ABSTRACTS

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30TH ANNUAL MEETING OF EUROPEAN ENVIRONMENTAL MUTAGEN SOCIETY

"CHALLENGES OF MUTATION RESEARCH FOR THE XXIst CENTURY"

AUGUST 22-26, 2000

BUDAPEST, HUNGARY

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Opening lectures

O/1 - O/2

DISCOVERING THE HUMAN GENOME: BENEFITS, DANGERS AND OBLIGATIONS

Árpád Gógl, Minister of Health of Hungary

By the end of the 20th century the genetic code system, determining and steering the construction and function of living organisms, has been available to immediate examination of unlimited extent. The greatest enterprise of human cognition approaches its success: the total discovery of the human hereditary material. The mapping of the total human genome, and the capacity to examine the hereditary qualities of the individual offers possibilities and involves severe dangers, hence assigns capital obligations on those dominating the collection and use of this kind of information.

The detailed knowledge of the genotype of individuals in large populations will introduce a new paradigm in medicine and public health care, giving rise to the predictive medicine, in front of the preventive and curative medicine.

Genetic knowledge is going to serve health protection and primary prevention. One will be aware of his hereditary susceptibility to some specific illnesses the manifestation of which can be prevented or delayed. This means measures taken in healthy state, before any structural or functional alteration or symptoms exist, just by knowing one's individual health risk. Predictive medicine will thereby diminish the share of curative medicine, lengthening the healthy life span and quality of life at the level of the society.

An established genome gives information on hereditary features of the relatives of the subject examined as well, raising the level of genetic counselling. Gene-level laboratory methods developed for mass examinations will enable screening for the hereditary share of population-wide diseases-such as atherosclerosis, cancer, diabetes, allergy and so on. They may help in establishing diagnoses before birth or before the manifestation of symptoms (prenatal and presymptome diagnoses), and may alert on the increased risk for an otherwise acquired illness (predictive diagnosis). They may also help the selection of the right embryo during assisted reproduction (preimplantation diagnosis).

The dangers, and the need for additional juridiction and ethical considerations issue from the fact that an unveiled genome determines unequivocally the identity of the person examined involving his ancestors and the risks of his descendants. One can use as well as misuse this information which has been the deepest secret of one's personality, unknown even by him- or herself.

A great challenge is for law and medical ethics to determine the level of privacy of the genetic information: what should be available to the subject himself, to the relative (eventually endangered), to the employer or assurance company, or even to the court.

Any tissue or blood sample of a living or dead person bear the whole genetic information. This requires the quality and legal control of all the laboratories getting samples from known individuals and being able to perform the genome analysis.

The possibility of individualization of environmental hazards is of value for the personal prevention but needs new legal approach if such a condition has led to harm to the person hereditarily inclined.

Finally, an important issue is the possibility of arteficial selection among members of the society, which implies the danger of genetic discrimination.

Industrial development has tought us that technological progress may proceed with such a speed that the society is unable to cope with it in respect of legal, moral and social development. The accessibility of the human genome, brought about by the superspeed development of molecular biology, is a consequence unseparable from the identity of the human being. By the time it becomes property of the society its legal and moral consequences must be developed in a consistent system or the deficiency will attack the fundamentals of human right.

O/2

THIRTY YEARS IN MUTATION RESEARCH

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The field of mutagenesis research is actually older than 30 years; it came into being in the late 1920s with the demonstration by Muller of the mutagenic effects of X-rays in Drosophila and by Auerbach's discovery in the mid-1940s of the mutagenic effects of mustard gas. However, the explosive developments in molecular genetics and biology particularly in the past three decades permitted in depth insight into the fundamental mechanisms of the action of radiation and chemicals as present in our environment. Among the advances in mutagenesis research are: the recognition that electrophilic mutagens are potential carcinogens, the development of a wide variety of test systems to determine mutagenicity, the need of metabolic activation to convert many chemicals in mutagens /carcinogens, the unraveling of DNA repair pathways and the uncovering of intimate links between DNA repair, transcription and replication, the development of knowledge-based approaches for mutagenicity and/or carcinogenicity prediction from chemical structure and the practical application of biological DNA and protein adduct dosimetry in biological monitoring procedures. This list is not meant to being exhaustive but reflects some major global scientific enterprises to cope with the risk of exposure of humans to radiation and a myriad of chemicals in our environment. Currently, the developments in genomics and proteomics promises to be valuable future tools to obtaining quantitative information on the carcinogenic and genetic risk of mutagens and carcinogens. In this presentation an attempt will be made to explain the need of these new technologies in explaining the worries of the past.

Plenary lectures

PL/1 - PL/6

PL/1

CURRENT TRENDS IN CANCER EPIDEMIOLOGY IN EUROPE

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An epidemiological assessment of the status of cancer clearly indicates that Europe is still divided by the differences in the probability of dying of cancer. Since the early 1990s, the cancer mortality rates have been slightly decreasing in Europe as a whole, but not to an equal extent for all types of cancer, and not in all geopolitical regions. The gap between the best and worst cancer patterns is getting wider. In one hand, in the majority of the European countries all the preventable exposures have been forcefully attacked in three major areas: action against tobacco, promotion of healthy nutrition and control of environmental hazards; the potential of secondary prevention is fully exploited, and, as a result, the treatment results are improving. On the other hand, the burden of cancer that the societies of the countries of the Central-Eastern European region have to carry is still heavy, and the mortality has continued to increase. This is most likely because of the considerable differences in the prevalence of lifestyle-related and other environmental exposures, which are having their roots in the social, political, economic inequalities that still exist. Even if attempts at prevention are eventually a total success, a major cancer load will persist in the decades to come.

PL/2

CHEMICAL MUTAGENESIS AND CARCINOGENESIS: INCORPORATION OF MECHANISTIC DATA INTO RISK ASSESSMENT

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The current understanding of cancer as a genetic disease, requiring a specific set of genomic alterations for a normal cell to form a metastatic tumor, has provided the opportunity for mechanistic data to be considered in the cancer risk assessment process. For some tumors, the specific steps and accompanying genetic alterations have been provisionally identified. However, the rate-limiting step(s) for tumor development remain to be elucidated. Such information is essential for establishing the shape of a tumor dose-response curve at exposure levels below that at which tumor incidence can be reliably assessed. The ultimate drivers of chemical carcinogenesis are DNA replication and cell division, but the substrates for these processes to give rise to errors are, for example, DNA damage, altered transcription, and cell cycle dysregulation. The protectors against carcinogenesis are, for example, DNA repair, cell cycle checkpoints and apoptosis. The probability of tumor development is, in part, a consequence of where the balance lies between drivers and protectors. Recent information has provided a high degree of understanding of how these fundamental cellular processes function. This information can be incorporated into cancer risk assessment for considerations of high to low dose extrapolation, interspecies extrapolation, chronic vs. acute exposures, susceptible subpopulations, and sensitive subgroups (e.g. children). The end product will be a reduction in the uncertainty for the cancer risk assessments derived from the various approaches employed. [This abstract does not necessarily reflect U.S. EPA policy.]

PL/3

ETHICAL CHALLENGES OF GENETIC TECHNOLOGIES IN EUROPEAN RESEARCH

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Genetic technologies are in the forefront of all debates on the potentials, benefits and risks involved with future progress of science. Science is getting a new social contract with the society, which has an effect on its directions, priorities and funding.
This is a new challenge also to fundamental research and the whole scientific community to communicate their thoughts and results also with the general public - which needs to be involved in decisions which might concern also their futureand wellbeing.
Bioethics has grown from the public awareness that scientific and technological progress has important human and social implications which raise important ethical issues. Simultaneously, various international, European and national legal and advisory instruments have been prepared for guidance of research, researchers and policy makers, among them the Council of Europe Convention on Human Rights and Biomedicine (1997) and UNESCO Universial Declaration on Human Genome and Human Rights (1997) have direct effects on genetic research. Recently, the European Union has issued Directives e.g. on (1) Data Protection (1995), referring to the principle of respect for privacy, (2) Protection of Biotechnological Inventions (1998), referring to morality, and (3) In-vitro Diagnostic Medical Devices (1998), referring to the protection of integrity of human persons. These directives are legally binding in the member states and also have indirect consequencies to decisions relating to genetic research.
The 5th Framework Programme of research and technological development of the EU clearly states that all research activities shall be carried out “in compliance with fundamental ethical principles”. These principles have been characterized in the opinionsgiven out by the European Group on Ethics on Science and New Technologies (EGE).

