9 μg/m^3 (23.8%). The total DNA adduct levels induced by 10 μg EOM/ml were 17.7; 37.2 and 19.5 adducts/10^8 nucleotides in summer, autumn and winter, respectively. However, when the EOM quantities per m^3 were taken into the consideration summer sample exhibited 3-fold lower genotoxicity than those of autumn and winter, while difference between autumn and winter samples was not significant: 88.6, 249 and 291 adducts (in relative units), respectively. Higher concentrations of EOMs induce toxic effects. Although concentration PM10 and EOM content in

autumn were significantly lower than in winter, genotoxic potential of ambient air in autumn and winter was almost equal. There are significant positive correlations between B[a]P and c-PAH contents in EOM from various sampling periods and total DNA adduct levels detected in the EOM treated samples. These findings support hypothesis that B[a]P and c-PAH contents in EOMs are the most important factors for their genotoxic potential. Thus, estimation of the genotoxic potential of ambient air and prediction of health risk should be based mainly on concentration of c-PAHs and biological activity of extracts, while amount of particles and EOM content are not crucial determinants. Supported by Czech Ministry of Environment grant VaV-SL/5/160/05 and Academy of Science of the Czech Republic grant 1QS500390506.

P#10

Improvement of ^{32}P-Post-labelling method for the detection of Ochratoxin A-DNA adducts

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A recent EU-funded research programme on mechanisms of renal carcinogenicity of ochratoxin A (OTA) has addressed the genotoxicity debate because of dispute over the DNA adducts that have been demonstrated by ^{32}P-post-labelling methodology for the toxin in French laboratories in the past 20 years, and the failure to reproduce such evidence elsewhere. DNA adducts of some genotoxic chemical carcinogens are readily demonstrated in many laboratories, but demonstration of the low maximum incidence associated with exposure to ochratoxin A (~100/10^9 nucleotides) is dependent on adherence to strict protocols for all the stages of DNA extraction, purification and the complex steps and specific reagents in ^{32}P-Post-labelling. Demonstration of adducts in renal DNA from rats exposed to very small amounts of ochratoxin A, more representative of natural exposure than of the doses necessary for maximum expression, require an even greater attention to analytical detail. DNA from rats treated by OTA, synthetic dG-OTA adduct and DNA from rat treated by aristolochic acid have been analysed by ^{32}P-post-labelling technique (contact transfer or multidimensional) using different DNA extraction and chromatographic conditions. This allows us to demonstrate that the lack of OTA-DNA adduct detection by some teams is due to methodological problems, including incomplete hydrolysis and inappropriate solvent of migration.

P#11

DNA adduct formation by genotoxicants from *Brassicaceae*

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Plants have to defend themselves against infecting microorganisms and herbivores animals. We hypothesized that reactive chemicals released after damage of the plant would be effective against all kinds of enemies; some of these reactive molecules might also damage DNA. To test this hypothesis, we developed a simple test system: we homogenised plants, incubated the homogenate for varying times and then analysed the endogenous DNA – as a surrogate target – for the presence of adducts using the ^{32}P-postlabelling assay. Under these conditions, a time-dependent formation of adducts was detected with various plants. Here, we present data from *Brassicaceae* plants. Four major and some minor adduct spots were distinguished in Arabidopsis. We found a similar DNA adduct pattern in other *Brassicaceae* plants such as broccoli, cauliflower and rape, suggesting the occurrence of the same or similar genotoxic compounds in many members of this plant family. Subsequently, we treated mammalian cells in culture as well as bacteria (Ames's *Salmonella* strains) with plant homogenate. This led to the formation of the same patterns of adducts as detected in the endogenous DNA. Moreover, gene mutations were induced in these model systems. Finally, we conducted studies in human volunteers chewing broccoli. Whereas no adducts were detected in buccal mucosa cells taken before chewing raw broccoli, very high levels of *Brassicaceae*-typical adducts (up to 1800 per 10^8 nucleotides) were detected afterwards (at least for 9 h, the latest time point analyzed). Negligible levels of adducts were found when steamed rather than raw broccoli was consumed. We are conducting feeding experiments in laboratory animals with raw broccoli to determine the tissue distribution of the adduct formation. This work is financially supported by BMBF (grant PTJ-BIO/0313053A).

P#12

Markers of inflammation and aberrant CpG-island methylation as biomarkers of vulnerability to colorectal cancer

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The *adenoma-carcinoma sequence* leads to formation of localised precancerous lesions (adenomatous polyps). These events are associated with abnormalities of gene expression caused by specific mutations and epigenetic changes, notably aberrant CpG-island methylation. In our studies we are seeking to identify molecular abnormalities in the colorectal mucosa that can be used as biomarkers of susceptibility to neoplasia. The potential of sub-clinical levels of colorectal inflammation playing an important role in sporadic colorectal cancer has recently been examined (Erlinger et al., 2004). Furthermore, it appears that there may be a direct association between markers of systemic and colonic inflammation (Poullis et al., 2003). In the current study we have recruited 100 patients proven at endoscopy to be free of colorectal neoplasia and are recruiting a second group of 100 patients with a history of adenomatous polyps. We are recording anthropometric data (height, body-weight and waist and hip circumferences), physical activity via a lifestyle questionnaire, and nutritional data via a food frequency questionnaire and blood and urine analyses. C-reactive protein is being measured as a systemic marker of inflammation in serum (using hs-CRP) and faecal calprotectin and IL-6 as markers of colorectal inflammation. This should allow us to determine whether systemic inflammation (associated with high body-weight and adiposity) is linked with low-grade colorectal mucosal inflammation, and if this condition is associated with higher vulnerability to colorectal neoplasia. If proven, plasma and faecal markers of inflammation may be useable as surrogate biomarkers of early neoplasia. The methylation status of the promoter regions of a panel of genes will be quantified (Belshaw et al., 2004), and the relationship between inflammation and gene methylation investigated. This study will help define the links between colorectal inflammation and gene silencing through CpG-island methylation and determine whether the effects are related to diet, lifestyle and body fatness. This project is funded by the Food Standards Agency (Project No. N12016).

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P#13

Development of an LC-MS/MS method to measure DNA adducts formed by platinum based chemotherapeutic agents: role of intrastrand crosslinks in resistance

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Cellular resistance, both intrinsic and acquired, poses a problem in the effectiveness of platinum based chemotherapy. The cytotoxic activity of platinum based chemotherapeutic agents is derived from their ability to react with cellular DNA. Cisplatin and oxaliplatin bind to the N7 position of the purine DNA bases forming mainly intrastrand crosslinks between adjacent guanines (GG) (~ 65%) and between an adjacent adenine and guanine (AG) (~25%) and a small percentage of monofunctional adducts, DNA-protein adducts and interstrand crosslinks. Several mechanisms have been proposed to account for the resistance of some tumours to platinum based therapies including increased DNA repair, increased lesion tolerance, defective apoptosis response, increased deactivation by intracellular thiol containing molecules and decreased intracellular accumulation of the drug. We report the development of an LC-MS/MS method for measuring GG and AG intrastrand crosslinks formed by cisplatin and oxaliplatin and compare the formation and persistence of intrastrand crosslinks between cell lines known to have high and low resistance to these drugs. Wild type

and glutathione S transferase P1 null mice (GSTP1 null) were treated with oxaliplatin (10 mg/kg). No significant difference was observed in the level of intrastrand crosslinks formed by oxaliplatin between the mice strains in liver, kidney and lung DNA. Adduct levels were greatest in liver and lowest in lung tissue.