PL/4

THE MUNICH-ENU-MOUSE-MUTAGENESIS SCREEN - DEVELOPMENT OF MOUSE MODELS FOR INHERITED DISEASES IN MAN

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Mutants are the most important tool to obtain insight into biological function of genes. Due to the similarity in genomes, developmental, biochemical and physiological pathways , the mouse has become the model of choice for the study of inherited diseases in man. We have established a research center that carries out a large scale ENU-mouse mutagenesis screen. A number of participating research groups screen the mice for specific abnormalities, i.e. congenital malformations, clinical-biochemical alterations, immunological defects and complex traits. Emphasis is on phenotypes that are relevant for the pathogenesis of human diseases. Base line levels for over 150 parameters/phenotypes were established for C3HeB/FeJ and C57BL/6JIco mice In screening over 14,000 mice for a large number of clinically relevant parameters, we recovered 182 mouse mutants for a wide variety of phenotypes. In addition, 247 variant mouse mutants are currently in genetic confirmation testing and will result in additional new mutant lines. The mutants are analyzed by backcross mapping and genome wide microsatellite typing in order to chromosomally map the mutations. Conserved synteny between mouse and human genome are used to check for the potential homologous human disease. Several lines are currently used in the scientific community for further analysis of gene function.
www:HTTP://www.gsf.de/isg/ENU.index.html

PL/5

MUTATIONAL STUDIES TO GENETICALLY DISSECT SIGNALLING PATHWAYS IN ARABIDOPSIS

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The transferred DNA (T-DNA) of Agrobacterium is a unique insertion element that is transferred by a conjugation-like process into plants and integrated by illegitimate recombination into the nuclear genome of infected cells. The transferred single-stranded form of T-DNA is covalently attached to a pilot protein, VirD2, that is phosphorylated by a plant protein kinase in a cell cycle-dependent manner. The VirD2-kinase is an ortholog of Cdk-activating kinases (CAKs) that phosphorylates both cyclin-dependent kinases and the C-terminus of RNA polymerase largest subunit as its yeast and animal orthologs represented by subunits of RNA polymerase II modulator and TFIIH complexes. The T-DNA integration is not sequence specific, but occurs preferentially at chromosomal loci that are potentially transcribed. The T-DNA is an efficient mutagen that is widely used for genetic identification of plant gene functions. In combination with the analysis of protein interactions in the yeast two hybrid system, the T-DNA insertion mutagenesis provides a powerful tool for reverse genetic dissection of signalling pathways. This approach is illustrated by studies of a mutation in the Arabidopsis Pleiotropic Regulatory Locus (PRL1) which drastically reduces the frequency of T-DNA integration. PRL1 encodes a regulatory WD-protein that interacts with multiple signalling factors, including plant orthologs of AMP-activated protein kinases. PRL1 participates in the control of cell elongation, cell cycle, RNA processing and protein degradation modulating several sugar, hormone and stress signalling pathways in plants.

PL/6

CHALLENGES FOR THE FUTURE IN ENVIRONMENTAL MUTAGENESIS

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Our rapidly growing understanding of the structure of the human genome is forming the basis for numerous new molecular approaches to identify human responses to environmental chemicals and pharmaceuticals. Gene expression data can be produced using technologies such as DNA microarrays, reverse transcript imaging, amplified fragment length polymorphism, serial analysis of gene expression and others. Gene expression profiling will dramatically increase our understanding of human exposure and susceptibility and will directly contribute to defining the mechanism of action of chemicals and drugs. Proteomics will quantify protein expression, providing a down-stream snapshot of gene regulation, protein synthesis and stability in the control of cell function. Genomics and proteomics research by definition require an understanding of biological molecules of interest in the context of many thousands of others. Unlike traditional study of one gene, gene product, or process at a time, the researcher will generate a large set of molecular data, perhaps with limited ability to predict its utility or application. The challenge then is to select and to monitor the right genes and to properly understand the relationship between genotype and phenotype. Strategies must be developed to do this. DNA sequence polymorphisms in genes that can determine human susceptibility and sensitivity are being mapped and characterized in large numbers. Some of these polymorphisms have little, if any, effect on the expression or function of a protein, while others dramatically alter protein activity and human response to chemicals and pharmaceuticals. Risk assessments and drug regimens that incorporate this new information can be used to better protect human populations. Unfortunately, the new technological approaches being applied in genomic and proteomic analyses can and will overwhelm the current information infrastructure. The challenge is not simply to manage the data flow that will be generated by these new approaches but to properly interpret the information in the light of current knowledge. To do this effectively will require new information systems and new biological models. Our goal should be not only to produce the data but also to develop new analytic strategies and tools to interpret it. [This is an abstract of a proposed presentation and does not necessarily represent EPA policy.]

Workshop A

Workshop on Eco-genotoxicology

WA/1 - WA/6

WA/1

NATURAL SELECTION IN CONTAMINATED HABITATS: CASE STUDIES FROM THE AQUATIC EVIRONMENT

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Selection of resistant genotypes in pollutant-exposed populations is relevant to ecological risk assessments for a number of reasons. First, such selection may reduce variation in the population, limiting the ability to respond to environmental variation. Second development of tolerance has associated costs (reduction in fitness, recruitment into populations). Finally, the ability for adaptation in opportunistic generalists and not in specialists may be the mechanism for simplification of communities in contaminated habitats. Differences in genetic composition between populations may suggest selection for specific genotypes, but in order to validate this hypothesis differential fitness (as reflected by fecundity and/or survival) and possible biochemical or molecular mechanisms for differential responses to toxicants must be shown. To do this, populations of Gambusia affinis were examined using the randomly amplified polymorphic DNA (RAPD) technique. In this work, three previous studies are summarized and integrated. The first study discovered RAPD markers that were present at an increased frequency in radionuclide-contaminated populations. It was found that fish in the contaminated ponds that displayed these bands had higher fecundity that fish that did not. In a second study fish were collected from a non-contaminated site and caged in a contaminated one. Fish that displayed these bands had a higher survival rate than fish that did not. In the third study, the amount of DNA damage was determined for the diffreent genotypes. It was found that fish with the contaminant-indicative bands had fewer strand breaks than did fish without such bands. These results suggest that these markers could be used for monitoring the effects of contaminant-mediated selection.

WA/2

DNA STRAND BREAKAGE IN CELLS OF LABORATORY- AND FIELD-EXPOSED AQUATIC ORGANISMS

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In the monitoring of organisms for evidence of genetic toxicity, it is valuable to have sensitive, non-specific assays indicative of a wide range of DNA damage mechanisms. Further specific analyses can then be used to determine the precise nature and extent of damage. DNA strand breaks as measured by the Comet assay offers such an end-point. A range of genotoxic chemicals , acting directly or through metabolites, cause DNA strand breaks in cells of aquatic vertebrate and invertebrate organisms. Laboratory studies have aided the identification of the metabolic mechanisms, including cytochrome P450 and free radical involvement, operative in mussels and fish for the activation of genotoxic chemicals. Furthermore, a number of field studies have determined the extent of DNA strand breakage in response to different environmental exposures including the study of mussels transplanted between sites. For this workshop an emphasis will be given to important issues that need consideration for the future including the use of blood or haemolymph as a surrogate target, variability between cells, individuals and seasons, and the relative role of pollutant versus “natural” stimuli.

WA/3

GENOTOXICITY OF EXPOSURE TO HEAVY METALS IN LAPLAND NATURE RESERVE

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The project aims at investigating the genotoxic effects of long-term exposure to heavy metals on plants and small mammals in the Lapland Biospheric Reserve. The study area in the reserve, which is situated in the Kola Peninsula (Murmansk Region) Russia, is about 5 000 km2. The area has a high level of heavy metal pollution and stretches from an area where the ecosystem is completely destroyed and through to areas which seem to be untouched by pollutants. In this area the Severonickel Smelter Complex, operating since 1938, is the only local source of atmospheric industrial pollution. Emissions from the Severonickel Smelter complex include 220-240 000 tons sulphur dioxide, 4 000 tons nickel and 4 000 tons copper annually. 60 years of production has had a catastrophic effect on the surrounding area. Plant material has been collected over a period of five years and small mammals were collected in two years. Levels of metals have been measured in plant species (Cu, Ni, Zn) and tissues from small mammals (Cu). The frequency of chromosome aberrations was examined in lymphocytes from Clethrionomys rufocanus (grey-sided mouse) and in root-tips from Picea abies (spruce) to study the effect of pollution on cell division. The results from the study of small mammals are consistent with a concentration/effect relationship between chromosome aberrations in lymphocytes and distance from the smelter stacks. The results from the spruce, so far, do not show the same trend.

WA/4

DNA DAMAGES IN MUSSELS AND LEVELS OF SOME XENOBIOTICS IN SEDIMENTS

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The science of eco-genotoxicology has many challenges. Primarily, it is necessary to find the most suitable species for biomonitoring the genotoxic effects caused by pollutants in various recipients. Secondly, it is also important to show that a genotoxic response in an organism can be correlated with specific chemicals in the environment. In studies of pollutant problems in the aquatic environment mussels have very often been the preferred organisms, mainly because they have a sessile life style and are filter feeding, which means that the exposure to water soluble chemicals is very extensive. In the present study the blue mussel, Mytilus edulis, was used for the detection of genotoxic damages in respectively hemolymph and gill cells examined for DNA strand breaks in the alkaline single cell gel (comet) assay. The mussels were exposed to the marine environment under natural conditions and were sampled from the beach at five stations in Koge Bay, a highly industrialised area south of Copenhagen receiving wastewater from four municipal wastewater treatment plants and several industrial outlets. The monitoring took place during the years 1998 and 1999. In the first year only hemolymph cells were examined, but in 1999 the gill cells were included as well. Moreover, sediments from the five stations were collected on the three last sampling dates in 1999 and analysed for chromium, nickel, cadmium and mercury. In spite of the fact that the levels of these heavy metals were relatively low, it was found that for some of the sampling dates there was a good correlation between metal concentrations and genotoxicity. Other xenobiotics as PAHs, PCBs and DEHP will also be measured in the sediments.

WA/5

BIOMARKER-BASED MONITORING OF THE LIGURIAN COAST: NATIVE VERSUS CAGED MUSSELS

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The impact of pollutants on the marine environment produces multiple consequences at organism, population and ecosystem levels. Marine animals are particularly sensitive to DNA alterations from xenobiotics, perhaps due to their usually high level of exposure and relatively inefficient DNA repair. A biomonitoring programme in native as well in caged mussels (Mytilus galloprovincialis) was carried out in 4 contaminated sites and in a reference area along the Ligurian coast (Italy) using a multimarker approach. Mussels of a very narrow size range were left in situ for 30 days. Adult specimen of mussels from natural substrates were collected in the same areas. Micronucleus frequency and DNA single strand breaks, evaluated by the alkaline elution, were used as biomarkers of genotoxicity. Lysosomal membrane stability was applied to evaluate the physiological status of the animals. Mussel tissue were also analysed for polycyclic aromatic hydrocarbons and heavy metals (Hg and Cd). A clear distinction among the sites was demonstrated by the analysis of genotoxicity parameters. The obtained results evidenced a higher extent of chromosomal damage with respect to single strand breaks. Micronuclei frequency ranged from 1.78±1.04 in control animals to 24.4 ±12.9 mussel from the most polluted site. Physiological status was significantly altered in mussels from polluted compared to those at the reference area although no clear difference among the sites was demonstrated.
A correlation between genotoxic effects and chemical contaminants was observed in native as well as in caged mussels. Native mussels accumulate much higher concentrations of chemicals and facilitate quantifying both the exposure and effects.

WA/6

CAN ENDOCRINE- DISRUPTING CHEMICALS SIMULTANEOUSLY ACT AS GENOTOXIC AGENTS? AN INTEGRATED STUDY EVALUATING THE EFFECTS OF TRIBUTYLTIN ON EMBRYO-LARVAL STAGES OF MARINE INVERTEBRATES

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In recent years there has been considerable concern over the potential of chemicals in the environment to disrupt the normal functioning of the endocrine systems with respect to both human and wildlife. The effects of tributyltin, an ingredient of antifouling paints, on certain marine gastropods represents a clear example where population declines have resulted from exposure to endocrine-disrupting chemicals. In this context, it is now emerging that some steriod hormones (or their metabolites) which are well known to act as cellular proliferators (cancer promoters) can also induce damage to the genetic material and, therefore can initiate carcinogenesis. In view of these observations, it is perhaps not surprising that the presence of environmental contaminants that inadvertently influence the hormonal metabolism has caused concern with respect to increasing evidence of some cancers, damage to reproductive systems, and developmental problems reported in both humans and wild life. This aspect of genotoxic potential of hormone-mimicking agents has not been properly addressed for aquatic organisms. Adopting an integrated approach and linking different levels of biological organisations, in this study, we have evaluated the genotoxic, cytotoxic and developmental effects in embryo-larval stages of two ecologically relevant marine invertebrates: Mytilus edulis (edible mussel) and Platynereis dumerilii (rag worm). Following determination of the maximum tolerated dose (MTD) in terms of developmental and survival effects, the embryo-larval stages of these organisms were exposed to a range of concentrations of TBT, and analysed for cytotoxic (proliferation rate index) and genotoxic (sister chromatid exchanges and chromosomal aberrations) effects. The study suggested that (a) TBT is both toxic and genotoxic to embryo-larval stages of both the species (b) at comparable concentrations, for developmental and genotoxic effects, P. dumerilii (non-target species) is more sensitive compared to M. edulis (target species) and (c) genotoxic effects are more closely tied with the developmental and survival of the organisms. The study emphasises the need of the evaluation of genotoxic potential of other endocrine- disrupting agents where mechanistic links with exposures have not been established.

Workshop B

Workshop on New developments in test methods

WB/1 - WB/6

WB/1

A SFTG COLLABORATIVE INTERNATIONAL STUDY ON IN VITRO MICRONUCLEUS TEST, USING HUMAN LYMPHOCYTES AND CHO, CHL AND L5178Y CELL LINES: RESULTS AND CONCLUSION

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This study, coordinated by the French branch of EEMS (SFTG), gathered 37 participants from Europe, Japan and USA. Negative and positive compounds (clastogens, aneugens and polyploidy inducers) were tested on lymphocytes, CHO, CHL and L5178Y cells using various treatment-harvest schedules, and with or without cytochalasin B when cell lines were used.
Negative compounds were always found negative. Mitomycin C, bleomycin and colchicine were found positive in all treatment-harvest conditions.
The preliminary study conducted on mitomycin C by all laboratories using cell lines provided useful information on inter-laboratory variability among cell types. For some compounds, e.g. for clastogens of the antimetabolite-type and some aneugens, differences were seen between the different treatment-harvest schedules. In conclusion, the results obtained in this study together with the already published data will give information on the most appropriate treatment-harvest schedule and the need for cytochalasin B with cell lines, and will therefore contribute to the elaboration of an optimal protocol for the detection of both clastogen and aneugen compounds.

WB/2

IN VIVO TRANSGENIC MUTATION ASSAYS : NEW DEVELOPMENTS

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The most widely used transgenic mutation assays are the Muta™Mouse, and the BigBlue® mouse and rat in which the bacterial target gene (lacZ and lac I, respectively) are incorporated in a recoverable lambda vector. The large number of studies recently conducted confirmed 1) their ability to detect the great majority of genotoxic carcinogens in a tissue/sex/species specific manner, and 2) their usefulness for the follow-up of in vitro positive studies, for the detection of compounds active at the site of contact tissues and for mechanistic studies. However, no optimal protocol has clearly been defined to conclude that a compound is negative , the main issue being the treatment duration (14 to 90 days). The measurement of mutant frequency is more and more often completed by the analysis of mutational spectra which helps in the confirmation of weak mutagenic effects and gives information on the mechanism of mutation formation. While the validation of the previous models is being enlarged, new versions of existing systems and new models are under development and evaluation. Among them is the use of the lambda CII reporter gene in the Muta™Mouse and the BigBlue® models. The main advantage is to provide a positive selection system for the detection of mutants in BigBlue® models and to facilitate the mutant sequencing in both models (only 294 bp). Moreover, systems able to detect large deletions in addition to point mutations (e.g. lacZ plasmid-based transgenic mouse, and gpt transgenic mouse using 6-thioguanine selection for the point mutations and spi - selection for the large deletions) are being evaluated. In vivo transgenic mutation assays are now accepted as additional tests when more information on in vivo genotoxicity is needed. The appropriate strategic use of these models in the mutagenicity/carcinogenicity risk assessment remains to be clarified.

WB/3

NEW DEVELOPMENTS IN THE COMET ASSAY

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The Single Cell Gel/Comet assay is a rapid and sensitive technique for detection of DNA damage. During the last decade, this method has been developed into a basic tool for investigations in research areas ranging from human and environmental biomonitoring to DNA repair processes to genetic toxicology. The advantages of the technique compared to other assays, include: (1) its demonstrated sensitivity for detecting low levels of DNA damage; (2) the requirement for small numbers of cells per sample; (3) its flexibility; (4) its low costs; and (5) its ease of application. The Comet assay has been used in various mechanistic studies: (1) to discriminate between strand breaks and alkali labile sites by using different pH conditions during electrophoreses (2) to detect specific DNA damage by using repair enzymes or antibodies; (3) to identify apoptotic/necrotic cells by using the neutral diffusion assay; and (4) to evaluate the ability of a test substance to interact directly with DNA by using an acellular Comet assay. The assay has mostly been used in genetic toxicology. Attractive uses of the assay in this field include: (1) as a potentially high-throughput screening assay; (2) in mechanistic studies to distinguish between genotoxicity- versus cytotoxicity-induced chromosomal damage; (3) in mechanistic in vivo studies to distinguish between genotoxic versus non-genotoxic carcinogens; and (4) potentially, as part of a battery of in vitro/in vivo assays used for regulatory submissions. However, comprehensive in vitro and in vivo data is needed before the assay can be critically evaluated for its utility in genetic toxicology (Tice et al (2000). Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu J-C, Sasaki YF. 2000. The Single Cell Gel/Comet Assay: Guidelines for In Vitro and In Vivo Genetic Toxicology Testing. Environ Mol Mutagen 35:206-221.