P#14

DNA adduct formation by diet induced endogenously formed nitroso compounds

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Red and processed - but not white meat and fish - are associated with colorectal cancer. The consumption of red and processed meat causes a dose-dependent increase in endogenously formed faecal nitroso compounds (ATNC) and these compounds may be the chemical link between red meat and cancer. Nitroso compounds (NOCs) are known carcinogens and can cause the formation of DNA adducts. Recently, we showed a link between meat consumption, faecal ATNC and the NOC-specific DNA adduct ^{6}O-carboxymethyl-2'-deoxy-guanosine (^{6}O-CMG) (LEWIN *et al.*, 2006). To elucidate this link further, it is important to identify these compounds, to investigate their formation and mode of action *in vivo*. In a controlled study, twelve healthy volunteers were fed three different diets containing different amounts of red meat and fibre (vegetarian, high red meat and high fibre, high red meat). Faecal samples were collected and analysed for total nitroso compounds, nitrosyl iron, nitroso thiols and haem content. Approximately 60% of the total amount of nitroso compounds formed following a meat diet consisted of nitrosyl iron compounds, approximately 30% of nitrosothiols; by difference, *N*-nitroso compounds only contribute 10%. ESR spectra showed that nitrosylated haem, e.g. nitroso-haemoglobin or nitroso-myoglobin, are found in faecal homogenates following a high meat diet. Nitrosylated and non-nitrosylated haem may facilitate the formation of pro-mutagenic adducts such as ^{6}O-CMG in the human gut.

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P#15

Influence of metabolic modifiers on the DNA adduct formation of 1-hydroxymethylpyrene and 1-hydroxymethyl-8-methylpyrene in the rat

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Some alkylated polycyclic aromatic hydrocarbons (alk-PAH), such as methylpyrenes, are much more abundant in the environment than the reference PAH benzo[*a*]pyrene. Two of these compounds, 1-methylpyrene and 1,8-dimethylpyrene, are converted via benzylic hydroxylation and subsequent sulfonation to the corresponding reactive sulfuric acid esters, 1-sulfooxymethylpyrene and 1-sulfooxymethyl-8-methylpyrene, which are mutagenic and form DNA adducts *in vitro* and *in vivo*. Sulfonation of the benzylic alcohols 1-hydroxymethylpyrene (1-HMP) and 1-hydroxymethyl-8-methylpyrene (1-HM-8-MP) as the critical activation step carried out by sulfotransferases competes with other reactions like oxidation by alcohol and aldehyde dehydrogenases that convert benzylic alcohols to stable, excretable carboxylic acids thus mediating an important detoxification reaction. To study the influence of several metabolic modifiers (pentachlorophenol, quercetin, 4-methylpyrazole, ethanol and disulfiram) on sulfotransferase-mediated bioactivation of 1-HMP and 1-HM-8-MP male Wistar rats received a concurrent treatment with the benzylic alcohol and one of the metabolic modifiers, whereas control animals received the benzylic alcohol and the solvent only. DNA isolated from liver and kidney was then analysed for DNA adducts by ^{32}P-postlabelling and DNA adduct levels of treatment and control groups were compared. Initial results demonstrate clear effects of the applied modifiers on DNA adduct levels caused by 1-HMP and 1-HM-8-MP due to alterations of the sulfotransferase-mediated bioactivation to reactive DNA binding species. A concurrent treatment of rats with benzylic alcohols and pentachlorophenol or quercetin, respectively, led to a decrease in DNA adduct levels compared to rats that only received the benzylic alcohol due to inhibition of sulfotransferase activity. In contrast, a concurrent treatment of rats with benzylic alcohols and 4-methylpyrazole, ethanol or disulfiram, led to increased DNA adduct levels due to inhibition of the oxidative detoxification pathway. Thus we conclude that the toxicological effects of some alkylated polycyclic aromatic hydrocarbons may be strongly affected by interaction with other chemicals that might be components of human nutrition (ethanol, quercetin) or used as drugs (4-methylpyrazole).

P#16

Reduction of oxidative DNA damage by a anthocyanin/polyphenolic rich fruit juice in an intervention study with patients on hemodialysis

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Background: Patients with chronic renal failure undergoing hemodialysis (HD) are considered to face an elevated risk for cancer, artheriosclerosis and other diseases. This has been attributed in part to increased oxidative stress, resulting from bioincompatibility of the extracorporal blood circuit, malnutrition and other factors. Uptake of fruit juice with especially high flavonoid/polyphenol content has been shown to reduce oxidative cell damage in healthy probands (Weisel et al. Biotechnol. J., 1, 388, 2006) and might also be utilized as preventive measure in HD-patients.
Methods: An intervention study is performed with mixed fruit juice (total phenols: 3478 mg/L). After a three week run-in phase, 20 HD-patients (non-smokers, stable HD) consume for 4 weeks 200ml/d of the juice, followed by a three week wash-out phase. Blood sampling is performed weekly. DNA damage (COMET assay with/without formamidopyrimidine-DNA-glycosylase) and glutathione (kinetic photometric assay) in whole blood are monitored. Additionally, lipid peroxidation (HPLC/fluorescence of malondialdehyde) and the trolox equivalent antioxidant capacity (TEAC) are determined in plasma; DNA binding capacity of the transcription factor NfκB (ELISA) is monitored in primary blood mononuclear cells.
Results: First results (obtained from 13 patients) show a strong decrease of total DNA damage (with FPG) during juice uptake (mean TI%: run-in: 5.3/juice uptake: 3, $p<0.0005$; juice uptake: 3/wash-out:, $p<0.0005$), basic DNA damage (without FPG) was also reduced (mean TI%: run-in: 0.61/juice uptake:0.51, $p<0.05$; juice uptake: 0.51/wash-out: 0.44, $p=0.052$). Correspondingly, an increase of total glutathione and of glutathione status was observed during the intervention, whereas modulation of the other markers was not apparent at present.
Conclusions: The red fruit juice clearly shows a marked potential to reduce (oxidative) DNA damage in HD-patients. This effect can bona fide be attributed to its high content of flavonoids/polyphenols. (Supported by K. Nolte Foundation)

P#17

***CYP1A1* up-regulation in MCF-7 cells following benzo[*a*]pyrene treatment occurs primarily during S-phase: a significant modulator of consequent genotoxicity**

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Pro-carcinogens, e.g., benzo[*a*]pyrene (B[*a*]P), may act as exogenous ligands of the aromatic hydrocarbon receptor and through this interaction modulate the susceptibility of target cell populations via the receptor-mediated up-regulation of cytochrome P450 (*CYP*) mixed function oxidases. Using an approach to concentrate oestrogen-receptor positive breast carcinoma MCF-7 cells in different phases of the cell cycle whilst in culture, we examined whether this might serve to determine the level of B[*a*]P-induced up-regulation of *CYP1A1*, *CYP1A2* or *CYP1B1*, and if this could then influence subsequent levels of DNA damage measurable either as single-strand breaks (SSBs) or B[*a*]P-DNA adducts. Cell cultures were manipulated to be G_0/G_1-phase concentrated, S-phase concentrated, or G_2/M-phase concentrated prior to being treated with B[*a*]P and the expression levels of *CYP1A1*, *CYP1A2*, *CYP1B1*, *CDKN1A* ($P21^{WAF1/CIP1}$), *BCL-2*, and *BAX* levels were determined using quantitative real-time RT-PCR. Levels of DNA damage were measured as DNA SSBs by the alkaline single cell-gel electrophoresis (comet) assay or as B[*a*]P-DNA adducts by ^{32}P-postlabelling analysis. B[*a*]P-induced up-regulation of *CYP1A1* was >100-fold in S-phase concentrated cells, but in G_0/G_1-phase or G_2/M-phase concentrated cultures up-regulation occurred to a significantly lower extent; in fact, CYP1A1 protein was undetectable in G_0/G_1-phase concentrated cultures by immunoblot analyses of whole-cell lysates with or without 24-h B[*a*]P treatment in contrast to a distinct band associated with S-phase concentrated cells. Consistent with this, B[*a*]P-treated S-phase concentrated cultures exhibited markedly up-regulated $P21^{WAF1/CIP1}$, higher levels of dose-related increases in DNA SSBs, and increased DNA adduct levels presumably as a result of CYP1A1-mediated activation of B[*a*]P to B[*a*]P-diol-epoxide compared with the cultures enriched for the other cell cycle phases. Whether cells cycle faster or are more quiescent *in vitro* may pre-determine susceptibility to activation of exogenous pro-carcinogens in short-term test systems and such findings have important implications when assessing risk to a particular target cell population *in vivo*.

P#18

DNA adduct formation by benzo[a]pyrene in target and non-target mouse organs

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DNA adduct formation is an early crucial event in the process of carcinogenesis by which many chemical carcinogens exert their biological effects. Correlations between DNA binding in target tissues and carcinogenic potency for some carcinogens are well established. However, it is more complex when considering target and non-target tissues and it is often not possible to predict accurately the target organs in humans from animal studies. This study has used the ^{32}P-postlabelling method to measure DNA adduct levels in Balb/c mice after exposure to the carcinogen benzo[a]pyrene (BaP) and the non-carcinogen benzo[e]pyrene (BeP) (125 mg/kg body weight/day by gavage). Target organs lung, spleen, forestomach and non-target organs liver, colon and glandular stomach were removed after 5 and 10 days of treatment, respectively. DNA adduct analysis showed that BaP forms DNA adducts time-dependently whereas BeP does not form adducts. Levels of BaP-DNA adducts were in the order spleen = liver > lung > colon = glandular stomach > forestomach. No significant difference in DNA adduct formation was seen between male and female mice. Collectively, DNA adduct levels were not significantly different between target and non-target organs. Our results suggest that events subsequent to DNA damage and mutations may be important in determining the organotropism of chemical carcinogens. Therefore, in future studies cDNA microarray will be used to identify critical changes in gene expression in mouse organs that may distinguish between target and non-target tissues for tumour formation.

P#19

Levels of the DNA adduct, N7-methyldeoxyguanosine, are associated with increased risk of failure of treatment of cervical intraepithelial neoplasia

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After conservative treatment of cervical intraepithelial neoplasia (CIN), the risk of invasive cervical cancer among these women is about five times greater than that among the general population of women. Treatment failure has been associated with numerous factors including persistent HPV infection and smoking. As tobacco specific nitrosamines are more prevalent in cervical mucus samples from smokers than non-smokers we have investigated whether exposure to these agents, as measured by N7-methyldeoxyguanosine (N7-MedG) levels in cervical DNA, are higher in those women who experience treatment failure than those who do not. 958 women attending for colposcopic examination after abnormal cervical smear test results were recruited into the cohort. Information on demographic factors, smoking and other risk factors was obtained and a pre-treatment biopsy was taken and stored at –70^{o}C. After follow-up, cases who had treatment failure of CIN within two years following treatment were identified (n=77) and matched to women with no treatment failure of CIN in this time period (controls, n=154). DNA was extracted from the pre-treatment biopsies and N7-MedG levels, were quantified as the ring-opened form of the base damage by a validated immunoslotblot assay. Sufficient DNA for N7-MedG analysis was extracted from 61 subjects corresponding to 20 matched case control pairs. N7-MedG was detected in cervical DNA with levels ranging from non-detected (<0.1μmol/mol dG) to 4.83 μmol/mol dG. N7-MedG levels were significantly higher in cases (geometric mean 0.99 μmol/mol dG) than controls (0.33μmol/mol dG; p=0.01). Log N7-MedG content, after adjustment for HPV status at time of treatment, was found to be significantly associated with increased risk of treatment failure (OR 5.74, 95% CI 1.05-31.23). The association between pre-treatment levels of DNA damage induced by methylating agents and subsequent treatment failure implicates methylating agent exposure as a causative factor in treatment failure.

P#20

N7-methyldeoxyguanosine (N7-MedG) levels in DNA from colorectal adenoma cases and matched referents

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The risk of developing colorectal cancer is influenced by dietary factors such as eating red meat but the mechanisms behind the variability in risk remain to be determined. Exposure to genotoxic carcinogens may be important as colon DNA contains damage induced by different carcinogens. Exposure to alkylating agents may be one such environmental determinant of colorectal cancer risk. If alkylating agent exposure is important, it is likely that the critical period is during the growth of a small adenoma into a large adenoma as this is when a key mutational event, often linked to alkylating agent exposure, occurs (i.e. a GC→AT transition mutation in *K*-ras). We have therefore measured, as an indicator of alkylating agent exposure, N7-methyldeoxyguanosine (N7-MedG) levels in colorectal DNA, in the normal tissue of patients with and without colorectal adenomas. Biopsies of normal colorectal mucosa were collected during colonoscopy from 85 patients with histologically proven colorectal adenomas (cases) and 85 patients free of gastrointestinal neoplasia (referents) matched by age, gender and biopsy location. N7-MedG levels were measured in DNA by a validated immunoslotblot procedure. N7-MedG levels were determined in DNA from 73 of the case-referent pairs. The majority of colorectal mucosal DNA samples contained detectable levels of N7-MedG (89% of cases and 93% of referents). Levels ranged from <0.1 to 7.7 in cases and <0.1 to 4.4 fmoles/ μg DNA in referents. N7-MedG levels were not associated with gender, age, the size of the adenoma, or with current smoking status (data not shown). Cases and referents had similar DNA-N7-MeG levels. N7-MedG levels were not associated with the presence of an adenoma suggesting that methylating agents are unimportant in colorectal adenoma risk. However, as human exposure to methylating agents is widespread, factors downstream of exposure such as DNA repair may be more important in influencing risk.

P#21

DNA adducts *versus* urinary metabolites of polycyclic aromatic hydrocarbons in healthy non-smoking females from Poland and Serbia

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Beside occupational exposure, dietary intake seems to be the most important source of PAHs and the main objective of the project was to trace the origin of high background levels of PAH-type of DNA adducts found in certain non-smoking individuals in the general population, while taking possible modulation by genetic factors and diet into consideration. The study population was 100 non-smoking women in each of the countries of Poland, Serbia and Italy. For all subjects, interviews about lifestyle and diet were performed, as well as genotyping of relevant detoxifying and DNA repair enzymes, determination of levels of PAH-DNA adducts and oxidative damages in DNA from white blood cells. Intake of PAHs was monitored by measurement of urinary PAH metabolites. DNA adduct levels were determined through sensitive dinucleotide/monophosphate version of the ^{32}P-postlabelling assay followed by TLC separation of 5´-labelled adducted monophosphates. Studies examining high exposure to PAH from smoking or occupation have shown that levels of urinary PAH metabolites as well as PAH-type of DNA adducts correlate with exposure to PAHs. However, contradictory results considering low exposure from air pollution have been found. Preliminary data from the women from Poland and Serbia showed that there was no correlation between levels of bulky DNA adducts and urinary PAH metabolites. The mean adduct level was statistically different between the two populations and correlated with the mean level of urinary PAH metabolites. Surprisingly, in both populations the 10 individuals with the highest adduct levels had very low levels of urinary metabolites and visa versa. Currently we can not explain these observations.