WB/4

RECENT DEVELOPMENTS WITH OTHER GENOTOXICITY TESTS

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Several recent workshops and collaborative trails have sought to provide advice on testing methods on a number of new assays, and to update testing methods for some established assays. In addition to the in vitro micronucleus test, transgenic mutation models and the comet assay, discussed in the preceding presentations, advice has been provided on tests for photogenotoxicity, the mouse lymphoma assay (MLA), the in vivo micronucleus test and detection of DNA adducts. In addition, discussions have taken place on the desired level of cytotoxicity for top dose selection in mammalian cell assays. Full reports on these methods as decided at the International workshop on Genotoxicity Tests held in Washington, D.C. in 1999 have recently been published in a special issue of Environmental and Molecular Mutagenesis (spring 2000). This presentation will therefore focus on 2 recent workshops to follow-up on the MLA and appropriate levels of cytotoxicity, which were held in New Orleans in April 2000. In the MLA further data were presented on appropriate measures of cytotoxicity (RS, RSG and RTG) with a view to recommending a single measure for all assays, and the sensitivity of the 24 hr protocol for detecting clastogens and aneugens. Further investigations on an appropriate level of cytotoxicity in the chromosomal aberration assay considered whether the 50% limit could be changed, and proposed approaches for evaluating the biological relevance of positive results only obtained at high levels of cytotoxicity. The outcomes of these workshops will be reported.

WB/5

A NEW METHOD TO AVOID INTERFERENCE BY APOPTOSIS IN IN VITRO CHROMOSOMAL ALTERATION TESTS

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Every new compound must be tested to search for potential genotoxic effect. The test battery used in mutagenesis intends to detect mutations at specific locus, chromosome breakages or loss of chromosome(s) (in metaphase analysis or in the micronucleus test) but the phenomenon of apoptosis, during which occurs DNA fragmentation, can interfer with tests designed to detect chromosomal alterations and give false positive conclusions.
For these reasons, we developed a modification of two mutagenicity tests: an in vitro micronucleus test and a metaphase analysis in vitro in CTLL-2 cells and CTLL-2 Bcl2 cells.
The CTLL-2 cells derived from a murine cell line. It is a subclone of Cytotoxic T Lymphocytes from C57bl/6 mouse . They need interleukin 2 to grow. They are sensitive to apoptosis.
In order to obtain CTLL-2 Bcl2 cell line, CTLL-2 celles were stably transfected by electroporation with pSFFV neo Bcl2 expression vector. It contains a 1,9 kb Eco RI insert encoding for Bcl2 and downstream the SFFV promotor. Moreover the plasmid contains the selective genes coding for ampicilline and geniticin resistance. This new cell line is unsensitive to apotosis due to the overexpression of Bcl2 proteine.
This test is, for the compounds studied, able to detect falsely positive results due to apoptosis phenomenon. For example dexamethasone, gliotoxin and methional that were not demonstrated as being genotoxic were positive only in non transfected cell line.
Moreover, this test can detect compounds which have a real clastogenic (methylmethanesulfonate, mitomycin C, MNU and MNNG) or aneugenic (griseofulvin, diazepam) potential without being apoptosis inducers in our conditions. The clastogen compounds as etoposide, genistein and cyclophosphamide or aneugens as taxol, nocodazole, diethylstilbestrol and chloral hydrate induced both apoptosis and micronuclei, but the genotoxic effects are weaker in Bcl2 transfected cell line than in CTLL-2 cell line. Such effects observed in the transfected cell line reflect then the real genotoxic potential .
Lastly, the test is sensitive and reproducible and with some of these compounds a good relationship with validated tests in human lymphocytes was demonstrated.

WB/6

AUTOMATIC ANALYSIS OF SLIDES PROCESSED IN THE COMET ASSAY

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During the last years, the comet assay (or single cell gel electrophoresis assay) has been established as a rapid and sensitive method for the detection of DNA damage. For early genotox screening of new chemical entities in industrial toxicology, the comet assay has proven to be suitable for the assessment of the DNA damaging potential of a test compound. In order to be able to increase the screening throughput of newly developed compounds, we established an image analysis system for fully automated measurement of microscopic slides processed in the comet assay.
Using the tail moment as the quantitative parameter for the comet formation, we found a very high correlation between our automatic image analysis system (Leitz MIAS image analyzer connected to a Leica DMR microscope with an 8-slide-stage) and a commercially available, but interactive system ("Comet Assay II" system, Perceptive Instruments, Haverhill, UK). We compared the data of several test compounds tested in different cell types such as V79 Chinese hamster fibroblasts, L5178Y mouse lymphoma cells, peripheral human lymphocytes and rat hepatocytes. It is possible to automatically preselect and then analyze 50 comets in about 8 minutes without any user interaction. The possibility to analyze around 50 samples (one slide carrying two samples) within one day and the high reproducibility of the results, make automatic image processing a powerful tool for the fast quantitative analysis of slide analysis and screening by means of the comet assay.

Symposium 1

MOLECULAR CHANGES RELEVANT TO MUTAGENESIS AND CARCINOGENESIS

S1/1 - S1/5

S1/1

DNA ADDUCTS: TOOLS FOR MONITORING HUMAN EXPOSURE TO CARCINOGENS AND FOR ELUCIDATING MECHANISMS OF CARCINOGEN BIOACTIVATION

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Because most chemical carcinogens exert their biological effects through covalent binding to DNA, the study of DNA adducts provides a means of assessing human exposure to genotoxic agents and of determining the pathways of metabolic activation in experimental systems. DNA adduct formation as a result of tobacco smoking has been much studied and has revealed that smokers have significantly higher levels of adducts in many target tissues for smoking-induced cancer. In a nested case-control study, smokers with elevated adduct levels in white blood cells were more likely to be diagnosed with lung cancer subsequently than smokers with lower adduct levels. The increased risk per unit increase in adducts was approximately 2-fold. The mechanism of activation of the antioestrogen tamoxifen, which is a potent carcinogen in rat liver, has been elucidated using ^{32}P-postlabelling to detect and to identify the DNA adducts it forms in rat liver cells. The proximate carcinogen is a-hydroxytamoxifen, which is converted to a reactive ester by hydroxysteroid sulphotransferase a (rHSTa). N-Desmethylation accompanies a-hydroxylation in the formation of some of the adducts, which are formed at the N^2-position of guanine and the N^6-position of adenine. All known human sulphotransferases have a much lower ability to carry out the activation step, accounting for the apparent lack of hepatocarcinogenicity of tamoxifen for humans and the general lack of tamoxifen-DNA adducts in human tissues. It also suggests that the mechanism of tamoxifen-associated endometrial cancer in humans may involve a non-genotoxic mechanism. rHSTa is expressed at much higher levels in female rat liver than in male liver, yet both sexes are equally susceptible to tamoxifen carcinogenesis. The reason is that tamoxifen induces rHSTa activity in male rat liver to levels similar to those in females, with concomitant increases in DNA adduct formation. Thus there is a close correlation between tumour formation, rHSTa activity and tamoxifen-DNA adduct formation.

S1/2

OXIDATIVE STRESS-RELATED DNA DAMAGE IN CANCER PROMOTION AND PROGRESSION

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Persistent oxidative stress and lipid peroxidation (LPO) cause DNA damage and disturbance of cell signaling pathways and are implicated in human cancers, neurodegenerative diseases and aging processes. Etheno (e) modified DNA bases (edA, edC, edG) are generated by reactions of DNA with LPO products (4-hydroxynonenal) derived from endogenous sources. Thus, these more stable secondary oxidation products are useful markers of oxidative stress-derived DNA damage. The recent development of ultrasensitive methods has made it possible to detect these (-adducts in vivo and to study their formation and role in experimental and human carcinogenesis. Highly variable background levels of e-adducts were detected in tissues from unexposed humans and rodents, suggesting an endogenous pathway of formation. The level of these DNA lesions in target organs was increased by several known cancer risk factors, e.g. by excess metal storage, high w-6 PUFA diet, chronic inflammatory processes, overproduction of nitric oxide (NO) or upregulation of COX-2 and LOX (emergency enzymes). Results will be presented to show that e-adducts are elevated in organs of cancer-prone patients and rodents (liver, pancreas, colon skin), suggesting that promutagenic e-adducts when formed by persistent oxidative stress play a role in the acquisition of genetic instability that could drive cells to malignancy (B. Singer & H. Bartsch (eds.): IARC Sci. Publ. 150, 1999). Therefore, identification of endogenous sources for DNA damage and the resulting oxidative modification of cellular components will give new insights into the carcinogenesis process and provide a better basis for cancer chemoprevention.
In part supported by EU contract ENV4-CT97-0505.