P#22

Urinary 1-hydroxypyrene as a biomarker of polycyclic aromatic hydrocarbon exposure in the general population and correlation between 1-hydroxypyrene and white blood cell DNA adducts - Results of our literature survey

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Urinary 1-hydroxypyrene (1-OHPY) is a widely used surrogate marker for biomonitoring of complex polycyclic aromatic hydrocarbon (PAH) exposure of humans. In the present study our interest was on two specific areas of the 1-OHPY literature, that is, 1-OHPY levels in the general population including comparison between adults and children, and the correlation between 1-OHPY and bulky DNA adduct level in white blood cells (WBC). The survey covers 32 papers published between 1988 and 2006. Urinary 1-OHPY levels were in the range of 0.03 and 1.16 μmol/mol creatinine in adults and between 0.08 and 0.74 in children. Urban residence was associated with significantly higher 1-OHPY level as compared to rural residence in both adults and children. 1-OHPY concentrations were higher in smokers than in non-smokers. ETS did not increase 1-OHPY concentration in children significantly. Significant age-dependent difference was observed recently by Huang et al (2006), in which 6-12 years old children had higher 1-OHPY levels than adolescents and adults. Females tended to have higher 1-OHPY levels than males, however, the effect was not significant. High dietary PAH exposure enhanced 1-OHPY excretion. The relationship between 1-OHPY and bulky DNA adducts was predominantly reported on occupational study populations. In some studies statistically significant correlation was found between 1-OHPY and WBC DNA adduct levels when the subjects were stratified for particular genetic polymorphisms, especially in coke oven workers with *CYP1A1* 462Val variant allele (Pan et al, 1998), in incinerator workers (Lee et al, 2002) and in aluminium plant workers (Schoket et al, 2001) with *GSTM1* homozygous deletion. The research project has been supported by the EU FP6 ECNIS NoE No. 513943.

P#23

Method development for ^{32}P-postlabeling of N7-guanine DNA adducts

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Detection and analysis of covalent DNA modifications is of great importance because a large body of evidence demonstrates that these DNA adducts are useful markers of carcinogen exposure, providing an integrated measurement of carcinogen intake, metabolic activation, and delivery to the DNA in target tissues. Monitoring DNA adducts also provides a means of investigating the correlation between occupational or environmental exposure in healthy individuals and cancer risk. The N7-position of guanine is the most nucleophilic position within the heterocyclic bases of DNA therefore the predominant reaction of many electrophilic compounds involves covalent bond formation at this site. These compounds include many structurally diverse alkylating agents like nitrosamines, epoxides and aflatoxins. The ^{32}P-postlabeling method is based on enzymatic hydrolysis of DNA to deoxyribonucleoside-3'-monophosphates with micrococcal nuclease and spleen phosphodiesterase. N7-guamine adducts are then enriched by an anion exchange chromatography step and subsequently converted to 5'-^{32}P-labeled 3',5'-bisphosphates by enzymatic derivatization involving T4-polynucleotidkinase. Emphasis of the method development is placed on glycidamide, the ultimate genotoxic product of acrylamide. The neurotoxic and probable carcinogen compound acrylamide was first reported in cooked foods in 2002, including bread, french fries and breakfast cereals. In animal models, exposure to acrylamide leads to DNA damage and, at high doses, neurological and reproductive effects. The International Agency for Research on Cancer has classified acrylamide as "probably carcinogenic to humans". Acrylamide is metabolized *in vivo* by CYP450 E1 to glycidamide which then forms DNA adducts at the N7 position of guanine and N1 position of adenine. Method development therefore includes the preparation of adduct standards, testing of postlabeling and enrichment conditions as well as development of different separation conditions using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Preliminary results show that separation from normal nucleotides is possible using a two-dimensional TLC system but adducts and normal nucleotides could not be separated using HPLC.

P#24

Separation and detection of fluorescence labeled RNA modifications by capillary electrophoresis with laser-induced fluorescence detection

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We investigated the separation and detection of the 5'-monophosphates of ribonucleosides selectively conjugated with BODIPY-FL-EDA (4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl ethylene diamine hydrochloride) at the 5'-phosphate group using capillary electrophoresis with laser-induced fluorescence detection (CE-LIF). BODIPY conjugates of the four common nucleoside-5'-monophosphates (guanosine-5'-monophosphate, adenosine-5'-monophosphate, cytidine-5'-monophosphate and uracil-5'-monophosphate) were prepared and subjected to CE-LIF to serve as standard compounds for peak assignment and to develop separation conditions for the analysis of RNA. Also, two products arising from the deamination of the purinic nucleosides (inosine-5'-monophosphate and xanthine-5'-monophosphate) were included in the above procedure. All BODIPY conjugates were detected and resolved by CE-LIF after digestion of RNA or oligonucleotides to 5'-monophosphates by nuclease P1 and fluorescence labeling without further purification step. Comparison with two previously developed assays with BODIPY-FL-EDA postlabeling for analysis of 2'-deoxynucleoside-3'- and -5'-monophosphates showed that all three versions were equally efficient and sensitive. Moreover, using our new assay we were able to analyze and quantify pseudouracil in samples from different types of RNA and RNA from different organisms. This indicates that fluorescence postlabeling of nucleoside-5'-monophosphates after digestion of RNA with nuclease P1 has the potential to measure naturally occurring modified bases and modifications from exogenous sources in RNA.

P#25

Urinary excretion of ethenoadenine and ethenocytosine by smokers and nonsmokers

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Tobacco smoking induces oxidative stress and lipid peroxidation resulting in the *in vivo* formation of promutagenic etheno DNA adducts. Etheno adducts are repaired by both base excision and nucleotide excision repair processes and excreted in urine as etheno-modified bases and nucleosides, respectively. It has been reported that smokers excrete elevated concentrations of $1,N^6$-ethenoadenine (etheno-A), $3\text{-}N^4$-ethenocytosine (etheno-C), $3\text{-}N^4$-ethenodeoxycytidine (etheno-dC), and $1,N^2$-ethenoguanine in urine. However, the relationship between urinary etheno adduct excretion and exposure to tobacco smoke has not been established. The excretion of both etheno-modified bases and nucleosides have the potential to serve as biomarkers of exposure to tobacco smoke and may also reflect oxidative stress and lipid peroxidation stemming from such exposure. An analytical method using gas chromatography with mass spectrometry (GC-MS) for the determination of etheno-C and etheno-A in human urine was developed and validated. This method was applied to the analysis of urine samples from 79 smokers and 24 nonsmokers collected under real-life conditions. Mean (± standard deviation) concentrations of etheno-C in urine were significantly increased in smokers compared to nonsmokers (0.35 ± 0.62 *vs*. 0.08 ± 0.08 nM; $p < 0.05$). Concentrations of etheno-A in urine, although higher in smokers compared to nonsmokers (0.57 ± 0.69 *vs*. 0.35 ± 0.20 nM), were not statistically significantly increased. Etheno-C excretion was also correlated with the molar sum of six urinary nicotine metabolites ($r = 0.228$; $p = 0.054$), but not with the number of cigarettes smoked per day ($r = 0.137$; $p = 0.250$), salivary cotinine ($r = 0.201$; $p = 0.091$), and CO in exhaled breath ($r = 0.150$; $p = 0.208$). Etheno-A excretion in urine was not correlated with any biomarker of smoking dose. We conclude that urinary etheno-C excretion may be a suitable biomarker for assessing smoking-related oxidative stress and lipid peroxidation.

P#26

Analysis of background DNA adducts in L5178Y mouse lymphoma cells by LC-MS/MS for the investigation of the biological significance of chemically-induced DNA damage

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The biological significance of DNA adducts for mutation and cancer could be investigated on the basis of exposure-related increments over background. For this purpose, analytical techniques are required for sensitive and specific quantification of background DNA adduct levels. Liquid-chromatography triple-quadrupole mass-spectrometry methods were developed and validated for the analysis of the 2'-deoxyribonucleoside adducts O^6-methyl-2'-

deoxyguanosine (O^6-mdGuo), 8-oxo-2'-deoxyguanosine (8-oxodGuo), 1,N^6-etheno-2'-deoxyadenosine (εdAdo) and for the purine adduct 7-methylguanine (7-mG). These adducts are formed both endogenously and exogenously by DNA methylation, direct oxidation of DNA, and reaction of DNA with lipid peroxidation products. O^6-mdGuo was synthesized as standard, deuterated O^6-[$^2H_3$]mdGuo was synthesized as internal standard. Using an on-line sample preparation with column switching, O^6-mdGuo, 8-oxodGuo, and εdAdo could be determined simultaneously with minimum workup after enzymatic hydrolysis of DNA samples. The limits of quantification (LOQ) for O^6-mdGuo, 8-oxodGuo, and εdAdo were 24, 98, and 48 fmol on column (o.c.), respectively. The analysis showed linearity in the range 0.24 to 125 pmol/ml, 0.98 to 125 pmol/ml, and 0.48 to 62.5 pmol/ml for the three adducts, respectively. The inter-day precision at 10-fold LOQ was 4.7% for O^6-mdGuo, 13.4% for 8-oxodGuo, and 5.9% for εdAdo. 7-mG was analyzed in a separate LC-MS/MS assay after neutral thermal depurination from DNA. The LOQ was 30 fmol o.c., the dynamic range was 0.03 to 30 pmol o.c., the inter-day precision at 10-fold LOQ was 3.9%. The method was applied to DNA isolated from large batches of L5178Y untreated mouse lymphoma cells. In samples of 450 μg DNA, O^6-mdGuo, 8-oxodGuo, εdAdo and 7-mG showed levels of 38, 850, 300 and 1200 adducts per 10^9 normal nucleotides, respectively. The data can now be used as a baseline for dose-response relationships and correlations with other in vitro endpoints of genotoxicity and mutagenicity available for mouse lymphoma cells (refer to oral presentation by W.K. Lutz).

P#27

Organospecific upregulation of 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate pyrophospho-hydrolase activity of mouse MTH1 protein by ionizing radiation-induced oxidative stress

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Mammalian MTH1 protein is an antimutagenic enzyme that prevents the incorporation of oxidatively modified purine nucleotides into nucleic acids. It decomposes a broad spectrum of 2'-deoxyribonucleoside and ribonucleoside 5'-triphosphates (dNTPs and NTPs) to the corresponding nucleoside 5'-monophosphates and inorganic pyrophosphate. Although canonical dNTPs and NTPs are also substrates for MTH1 protein, the enzyme decomposes most specifically the miscoding products of oxidative damage to purine dNTPs and NTPs (e.g. 8-oxo-dGTP, 8-oxo-dATP, 2-OH-dATP, 2-OH-ATP, 8-oxo-GTP) that may cause point mutations or transcription errors after the incorporation into DNA and RNA, respectively. There is a growing evidence for an overexpression of *MTH1* gene in the cells/tissues that undergo oxidative stress. Therefore, the increased expression of MTH1 mRNA and MTH1 protein was proposed to be a new molecular marker of oxidative stress. To test whether oxidative stress can indeed affect MTH1, we investigated the influence of ionizing radiation (source of oxidative stress) on the MTH1 8-oxo-dGTPase activity in mouse organs. Male BL6 mice, 6-wk old, were subjected to 0, 10, 25, 50, and 100 cGy of 137Cs gamma-radiation, 100 cGy/min, given in a single dose per whole body, and killed 4, 8, and 24 hrs later (three mice per each dose/kill time combination). The 8-oxo-dGTPase activity measurements were performed in 6 mouse organs. A clear induction of 8-oxo-dGTPase was noticed in mouse brains, testes, and kidneys, whereas lungs, hearts, and livers did not reveal any statistically significant effects of applied gamma radiation doses within 24 hours. Mouse brains, which demonstrated most dramatic 4.3-fold up-regulation of 8-oxo-dGTPase activity, were also shown to have significantly higher level of MTH1 protein as revealed by SDS-PAGE Western blot analysis. Our results indicate that comparative measurements of MTH1 8-oxo-dGTPase activity might be used as a marker of oxidative stress in certain types of cells or tissues.

P#28

Development and statistical analysis of the mouse lymphoma thymidine kinase locus gene mutation assay for correlations between DNA adducts and mutant frequency

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The L5178Y $tk^{+/-}$ mouse lymphoma assay is an established in vitro test for the induction of forward mutations. It is based on the selection of $tk^{-/-}$ mutants on the basis of their resistance to the toxic DNA precursor trifluorothymidine. The assay was adapted to large batches of cells, in order to get a sufficient amount of DNA for a concurrent adduct analysis by LC-MS/MS. Using this large scale, we (i) tested whether cleansing from background mutants resulted in improved sensitivity to low dose, (ii) defined the optimum time window for treatment after cleansing, and (iii) calculated confidence limits (CI) for the estimation of mutant frequencies based on the 96-well microtiter plate version. Results. (i) Cleansing clearly increased the sensitivity; treatment with 20 μM MMS immediately after cleansing resulted in a significant 2.9-fold increase in mutant frequency whereas no significant effect was observed in an uncleansed cell

population. (ii) The time window available for the investigation of small effects spanned 1 week after cleansing. Afterwards, the number of spontaneous mutants increased and sensitivity decreased. (iii) CI of the probable number of clones per well were calculated for all possible numbers of empty wells (EW). The minimum CI was seen for 21 EW. Based on 2000 plated cells per well, as recommended for mutation plates, the estimated cloning efficiency was [– ln (21/96)]/2000 = 0.000760, i.e. 760 per 10^6, with a 95% CI of 578 to 980 per 10^6. The ratio between the upper and the lower limits of the CI took values <1.7, <1.8, <1.9, and <2.0 for the ranges of EW 18-25, 8-44, 5-53, and 4-59, respectively. In order to minimize the statistical error, we consider it advisable to avoid large numbers of EW by adjusting the number of cells per plate.

P#29

Detection of phosphodiester adducts formed by the reaction of benzo[a]pyrene diol epoxide with 2′-deoxynucleotides using collision induced dissociation tandem mass spectrometry.

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To date the potential of polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (B[a]P) to form phosphor-triester adducts in DNA has not been clearly ascertained. Studies with alkylating agents have shown that phosphotriester adducts represent long lived biomarkers of exposure due to lack of efficient repair and it has been postulated that their formation may ultimately lead to adverse biological consequences, such as DNA strand breaks. PAHs are carcinogens that are ubiquitous environmental pollutants and are present in cigarette smoke, vehicle exhaust fumes as well as charbroiled food. B[a]P undergoes cytochrome P450 mediated metabolism *in vivo* resulting in the formation of benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (B[a]PDE), the ultimate reactive species that reacts with nucleophilic sites in DNA. In this study we investigated the products formed following the *in vitro* reactions of B[a]PDE with 2′-deoxynucleoside 3′-monophosphates of guanine, adenine, cytosine and thymine. The B[a]PDE plus 2′-deoxynucleotide reaction mixtures were purified using solid phase extraction (SPE) and subjected to HPLC with fluorescence detection. Fractions corresponding to reaction product peaks were collected and desalted using SPE. The purified reaction products were analysed for the presence of molecule ions corresponding to *m/z* 648, 632, 608 and 623 $[M-H]^-$ consistent with B[a]PDE adducted 2′-deoxynucleotides (either on the base or phosphate group) of guanine, adenine, cytosine and thymine, respectively, using liquid chromatography-negative electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) collision induced dissociation (CID). Reaction products were identified having CID product ion spectra characteristic of B[a]PDE adduct formation with the base of the 2′-deoxynucleotide. Product ions were observed at *m/z* 452 (guanine), 436 (adenine) and 412 (cytosine) corresponding to $[B[a]P\text{-triol+base-H}]^-$, resulting from cleavage of the glycosidic bond between the 2′-deoxyribose and the B[a]PDE adducted base. Further reaction products were identified having unique CID product ion spectra characteristic of B[a]PDE adduct formation with the phosphate group of the 2′-deoxynucleotide. The presence of product ions at *m/z* 399 and 497 was observed for all four 2′-deoxynucleotides, corresponding to $[(B[a]P\text{-triol+phosphate})\text{-H}]^-$ and $[(2'\text{-deoxyribose+phosphate+B[a]P-triol})\text{-H}]^-$, respectively. The absence of product ions in the spectra corresponding to B[a]PDE adducted bases provided further confirmation of the formation of phosphodiester adducts. In conclusion this work provides the first direct evidence for the formation of phosphodiester adducts by B[a]PDE and it is anticipated that similar reactions occur in DNA resulting in the formation of phosphotriester adducts.