S1/3

ENHANCED FREQUENCY AND SEQUENCE SPECIFICITY OF HEPATIC CELL MUTATIONS IN TREMATODE-INFECTED TRANSGENIC MICE

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Parasite infections in humans have long been associated with specific types of cancers. Schistosoma hematobium is a known bladder carcinogen, Helicobacter pylori is a gastric carcinogen, and hepatitis B and Opisthorchis viverrini are causative agents of liver cancers.
Another fluke, Fasciola hepatica, has also been identified as a neoplastic risk agent, mainly in mammals. We used F. hepatica as a model agent to determine if the presence of an agressive liver fluke cound induce mutagenic events in mammalian tissue. Using the Big Blue transgenic mouse assay we found a two- to three-fold increase in lacI mutations in hepatic tissue cells harvested from mice harboring F. hepatica worms when compared to uninfected control tissue. Sequence analysis of these mutations indicate an enhanced level of complex mutations when compared to the distribution of mutation types found in spontaneous mutations from control animals. Collectively these data suggest that biological infections can cause increased genetic damage in surrounding host tissues. This research was supported by a grant from the National Science Foundation (NSF-REU 9322220) and by a grant from the Howard Hughes Medical Institute. (71191-528501).

S1/4

SEQUENCE-SPECIFIC DETECTION OF ARISTOLOCHIC ACID-DNA ADDUCTS BY TERMINAL TRANSFERASE-DEPENDENT PCR IN THE HUMAN P53 GENE

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Chinese herbs nephropathy (CHN), a unique type of nephropathy, associated with the prolonged intake of Chinese herbs during a slimming regimen, has been related to the toxic effects of Aristolochia species, containing carcinogenic aristolochic acid (AA). Recently increased numbers of urothelial carcinomas associated with a p53 overexpression were observed in these CHN patients indicating that AA is also implicated in CHN-associated urothelial malignancy. Human MCF-7 DNA was modified in vitro with AAI and AAII, the two main components of the natural plant extract, by chemical activation with zinc and AA-DNA adducts were determined by the ^{32}P-postlabeling method. To investigate the relation between AA-DNA adduct formation and possible p53 mutations, we mapped the distribution of AA-DNA adducts along exons 5-8 of the p53 gene by adduct-specific polymerase arrest combined with a terminal transferase-dependent polymerase chain reaction to amplify DNA fragments. Arrest bands varied in intensity, indicating that some sites in the sequence were more readily adducted than others. Strongest signals were seen at codons 154-167 for exon 5, at codons 196-199, 202, 209, 214 and 219-220 for exon 6, at codons 234-237 and 248-249 for exon 7 and at codons 283-284 and 290-291 for exon 8. Most of the arrests were associated with adenine and guanine residues, the preferential reaction sites for both AAs. In CHN patients the major adenine adduct of AAI was detectable up to 7 years after interruption of the slimming regimen in high levels and seems also to be the adduct responsible for the carcinogenic process. Many adduct hotspots are associated with adenine residues. In an ongoing study the comparison of the AA-DNA binding spectrum in the human p53 gene with the p53 mutational spectrum found in tumors of CHN patients may help to understand the molecular mechanism leading to cancer by AA in humans. (This work was supported by a stipend of the Boehringer Ingelheim Fonds to V.M. Arlt)

CHROMOSOME ALTERATIONS IN PROGRESSION OF TOBACCO SMOKE-INDUCED LARYNGEAL CANCER

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A genetic progression model for head and neck squamous cell carcinoma provides an accumulation of chromosome alterations in relation to disease evolution (1). The aim of the work was recognition of chromosome alterations specific for a stage of metastasis to adjacent lymph nodes in laryngeal cancer (2,3). Comparative genomic hybrydization (CGH) was applied to detect differences of genome imbalances in two series of clinical material: localised tumour v. tumour with metastases (material derived from 39 subjects) and primary tumour v. adjacent metastatic lymph nodes (material from 20 subjects).
Rearrangements of chromosomes were observed in all studied samples. The most frequent changes were found at chromosomes 3p, 3q, 5p, 9 and 13. Losses of DNA copy number were found to be more frequent than amplifications. The changes of DNA copy number tended to increase with the disease progression. A profile of losses in localised tumours differed from that in tumours with metastases that indicate for a significance of certain chromosome alterations in metastasis. In the separate series of experiments with FISH technique a frequent loss of chromosome Y in tumour cells was demonstrated.
Chromosome alterations studied by CGH and FISH seem to be a promisive tool in prognostic diagnosis (4).

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Symposium 2

Human biomonitoring

S2/1 - S2/6


EEMS • 2000
22–26 AUGUST, 2000, BUDAPEST, HUNGARY

S2/1

FACTORS AFFECTING BACKGROUND VARIATION OF BIOMARKERS OF GENOTOXICITY IN A POPULATION EXPOSED TO LOW LEVELS OF GENERAL ENVIRONMENTAL POLLUTION - EXPERIENCES FROM THE AULIS PROJECT

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Past studies have shown that exposure to high levels of urban air pollution is associated with increased levels of biomarkers of genotoxicity as well as increased risk of lung cancer. Whether analogous effects are induced by lower air pollution levels such as those normally found in western European cities is currently uncertain. The AULIS project (a multicentre molecular epidemiology project) aimed to investigate this question by examinine personal exposures to airborne PAH, and a range of biomarkers of genotoxicity, in 194 non-smoking technical institute students living in the city of Athens or in and around the nearby provincial town of Halkida. Personal exposures to PAH were found to be generally moderate to low, tending to be lower in Halkida and during the summer. Furthermore, the levels of biomarkers of exposure (urinary 1-hydroxypyrene, lymphocyte bulky DNA adducts measured by ^{32}P-postlabelling) were very low as compared to those reported in most other analogous studies, while biomarkers of effect (HPRT mutations, chromosome aberrations and sister-chromatid exchanges) were close to levels expected for subjects of the corresponding gender and age-group suffering background genotoxic burden.
Analysis of the data using detailed questionnaire information regarding the subjects' activities, lifestyle and dietary habits revealed only limited effects on the biomarkers measured. After controlling for other parameters, the main factors found to influence the levels of DNA damage were gender (males>females) and recent physical exercise, while no significant effect of ambient air pollution was detected. Highest levels of genetic damage were consistently observed among students living on the Halkida Institute campus, located in a rural area with minimal ambient air pollution. In this subcohort, biomarker levels consistently correlated with a series of markers of exposure to environmental tobacco smoke, suggesting that exposure to ETS may be an important factor influencing genetic damage in populations suffering otherwise low exposure to genotoxins.
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S2/2

FHIT AND NF2 GENE ALTERATIONS IN PULMONARY CANCER

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The pathogenesis of cancer is a multistep process involving alterations in many different genes. One of the frequently detected alterations in human lung cancer is loss of heterozygosity (LOH) at chromosome 3p. This firmly suggests presence of one or several tumour suppressor genes important for development of lung cancer. One of the candidates is the recently cloned FHIT (fragile histidine triad) gene at 3p14.2 proposed to function as tumour suppressor based on frequent allele loss, homozygous deletions and aberrant FHIT transcripts found in many human cancers, including lung cancer. Furthermore, in lung cancer, LOH at the FHIT gene as well as loss of Fhit protein expression has been associated with exposure to major lung carcinogens. These observations have lead to the postulation that the FHIT gene may constitute an important molecular target for pulmonary carcinogens. With this background knowledge, we have investigated a set of lung cancers for FHIT alterations in search for correlation between gene alterations and tobacco smoking and asbestos exposure of the patients.
Another tumour suppressor gene candidate is the NF2 gene (22q12), which predisposes through its germline inactivation and loss of the gene product to multiple nervous system tumours. NF2-associated tumours also occur sporadically and they are known to harbour NF2 gene mutations as well. A broader tumour suppressor function has been suggested for NF2, because NF2 mutations has also been identified in other tumour types including malignant mesothelioma (MM), a tumour of the pleura and peritoneal cavities. Molecular pathology of MM and associations between molecular changes and the main etiological factor of the disease, occupational exposure to asbestos, are still largely unknown. To explore this, we examined tumour specimens and cell lines from human MM for LOH at the NF2 gene region.

S2/3

THE USE OF BIOMARKERS AS PREDICTORS OF RISK (ADVERSE HEALTH EFFECTS) IN HUMAN STUDIES

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The use of biomarkers to predict the future occurrence of diseases in human population is a recurrent topic in many editorials and commentaries, in specialized literature as well as in leading biomedical journals. The reason of this recent interest is the increasing awareness that new endpoints to be utilized as index of adverse health effects in human population studies are urgently necessary. The complex pattern of most modern exposures, characterized by low doses and mixtures of various agents, requests a better understanding of the causal pathways of diseases, with the leading priority of validating biomarkers of intermediate steps as surrogate endpoint of disease. Due to the biological complexity and the multifactorial etiology of most chronic diseases this process is challenging, and so far only a few examples can be reported, such as colorectal polyps in large bowel malignancies or HPV infection in cervical cancers. The use of a biomarker as surrogate endpoint of disease has different aims and applications in clinical trials and in population studies. As regards this second aspect the major interest is focused into markers of early effect, whose impact in prevention is far more effective despite the weaker association with the final outcome. Examples in the field of human cancer are DNA adducts, somatic mutation and cytogenetic biomarkers, and the validation process of these biomarkers will be discussed. Special emphasis will be given to chromosomal aberrations, which have been found to be associated with the risk of cancer in healthy subjects in independent cohort studies. The design of human biomonitoring studies using surrogate endpoints of disease should necessary consider ethical implications and requests the application of preventive policies whenever a significant increase of the biomarkers is found in the study group.