P#30

Investigations on the mutation signature of 3-nitrobenzanthrone in the human p53 sequence

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To test hypotheses on the origins of p53 mutations in human tumors, novel strategies are needed for generating mutation spectra experimentally. Recently, an assay employing Hupki (Human p53 knock-in) mouse embryonic fibroblasts (HUFs) has been developed. Utilizing this assay, we examined p53 mutations induced by 3-nitro-benzanthrone (3-NBA), a carcinogen and environmental pollutant. Seven immortalized cultures (cell lines) from 26 HUF primary cultures exposed at passage 1 for 48 hrs to 7.5 µM 3-NBA harboured mutations in the human DNA binding domain sequence of the Hupki p53 tumor suppressor gene or its splice sites. DNA was isolated, amplified and sequenced via the dye-terminator method to reveal manifest point mutations in the coding region of exons 4–9. Preliminary results indicate mostly A to G transitions, next to mutations of the splice site preceding exon 8. The mutations found at codons 205, 259 and the splice site have not been observed in previous experiments. Apart from point mutations in the coding region (e.g. p53), a tumor suppressor can be inactivated through hypermethylation of the

promoter region, which silences the gene (e.g. RASSF1A). This often coincides with genome-wide DNA hypo-methylation. Thus, a second focus is on the DNA methylation status of the HUF cell-lines. To measure the degree of methylation, DNA was hydrolyzed to 3'-nucleotides, derivatized with a fluorescent dye and analyzed through micellary electrokinetic capillary chromatography with laser-induced fluorescence detection (CE-LIF). The degree of DNA methylation in HUF cell lines that were mutant in p53 was significantly lower (3.8%) than that of primary cultures (4.0%, $p<0.001$), while that of cell lines wild-type in p53 was even lower (3.6%, $p<0.001$).

P#31

Enzymes and conditions used for DNA hydrolysis affect the release of propano adducts: Comparison of LC-MS/MS using column switching with ^{32}P-postlabeling methods

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4-Hydroxynonenal-2'deoxyguanosine (HNE-dGuo) propano adducts are promutagenic DNA lesions originating from free radical-induced lipid peroxidation of polyunsaturated fatty acids. A sensitive LC-MS/MS method was developed to measure propano adducts, using online sample clean-up by column switching coupled with tandem mass spectrometry. Although high amounts of DNA (250 µg) were hydrolyzed and injected we were not able to confirm the levels of HNE-dGuo previously estimated by ^{32}P-postlabeling. We hypothesized that incomplete DNA hydrolysis to 2'-deoxyribonucleosides by the nuclease P1 (NP1) and alkaline phosphatase (AP) digestion system used might be responsible. Therefore, we investigated the effect of different DNA digestion systems on the release of HNE-dGuo and the normal 2'-deoxyribonucleosides. The enzymes tested were DNAse I, Snake Venom Phosphodiesterase (SVPDE), Phosphodiesterase from Bovine Spleen (SPDE), Micrococcal Nuclease (MN) and NP1 to release the 3`- or the 5`-monophosphatenucleotides followed by a subsequent treatment with alkaline phosphatase (AP). Results: Clear differences in the amount of HNE-dGuo detected were observed with the various digestion systems. No signal for HNE-dGuo could be found with the originally used NP1 hydrolysis system and the highest signal could be detected with a DNAse I+SVPDE hydrolysis system, whereas the amounts of the normal 2`-deoxyribonucleosides released from DNA were similar in all DNA digestion systems tested. Discussion: Combination of DNAse I+SVPDE+AP was the most effective DNA hydrolysis system for liberation of HNE-dGuo from DNA. The considerably smaller amounts of HNE-dGuo obtained with MN+SPDE+AP may indicate that the levels detected with our ^{32}P-postlabeling method were underestimated. Further studies must prove if this applies to other propano adducts as well.

P#32

European Standards Committee on Urinary (DNA) Lesion Analysis (ESCULA): Towards consensus for the measurement of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine

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There is growing evidence that, rather than simply being a non-invasive marker of whole body oxidative stress, measurement of urinary lesions, such as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), may, to some extent, reflect the repair of oxidised DNA and deoxynucleotides, although their precise provenance is far from clear. A discrepancy in basal urinary 8-oxodG levels has been noted when comparing chromatographic techniques (e.g. GC/MS following prior HPLC pre-purification, or LC-MS/MS, or LC-EC) with ELISA, although all techniques have been shown to discriminate between diseased and healthy subjects, and possess good within-technique agreement. ELISA has received widespread use, and is clearly amenable to the greatest number of laboratories, however, this discrepancy continues to raise questions regarding its utility. Understanding the basis of this discrepancy will aid our understanding of the significance of urinary lesions. Furthermore, performing inter-laboratory validation of assays for urinary 8-oxodG measurement would provide robust methods for widespread dissemination and application. Hitherto, this has been performed in a limited fashion, and the discrepancies remain undressed. Finally, there is growing clinical interest in the measurement of urinary 8-oxodG, as a means to determine the role of oxidative stress in disease, and evaluate intervention strategies. As with other clinical parameters, a reference range must first be determined. ECNIS support has been provided, enabling the formation of the European Standards Committee on Urinary (DNA) Lesion Analysis (ESCULA), to address these issues.

P#33

Genotoxicity of urban particulate matter is due to both organic and inorganic components

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We have examined the genotoxicity of urban particulate matter (UPM) using the comet assay, ^{32}P-postlabelling assay and supF mutation assay. Human lung epithelial A549 cells were exposed to size segregated UPM and DNA damage assessed by the comet assay. ^{32}P-postlabelling assay was used to confirm that DNA adducts were induced in the treated cells. In the comet assay, DNA strand breaks increased with exposure to decreasing size of UPM, with maximum damage induced by particles smaller than 2.5 μm diameter. About 75% of this DNA damaging ability was retained by an organic extract of the UPM. Using the plasmid strand break assay we demonstrated that free radicals were also generated by the UPM. To investigate the mutagenicity of this free radical generating capacity, we exposed pSP189 plasmid to the UPM and examined mutations in the supF assay. The mutation frequency induced by 1 μg/μl UPM was 4.99 mutants per 10^4 colonies, compared to a background mutation frequency of 0.35 mutants per 10^4 colonies. The UPM induced mutation frequency was reduced to 0.84 and 1.48 mutants per 10^4 colonies by addition of 1 mM mannitol or 1mM EDTA, respectively. Of the base substitution mutations, 88% where at GC pairs, with twice as many transversions as transitions. The types of mutations induced, the reduction of mutagenicity by the inclusion of the free radical scavenger, mannitol, or the metal chelator, EDTA, and the sequence context of the induced mutations all support the conclusion that the majority of mutations were induced by reactive oxygen species generated by metal ions present in the UPM. Taken together the results of our studies highlight the fact that both organic chemical carcinogens and metal ions contribute to the potential genotoxicity of UPM.

P#34

Smoking-related bulky DNA adducts and *TP53* mutations in lung adenocarcinoma and squamous cell carcinoma

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TP53 tumour suppressor gene mutations are common genetic alterations in human lung cancer. *TP53* has a regulatory role in various cellular mechanisms including cell-cycle control, DNA repair and apoptosis. The aim of the project was to study the associations between the levels of smoking-derived aromatic DNA adducts, mutations of *TP53*, smoking status and histological type of lung cancer in a Hungarian study population. Tumour and normal lung tissue samples were available from 104 adenocarcinoma and squamous cell carcinoma patients, who underwent lung resection. Aromatic DNA adducts were determined by ^{32}P-postlabelling. *TP53* mutations were detected in the tumour tissue in exons 5 to 9 and 11, and mutations were sequenced. Smokers had statistically significantly higher level of DNA adducts than non-smokers, and normal lung tissues had two-fold higher DNA adduct level than tumour tissues. There was a statistically significant correlation between DNA adduct levels of the normal and the tumour tissue ($r=0.64$, $p<0.0001$). Among smokers, *TP53* mutation carriers had similar DNA adduct level as the subjects with wildtype *TP53*, whereas in non-smokers mutation was associated with higher DNA adduct level ($p=0.076$), especially with mutation on exon 5 ($p=0.01$). G->T mutation was detected exclusively with smokers, and G->A mutation was predominant with non-smokers. Slightly enhanced DNA adduct level was observed with G->A mutation as compared to the wildtype ($p=0.18$). No conclusive difference was seen in DNA adduct levels according to the histological type. The results suggest that association between *TP53* mutation and aromatic DNA adduct level may be more apparent in non-smokers.

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P#35

Kinetic of induction and disappearance of DNA-strandbreaks and mutations of different alkylating agents: 3-nitrosooxazolidine-2-one (NOZ-2), 3-nitrosooxazolidin-5-one (NOZ-5) and glycidamide using comet-assay and *hPRT*-gene-mutation-assay in V79-cells

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Genotoxic N-Nitrosooxazolidinones have been reported to be generated from nitrosated amino acids in the presence of dietary aldehydes. These compounds can rapidly hydrolyse, generating highly reactive diazonium-ions supposed to covalently interact with nucleophilic centers in DNA. The induction and disappearance kinetics of DNA-strandbreaks was studied with the putative hydroxyethylating NOZ-2 and its carboxy-methylating/methylating analogue NOZ-5. Glycidamide (GA), a preferentially N^7-guanine-alkylating agent, was tested in comparison. Mutagenicity was determined by the *hPRT*-gene-mutation-assay. NOZ-2 strongly induced DNA-strandbreaks (SSB) already after 15' incubation (≥3µM). Additional treatment with the DNA-repair enzyme formamido-pyrimidine-DNA-glycosylase (FPG) did not substantially increase DNA-strandbreaks. In contrast, GA and NOZ-5 were only marginally active without FPG-treatment (≥300µM), but FPG-treatment elevated the response significantly (≥10µM). Moreover, whilst NOZ's induced DNA-strandbreaks very fast (15' incubation), GA reacted much slower (≥1h). Within 8h, GA-induced DNA-strandbreaks (300µM) disappeared nearly completely. In contrast, within the same time-interval the NOZ-2-induced DNA-lesions (30µM) were persistent. Both NOZ's were strongly mutagenic inducing significant *hPRT*-mutations already at ≥10µM. GA became significantly mutagenic only at about 80-fold higher concentrations. In terms of DNA-lesions, NOZ-2 is expected to hydroxyethylate, NOZ-5 to carboxymethylate/methylate DNA at various nucleophilic centers, including O^6 and N^7 of guanine. Predominantly however, alkylation at the phosphodiester groups of the DNA-backbone is to be expected. Since V79 cells are repair-deficient concerning O^6-alkyltransferase, the strong *hPRT*-mutagenicity of NOZ's might be ascribed to proportionate O^6-alkylation and to further more persistent lesions. Given, that N^7-adducts are subject to FPG-repair, the strong comet-signal-enhancement by FPG for NOZ-5 finds an explanation. In contrast, the FPG-effect on the NOZ-2-induced comet-signal is small, most probably because phosphate group hydroxyethylation triggers spontaneous SSB-induction. At variance to NOZ-2 however, phosphotriesters generated by NOZ-5 are not supposed to give rise to spontaneous SSB, thus allowing the FPG-effect to become detectable. In contrast to NOZ-induced SSB, GA-induced SSB are effectively removed within 8h in V79 cells which might explain in part the rather weak *hPRT*-mutagenicity.

P#36

DNA adduct formation capacity and induction of oxidative stress by the carcinogenic urban air pollutant 3-nitrobenzanthrone and its abundant isomer 2-nitrobenzanthrone, measured *in vitro* and *in vivo*

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Background: The carcinogenic vehicle emission product 3-nitrobenzanthrone (3-NBA) is known to rearrange in the atmosphere to the isomer 2-nitrobenzanthrone (2-NBA), hence 2-NBA but it exists in 70-fold higher concentration in ambient air.
Methods: The genotoxicity of 2-NBA and 3-NBA was studied both *in vitro* (A549 and human hepatic tumour cells HepG2) and *in vivo* (F344 rat) models, using the ^{32}P-HPLC and the Single Cell Gel Electrophoresis (Comet assay) method. *In vitro,* also the parent compound benzanthrone (BA) and the metabolite 3-aminobenzanthrone (3-ABA) were evaluated.
Results in vitro: 3-NBA gave highest levels of DNA adducts in the two cell lines, but significantly higher in HepG2 (~ 500 adducts/10^8 normal nucleotides (NN)) compared to A549 (~ 300 adducts/10^8 NN), whereas 2-NBA formed about 1/3 and 1/10 of the DNA adduct amount in A549 and HepG2 cells, respectively. 3-ABA formed significantly higher DNA adduct level only in the A549 cells, whereas BA did not give rise to any detectable amounts of DNA adducts. The DNA adduct patterns from 3-NBA were similar *in vitro* and *in vivo*, but differed somewhat for 2-NBA. The oxidative stress induced by BA was almost as high as what was observed for 3-NBA and 3-ABA in both cell lines, whereas 2-NBA induced lowest level of oxidative stress.
Results in vivo: The oxidative stress and DNA adduct level, in whole blood, was significantly increased by 3-NBA but not by 2-NBA. 3-NBA also formed the highest levels of DNA adducts in the nine tissues examined, compared to 2-NBA. While DNA adduct level in the 3-NBA exposed animals reached a peak around 1 and 2 days post instillation, 2-

NBA treated animals showed a tendency towards a continuing increase at the end of the study. This raises the question whether 2-NBA can be stored longer in the body compared to 3-NBA, or if it has the potential to bioaccumulate and thereby pose a greater and more long-term threat to health.