S2/4

COMPARISON BETWEEN SMOKING-RELATED DNA ADDUCT ANALYSIS IN INDUCED SPUTUM AND PERIPHERAL BLOOD LYMPHOCYTES

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We investigated the applicability of induced sputum (IS), a non-invasive derivative from the lower respiratory tract, for smoking-related DNA adduct analysis, and its comparability to peripheral blood lymphocytes (PBL). Lipophilic-DNA adducts were quantified by the ^{32}P-postlabeling assay in IS and in PBL of smokers (n=9) with stable smoking status at three time points (one week intervals) and non-smokers (n=9) at one time point. The success rate for sputum induction was 100% at all time points. There was no significant difference in total cell count, cell viability, cell differential and DNA yield between smokers and non-smokers and within the smokers throughout. A typical smoking-associated diagonal radioactive zone was observed in the adduct maps of IS and PBL of all and five smokers, respectively, and of none of the non-smokers. Lipophilic-DNA adduct levels in both IS and PBL of smokers were higher than those of non-smokers (3.7 ± 0.9 vs $0.7 \pm 0.2/10^8$ nt, P=0.0005 and 2.1 ± 0.3 vs $0.6 \pm 0.1/10^8$ nt, P=0.0001, resp). In smokers, the level of adducts in IS was non-significantly higher than that in PBL (3.7 ± 0.9 vs $2.1 \pm 0.3/10^8$ nt, P=0.1), whilst in non-smokers, the difference was not appreciable (0.7 ± 0.2 vs 0.6 ± 0.1/108 nt). Within the smokers, there was no significant change in the level of adducts at three time points either in IS or in PBL [CI: 34% and 29 %, resp]. Adducts level in IS at each time point was higher than that in PBL leading to a significantly higher overall (mean of three quantifications) level of adducts in IS than PBL (3.3 ± 0.2 vs $2.1 \pm 0.1/10^8$ nt, P=0.02). The overall levels of adducts in both IS and PBL were dose-dependently related to smoking indices. We conclude that IS is a preferable matrix as compared to PBL for molecular dosimetry of exposure to inhalatory carcinogens as both the existence and the magnitude of exposure can be more explicitly determined in its analysis.

S2/5

DNA AND CYTOGENETIC DAMAGES IN HUMAN MONITORING STUDIES

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The single cell gel electrophoresis (SCGE) assay is a rapid and simple technique for measuring levels of DNA damage in cells after treatment by various mutagenic agents. The assay could be used as a predictive assay or for the analysis of environmentally induced DNA damage, however the relation of damage detected to late biological effects is still unclear. Results of the studies in vitro and in vivo in which the genotoxic effects induced by radiation and various environmental mutagens in human lymphocytes were investigated are presented. Human lymphocytes were irradiated by X-rays and fast neutrons or treated with various chemicals and DNA damages were assessed using the SCGE assay or cytogenetically in the first and second mitosis after the stimulation of the division. Genotoxic potencies of the chemicals investigated have increased in the order reflecting predicted on its structure related capabilities. Comparison between DNA damage investigated and chromosomal damage induced in human lymphocytes revealed a strong correlation between: sister chromatid exchanges levels (SCE) or percentages of cells with aberrations on one hand and the DNA damages estimated by SCGE-assay on the other. Linear-quadratic dose-response relationship after irradiation with X-rays and almost linear relationship after irradiation with neutrons were observed. That was in a good agreement with the molecular theory of radiation action. The strong correlation was again observed between DNA and chromosomal damages induced by radiation. DNA and chromosomal damages induced in vivo by radiation or occupational exposure are compared. Correlation between individual sensitivity to challenging treatment and cellular repair capacity of the damage induced and cytogenetic biomarkers in healthy donors and cancer patients are discussed.
ACKNOWLEDGMENTS This work was supported by grants from Polish State Committee for Scientific Research PAA/NIH -97-308 and European Committee ERBIC 15 CT 960300.

S2/6

MEASUREMENT OF OXIDATIVE DNA DAMAGE IN CANCER PATIENTS UNDERGOING RADIOTHERAPY: ESTIMATION OF URINARY EXCRETION OF 8-OXODEOXYGUANOSINE (8-oxodG) AND 8-OXOGUANINE (8-oxoG)

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Exposure of cancer patients to therapeutic doses of ionizing radiation causes significant increase of the amount of 8-oxodG and other base modifications in DNA isolated from their lymphocytes, Olinski et al. (1996). The products of repair of this kind of DNA damage are considered to be excreted into the urine without further metabolism. There is common believe that the presence of the modified nucleoside (8-oxodG) in urine represent the primary repair product of the oxidative DNA damage in vivo, Loft, Poulsen, (1999). However, oxidatively damaged DNA bases are mostly repaired by the base excision repair pathway (BER) although the nucleotide excision repair pathway may also play a role in the repair of some oxidised bases in DNA, Boitoux, Biochimie (1997). In the present study we applied recently developed technique involving a HPLC prepurification followed by gas chromatography with isotope dilution mass spectrometric detection, Ravanat et al. (1999) for the detection of 8-oxodG and 8-oxoG in the urine samples of the patients undergoing radiotherapy. Patients have been irradiated with a single dose of 1000cGy. Urine samples were collected before radiotherapy (control sample) and during first (#1), seventh (#2), fourteenth (#3) and twenty first (#4) day after radiotherapy. In most cases significant increases of excreted 8-oxodG and 8-oxoG over their control values were observed in (#1) urine sample. However, in some cases the values of the both repair products did not returned to the control values even in the sample #4. These results suggest that in addition to repair mechanisms some other factors may influence excretion of both molecules.
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Symposium 3

Cell cycle and repair

S3/1 - S3/6

S3/1

IMPACT OF NUCLEOTIDE EXCISION REPAIR ON ACUTE AND LONG TERM EFFECTS OF DNA DAMAGE IN MICE

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Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are genetic disorders associated with defects in nucleotide excision repair (NER). Two NER sub-pathways have been identified either dealing with repair of lesions in the transcribed strand of active genes (transcription-coupled repair, TCR) or with repair of bulk chromatin (global genome repair, GGR). We have assessed the contribution of TCR and GGR to repair, mutagenesis and cancer in cells and transgenic mice with defined deficiencies in the NER sub-pathways employing different genotoxic agents. Human and mouse XPC fibroblasts are defective in GGR of UV photolesions, but exhibit a normal level of TCR. The key role of TCR in alleviating the adverse effects of DNA damage is illustrated by a 10-fold higher resistance of XPC mice to acute toxic effects (erythema, killing) of UVB irradiation and DMBA treatment when compared to XPA (defective in GGR and TCR) or CSB (defective in TCR) mice.). Upon UVB treatment, XPA and CSB mice revealed an extensive apoptotic response in the skin and a blockage of cell cycle progression of epidermal cells. Interestingly, the absence of this apoptotic response in the skin of XPC and wt mice coincided with the ability of epidermal cells to progress through S-phase. However, only epidermal cells of XPC mice subsequently arrested in G2-phase. Our data demonstrate that TCR (and/or restoration of UVB-inhibited transcription) enables damaged cells to progress through S-phase and prevents the induction of apoptosis. G2 arrest is only manifest under conditions of proficient TCR in combination with deficient GGR indicating that epidermal cells arrest in the G2-phase as a result of persisting damage in their genome.
In spite of the resistance to acute UVB effects, XPC are as prone as XPA mice to skin cancer induction by UVB irradiation whereas skin cancer develops more slowly in CSB mice This indicates that expression of GGR (and to lesser extent TCR) is critical for counteracting UVB induced skin cancer in mice. Treatment of XPC mice with DMBA resulted in a hypermutable phenotype. Unexpectedly, aging XPC mice show an highly elevated spontaneous Hprt mutation frequency in splenic T-lymphocytes in contrast to XPA and CSB mice. These results will be discussed in relation to endogenous DNA damage (requiring NER for its repair), inhibition of transcription, restoration of DNA damage-inhibited RNA synthesis and apoptotic response. Surprisingly, although during aging the spontaneous mutation frequency dramatically increased in XPC mice, the animals did not show elevated frequencies of cancer.

S3/2

INTRAGENOMIC HETEROGENEITY AND CHROMOSOME INSTABILITY: INSIGHTS FROM ADVANCED MOLECULAR CYTOGENETICS

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Over the last decade, a number of studies have addressed the fundamental question as to whether chromosome damage is randomly distributed, with special emphasis in chromosome length. However, chromosomes are heterogeneous not only in length but also in gene density, genetic activity, chromatin structure, replication timing, nuclear architecture, telomere length, etc. Some of these factors can modulate the induction, processing and/or persistence of chromosome aberrations. We have used advanced molecular cytogenetic techniques including (i) multicolour painting, (ii) immunocytogenetics combined with fluorescence in situ hybridization (FISH), (iii) quantitative FISH (Q-FISH), (iv) arm-specific painting in prematurely condensed (PCC-FISH) interphase and metaphase euchromatic and heterochromatic hamster chromosomes, and (v) interphase FISH with probes labelling heterochromatic (1cen-1q12) and euchromatic (17cen-p53) regions, to unravel new factors modulating the induction, processing and/or persistence of radiation-induced chromosome aberrations in mammalian cells, both in vitro (cell lines and splenocytes) or in vivo (lymphocytes and buccal cells from radioactive iodine exposed thyroid disease patients). Our results provide evidences that chromosome length, gene density and genetic activity do not modulate the induction and long-term persistence of chromosome aberrations in vitro or in vivo in a 4 years follow-up study. We will show recent experimental data supporting the view that nuclear architecture in chromosome territories modulates the processing of radiation-induced chomosome breaks and that telomeric length and chromatin structure could play a role in the induction and persistence of chromosome damage.

S3/3

INHIBITORY PATHWAYS IN CELL CYCLE REGULATION IN NON-TRANSFORMED HUMAN FIBROBLAST MODELS

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It is widely accepted that cell proliferation is regulated by the cyclin-dependent kinases (cdk-s) around the restriction point in the G1 phase of the cell cycle. The mechanism was clarified regarding the proliferating populations but almost nothing is known about the regulatory processes active in resting/G0 cell population, maintaining the resting state. We are studying the behavior of the cdk related regulatory pathways in non-transformed human cell systems (WI-38 and MRC5 cell lines). Both cell lines grow in monolayer, exhibit contact inhibition and their proliferation is serum dependent. Moreover, all studied proliferation regulatory pathways are wild- type (p53, cdk-s, cyclines, RB, cdk inhibitors). Using a proliferation panel composed of cultures in different (exponentially growing, serum starved and contact inhibited) proliferation states we examined p53, $p21^{WAF1}$, p15, p16 expression at both the mRNA and protein level in parallel cultures. Our results clearly shows that the regulatory mechanisms of proliferating cell are different from the control processes active in cell cycle arrest and/or in resting cell population. In our experimental system it seems, that the p53 protein levels do not activate the transcription of $p21^{WAF1}$ mRNA in silent, non-proliferating cells, and the p53 protein level does not correlate with the cellular p53 mRNA content. On the basis of our results we propose a p53 - independent control mechanism which keep cells in non-proliferating state in fibroblast-originated non/transformed models.

S3/4

CENTROSOME-MEDIATED CONTROL OF THE TRANSITION PROPHASE-METAPHASE: EXPERIMENTS WITH NORMAL AND TUMOUR CELLS

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Chromosomal instability (CIN) has been largely reported in many human tumour. The causes of CIN are largely unknown and an expected source for this phenomenon is related to defects in the mitotic apparatus functioning, in which centrosomes may play a major role. A G2-to-M transition checkpoint mediated by an impaired centrosome functioning, has been recently proposed. Interestingly, recent data show that centrosomes are frequently alterated in their number/morphology in hystochemical specimens of many human tumours.
To test the hypothesis of "centrosome functioning alterations", human primary fibroblasts and tumour cells (HeLa, MCF-7, Hep-2,Hep-G2,SiHa,Me180) were treated with Diazepam (DZ), a benzodiazepine known to arrest cells at the metaphase stage by interfering with centrosome functioning (i.e."maturation", separation). Cells incubated 6 or 12 h with 80 ug/ml DZ were immunostained with g- and a-tubulin, to monitor the presence of monopolar/bipolar spindles. Interestingly, 5 out 6 tumour cells, showed a high percentage of bipolar spindles with non-congressed chromosomes. The level of proteins involved in G2/M transition (cyclin-B1, p34CDC2) were also evaluated by immunoblot to check whether a less stringent control of centrosome splitting might be relate to overexpression of such proteins. In particular, the level of the kinase p34CDC2 appared higher in tumour compared to normal cells.
These results seem to support the hypothesis that centrosome functioning greatly differed between normal and maligniant cells.
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S3/5

HUMAN DNA BASE EXCISION REPAIR MEASURED WITH THE COMET ASSAY REVEALS INTER-INDIVIDUAL DIFFERENCES

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DNA repair plays a key role in preventing mutations arising from DNA damage, and differences in DNA repair capacity can help determine individual cancer risk. Inherited DNA repair disorders are associated with elevated cancer risk. But whether DNA repair activity in normal subjects varies significantly is not known, largely because of the lack of a convenient, sensitive assay. We have tested two variants of the comet assay to measure repair of oxidative damage.
1. Lymphocytes were treated with H_2O_2 and incubated; at intervals, samples were analysed for DNA breaks with the standard comet assay. After several hours, breaks were still present, suggesting slow repair, but analysis is complicated since further damage seems to be introduced by exposure to atmospheric oxygen.
2. To avoid this problem, we now measure the repair reaction in vitro. A DNA substrate containing 8-oxoguanine is prepared by treating cells with phosensitiser Ro 19-8022 (Hoffmann-La Roche) and visible light. The cells are embedded in agarose, lysed, and then incubated for different times with crude lymphocyte extract. Incisions at damage sites are measured. Extracts from lymphocytes from 5 individuals give reproducibly different time-courses of accumulation of DNA breaks. This new assay is a potentially valuable tool for molecular epidemiological studies.

S3/6

ANALYSIS OF THE MICROSATELLITE INSTABILITY IN DNA REPAIR MUTANTS OF DROSOPHILA MELANOGASTER

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Due to the homology between yeast and mammals, considerable advances have been made in the last years in the knowledge of the repair mechanism in eukaryotes. Nevertheless, the repair mechanisms in Drosophila are still not well known.
One of these mechanisms, the mismatch repair, has been associated to genomic instability, consequently the failure of mismatch repair increases microsatellite instability, both in bacteria and in eukaryotes. Recently, a new gene that is homologous to the yeast MSH2 gene and associated to microsatellite instability has been identified in Drosophila.
Around 30 different mutants, associated to repair deficiencies in the DNA, have been identified in Drosophila, but in many of them, the function of the gene product is still unknown. The aim of this work is to determine if some of these mutants present microsatellite instability, which could be associated to alterations in the mismatch repair process.
For this reason, we analysed the instability of 11 microsatellite sequences in individual offspring from males treated with acethylaminofluorene (AAF) crossed with females from different DNA repair deficient strains. Our results indicate that under these experimental conditions none of the strains, mus-201, mus-205, mus-207, mus-308, mei-9, mei-41 and spel1, show microsatellite instability induced by AAF.

Symposium 4

GENETIC SUSCEPTIBILITY TO ENVIROMENTAL TOXICANTS

S4/1 - S4/15

ECETOC IN BRIEF

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), was established in 1978 as a scientific, non-commercial association; it is financed by over fifty companies with interests in the manufacture and use of chemicals.

The main objective of our activities is to identify, evaluate and minimise any potentially adverse effects on health and the environment which might arise from the manufacture and use of chemicals.

To meet this objective, we facilitate the networking of suitably-qualified scientists from our member companies. The output of our activities includes Technical Reports and monographs reflecting the current state of the science for the issue under review.

An internal peer-review process has ensured that we have earned recognition and respect by external bodies for scientific integrity. We have become a valued partner with many other organisations and regulatory bodies, such as the World Health Organization (WHO) notably the International Programme on Chemical Safety (IPCS) and the European Commission (EC), in establishing a scientific foundation for the development of legislation on chemicals.

ECETOC MODUS OPERANDI

Board
ECETOC operates under the general direction of a Board comprised of up to twelve senior executives from member companies. The Board is responsible for the overall policy and finance of the association.

Scientific Committee
Crucial to the success of ECETOC in establishing and maintaining its authority and reputation as a source of sound scientific information and judgement, is its Scientific Committee. Composed of fifteen top industry scientists (mainly toxicologists, ecotoxicologists and physicians) the Committee is appointed by the ECETOC Board. Members are selected on the basis of their proven scientific expertise, thereby underpinning their role of assuring sound scientific standards and quality.

The Scientific Committee is responsible for the definition, management and peer review of the ECETOC work programme. A major part of this work programme is the production of ECETOC publications by Task Forces appointed by the Scientific Committee.

Task Forces
ECETOC publications are produced by Task Forces composed of appropriate experts drawn from member companies and other organisations as required. Although all member companies have the opportunity to nominate members to the Task Forces, their final composition is subject to endorsement by the Scientific Committee, taking into account the range of skills required to address the selected topic. The work of the Task Force follows the Terms of Reference established by the Scientific Committee and is directed by a Chairman who is appointed to the task by the Scientific Committee. Most but not all Task Force activities result in one or more ECETOC publications. The specific objectives of the other projects undertaken by Task Forces vary, and frequently involve activities with other organisations.