P#37

Inhibition of nucleotide excision repair by neutrophils: role of myeloperoxidase

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Inflammation has been recognized as an important factor in cancer development. However, the biological processes underlying this relationship have not been fully defined. In the lung, the influx of polymorphonuclear neutrophils (PMN) is considered as a specific etiological factor linking inflammation with carcinogenesis. More specifically, neutrophils are implicated in pulmonary carcinogenesis through their capacity to accelerate the metabolism of inhaled chemical carcinogens, causing enhanced formation of pro-mutagenic bulky DNA-adducts. We aimed to investigate the effect of neutrophils on the DNA repair processes involved in the removal of such adducts. Specifically, we tested the hypothesis that neutrophils are potent inhibitors of nucleotide excision repair (NER). Alveolar epithelial cells (A549) were co-incubated with PMA-activated human neutrophils. Phenotypical assessment of NER capacity in the exposed A549 cells was performed using a modified comet-assay. The co-incubated epithelial cells showed a PMN dose-dependent reduction of NER capacity. Moreover, we found that addition of the specific myeloperoxidase (MPO) inhibitor 4-ABAH abrogates NER inhibition by PMN. In further experiments we showed that the MPO-product HOCl also dose-dependently and persistently (24h) reduced DNA repair capacity in the A549 cells, indicating that NER inhibition by PMN is mediated by MPO-dependent formation of HOCl. Importantly, these effects were observed in the absence of cytotoxicity or loss of cellular ATP. Besides, no down-regulation of NER-relevant genes (ERCC-1, XPA, XPC, XPF, XPC) was observed. Further preliminary data suggest a role of protein modification in the inhibition of NER by HOCl. We finally showed by using ^{32}P-postlabeling that HOCl-induced inhibition of NER was associated with a delayed removal of DNA-adducts in benzo[a]pyrene-exposed A549 cells. Our data show that neutrophils are potent inhibitors of nucleotide excision repair through MPO-mediated formation of HOCl. Generally, these observations may provide a further biological explanation for the observed association between neutrophilic inflammation and lung cancer risk in smokers.

P#38

Determination of the DNA methylation level: An increase in sample throughput by an optimised sample preparation

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The epigenetic code, i.e. the methylation of cytosines, various histone modifications and DNA-binding proteins, is, unlike the genetic code, always being rewritten and erased. Epigenetic mistakes are involved not only in mutations in the genotype, but also in the development of abnormalities, cancer and other diseases. Using a capillary electrophoretic method we have previously shown, that the genomic methylation level can be determined very exactly. In the present work, this method was applied to study the degree of methylation in tissue samples of rats that had been treated with 3-NBA. In the various organs of treated animals, the degree of methylation was found to be different from those of control animals. For coping with the increased quantity of samples in an adequate time, we also present here a sample preparation that eliminates the surplus of fluorescence marker and carbodiimide used for the coupling reaction. With this method, sequences of up to 120 measurements can be carried out, which increased the sample throughput from 75 to 250 analyses per week.

P#39

Paternal exposures to benzo[a]pyrene and genetic risk in their offspring

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Polycyclic aromatic hydrocarbons (PAHs), like benzo[a]pyrene (B[a]P), are well known environmental and occupational pollutants. Subjects exposed to PAHs were found to be at risk to develop malignancies. Although PAHs induce without any doubt gene mutations in somatic cells and consequently somatic tumors, their role in germ cell gene mutation induction is largely unknown. No relevant human or limited laboratory animal studies on male reproductive effects have been done so far. Still though, it is likely to assume that paternal exposure to mutagenic chemicals may increase health risks in offspring individuals owing to induction of mutations in the DNA of gametes. Children may inherit gene mutations, which were induced upon exposure to DNA damaging agents during different stages of spermatogenesis. Next, there is some human evidence demonstrating that paternal exposure to PAHs causes gene mutations in sperm DNA, and animal studies using B[a]P as a challenging seem to support these human data. However, there is also evidence that germ cells are protected from paternal exposures by a complex network of molecular defence mechanisms. As such it is still far from clear yet what the human risk is for PAH-induced germ cell mutagenesis. Therefore, it is our intension to investigate reproductive effects of low dose pre-conceptional B[a]P exposure to further establish potential health risks in offspring. To be successful more sensitive methods are required (like DNA repair deficient mouse models) and will be developed in order to be able to detect heritable damage and germ line mutations at low doses.

P#40

Analytical characterization of azanucleosides as novel chemotherapeutic agents

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Epigenetic therapy plays an increasingly important role in the treatment of leukemia and other forms of cancer. Currently, the nucleoside DNA methyltransferase inhibitors Decitabine® (5-aza-2‘-deoxycytidine) and Vidaza® (5-azacytidine) represent the most advanced drugs. However, their physico-chemical characteristics and their incorporation rates into DNA have been poorly characterized. We are using capillary electrophoresis (CE) to analyze azanucleosides in various experimental contexts. In a first series of experiments, we have determined the stability of both Decitabine and Vidaza in their standard application concentrations. This revealed an unexpected half-life time of several hours up to 4 days at clinically relevant temperatures (20°C and 37°C). This finding should allow the application of the drug in a more patient-friendly way. Secondly, we took a closer look on the demethylating kinetics of the two methyltransferase inhibitors. The result was an interesting difference between Decitabine and Vidaza in the demethylating effect over time. A synthesis of a 3‘-phosphate standard of 5-aza-2‘-deoxycytidine was performed to unambiguously identify the compound peak during CE analysis of complex DNA samples and to optimise separation conditions. This will allow the identification of 5-aza-2‘-deoxycytidine 3‘-phosphate as a distinct signal among other genomic nucleotides. The aim will be to analyse DNA samples from drug treated cell lines and finally tumour samples from patients undergoing epigenetic therapy with regard to determine drug incorporation rates into genomic DNA and to potentially optimise treatment protocols according to individual incorporation rates.

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AIMS OF THE QUALIFICATION

❖To encourage new people to enter the field by providing background education in Genetic Toxicology and a structured route for further education.
❖To allow for the continued professional development (CPD) for those working directly or indirectly in the field (e.g. managers, regulators, clinicians etc as well as genetic toxicologists), via a range of qualifications up to a Masters degree.
❖To provide "stand-alone" modules for specific educational and training purposes.
❖To provide a mechanism for less experienced genetic toxicologists to learn from individuals with greater experience.

COURSE STRUCTURE

❖The modular course covers the essentials of Genetic Toxicology and Environmental Mutagenesis.
❖The course will be run at the University of Wales, Swansea and each module will delivered over 5 days.
❖The modules build together to form three different qualifications – Certificate, Diploma and MSc.
❖Modules can be built up over several years at times to suit the individual – they do not need to be taken sequentially.
❖Modules may also be taken individually for CPD purposes.

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(20 credits each, except B with 40 credits and H with 60 credits)
A) Mutations and Human Health.
B) 1. Testing Methods in Genetic Toxicology 1.
2. Testing Methods in Genetic Toxicology 2.
C) Lesions, Repair and Mutations.
D) Metabolism, human and animal.
E) Data Analysis.
F) Study design, quality and interpretation.
G) Molecular Epidemiology.
H) Research Project.

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UKEMS/UWS Diploma:- 120 credits (Modules A + B and three of C, D, E, F or G).
Master of Science (University of Wales Swansea) : 180 credits. (Modules A+ B and three of C, D, E, F or G PLUS Module H). ***Customised Certificate*** : Available on request, combination of modules providing 60 credits.
Reduced Module C : Provides CPD points only. Intended for people working outside of field such as regulators and project leaders.

2006/07 MODULES (5 days each) (Provisional dates)

Module A : Sept 25th 2006
Module B1 : Dec 11th 2006
Module B2 : Jan 5th 2007
Module C : May 14th 2007
Module D : Jan 22nd 2007
Module E : Nov 13th 2006
Module F : May 21st 2007
Module G : To be arranged

WHERE?

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APPROXIMATE COSTS

20-credit Modules = £1000-£1500
40-credit Module = £2000-£2500
(Accommodation and breakfast/evening meals not included).

CONTACT

If you require registration forms or would like more information on specific modules, please contact Prof Jim Parry (JMP@swansea.ac.uk).

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