Secretariat
The Board, Scientific Committee and Task Forces are supported and assisted in their activities by a small team of scientists with administrative support, led by the Secretary General.

Programme Selection
A topic for consideration by ECETOC may be proposed by any member company or any other organisation whether trade association, academia or regulatory authority. For the proposal to be progressed it must be supported by at least two member companies; in addition it must be judged to meet the scientific standards required by the Scientific Committee. Provided these criteria are met, specific Terms of Reference are drawn up and endorsed by the Scientific Committee prior to selection of Task Force members.

Publications
The main output of ECETOC's Task Force activities is the publication of a range of reports varying in scope from the 'JACC' reports on specific chemicals to 'Monographs', dealing with the fundamental principles underlying the various branches of science in toxicology and ecotoxicology. All reports are published following peer review by the Scientific Committee and copies are sent to all member companies and to other interested parties, such as the various regulatory authorities, international organisations and academic groups, for use as required.

Website
The ECETOC Website communicates the goals, objectives, output and activities of the Association to the outside world, provides an additional information source and point of contact to our members and supports the Committees and Task Forces in developing their outputs.

Representation
ECETOC regularly receives invitations to send representatives and observers to a variety of fora, such as the IPCS, OECD, IARC and the EC groups, where the health and environmental effects of chemicals are discussed and evaluated.

Workshops and Seminars
Workshops and seminars are convened, often in partnership with other interested parties and groups, in order to develop and communicate understanding and counsel on the key issues affecting the responsible environmental management of chemicals.

S4/1

GENETIC SUSCEPTIBILITY: CURRENT AND FUTURE PERSPECTIVES

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Disease susceptibility to environmental and dietary chemicals is often determined by heritable differences in polymorphic genes that encode enzymes that either metabolically activate or detoxify these agents. Genetic polymorphisms, which are usually defined as genetically-determined differences in >=2% of the population, may be due to gene deletions or truncations, but they most commonly arise due to point mutations in coding or non-coding regions of the gene. These variations result in altered catalytic activity, and/or stability or expression of the enzyme or mRNA. Some of the enzyme systems that have been the most widely studied include the cytochromes P450 (CYPs), the N-acetyltransferases (NATs), sulfotransferases (SULTs), glucuronosyltransferases (UGTs), and the glutathione S-transferases (GSTs). Recent examples from our laboratory on genetic polymorphisms for CYP3A4, NAT1, NAT2, SULT1A1, GSTA1, and GSTA2 in relation to adverse health outcomes will be presented. In addition, the development and application of DNA microarray chip technology, which utilizes allele-specific PCR with an immobilized probe, for high throughput genotyping will also be described.[In collaboration with Mary Wolff, Trudy Berkowitz, Robert DeLongchamp, Kristin Anderson, Myron Gross, Christine Ambrosone, Susan Nowell, Nicholas Lang, Elena Martinez, David Alberts, Brian Coles, Charles Wang, Patricia Thompson, Michael Hogan].

S4/2

CYP-FAMILY OF ENZYMES

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The majority of human P450-dependent xenobiotic metabolism is carried out by polymorphic enzymes which can cause abolished, quantitatively or qualitatively altered or enhanced metabolism. The latter situation is due to stable duplication, multiduplication or amplification of active genes, most likely in response to dietary components that have resulted in a selection of alleles with multiple non-inducible genes. At present functionally important polymorphic CYP variants have been described for CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 in addition to CYP17, CYP19 and CYP21 active in steroid biotransformatiion. An updated list of variant CYP alleles is always present at the Home Page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (http://www.imm.ki.se/CYPalleles/). At present more than 80 different allelic variants for CYP2D6 have been described and with respect to CYP1B1, five common functional mutations are present in the gene yielding 25 possible different forms of the enzyme, although only 9 have yet been proven to exist. Several examples exist where subjects carrying certain alleles suffer from a lack of drug efficacy due to ultrarapid metabolism or, alternatively, adverse effects from the drug treatment due to the presence of defective alleles. With respect to the metabolism of precarcinogens the functional evidence for the role of CYP polymorphism in the susceptibility for carcinogenic or toxic susceptibility is currently less convincing, although being a subject for intensive research.

S4/3

GST FAMILY OF ENZYMES

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The glutathione S-transferase (GST) supergene family encodes proteins expressed in tissue cytosols or, cell membranes. They catalyse the detoxication of a variety of electrophilic compounds including many products of oxidant stress. Polymorphism has been identified in various GST genes (including GSTM1, GSTM3, GSTT1, GSTT2, GSTP1). The possibility that particular alleles, particularly those associated with impaired enzyme activity (GSTM1*0, GSTT1*0), confer altered disease susceptibility and outcome has attracted much interest. We have focused on high-risk subgroups in several disease cohorts in the expectation that the influence of polymorphism will be most evident in such patients. Thus, we found strong associations between GSTP1 genotypes and bronchial hyper-responsiveness; GSTP1 Val^{105}/Val^{105} was significantly lower in asthmatics than controls. Indeed, this genotype conferred a 6-fold decrease in asthma risk compared with GSTP1 Ile^{105}/Ile^{105}. This risk was reduced to 9-fold after correction for atopic indices, age and gender. These data suggest this gene is an alternative asthma candidate on chromosome 11q13. Similarly, we found in basal cell carcinoma cases, significant associations (with large odds ratios) between GSTT1 null and phenotypes associated with large numbers of tumours. In particular the genotype was associated with presentation with multiple clusters of primary tumours. We have little data on how and in what numbers, protective and risk genotypes interact to determine a phenotype. Further, the relationship between genotype and expression may be unclear given the possibility of epigenetic changes (eg methylation).

Acknowledgements.
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S4/4

MOLECULAR BIOLOGY OF NAT2 AND ITS ROLE AS RISK FACTOR

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A large body of experimental and epidemiological literature has documented the relationship between exposure to homocyclic and heterocyclic amines and the development of tumors at a variety of tissue sites. These chemicals are not carcinogenic per se, but require metabolic activation by enzymes of xenobiotic biotransformation to electrophiles that bind to nucleophilic sites on DNA bases and produce mutations that advance cells to a more malignant phenotype. Among the enzymes known to take part in competing pathways of aromatic amine activation and detoxication are the arylamine N-acetyltransferases NAT1 and NAT2. NAT2 is the site of the classical isoniazid acetylation polymorphism, while NAT1 has recently also been shown to exhibit genetic variation in human populations. The molecular basis and functional consequences of the phenotypic variations in NAT1 and NAT2 have been extensively studied. Epidemiological studies have also provided many clues concerning the importance of variations in both NAT1 and NAT2 in altering risk for cancers following aromatic amine exposure. For instance, the NAT2 slow acetylator phenotype has been associated with an increased risk for bladder cancer in many studies. On the other hand, the link between NAT2 acetylation status and breast cancer is more controversial, with variable results that may reflect differences in types and levels of arylamine exposures in the various studies. The NAT2 rapid acetylator phenotype has also been associated with an increased incidence of lung and laryngeal cancers in smokers, who are exposed to compounds such as 4-aminobiphenyl. On the other hand, no association has been observed between NAT2 acetylator phenotype and risk for prostate cancer. Current and future studies will need to take into account the interaction of multiple metabolic and cellular response factors, each of which may exhibit genetic variation, in elucidating the true impact of each in producing a toxic endpoint such as tumor formation.

S4/5

THE GENETICS OF SULPHOTRANSFERASES

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Cytosolic sulphotransferases (SULT) play an important role in the regulation of various hormones as well as in the biotransformation of xenobiotics. SULT, like other conjugating enzymes, have been associated primarily with detoxification. However, it is now evident that SULT are also able to activate a large number of promutagens. In human, eleven different forms of SULT have been detected. For two forms (SULT1A1 and SULT1A2), functionally important genetic polymorphisms are known; most of the other forms have not yet been studied to a significant extent with regard to polymorphisms. We have individually expressed all human SULT forms and their known allelic variants, as well as various rat and mouse SULT forms, in Ames's Salmonella typhimurium strains and we have characterised their substrate specificity towards promutagens. These substrate specificities differed strongly even between allelic variants of the same SULT form. In addition we have genotyped about 2000 subjects. Associations were detected between the SULT genotype and obesity as well as certain types of cancer. Some data from our and other laboratories also suggest a possible association between SULT genotype and longevity.
Supported by Deutsche Forschungsgemeinschaft (INK 26) and BMBF (grant 0311241).

S4/6

INFLUENCE OF HUMAN GST POLYMORPHISMS ON THE METABOLISM AND TOXICITY OF ETHYLENE OXIDE AND ACRYLONITRILE

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As part of a medical surveillance programme of 59 individuals having occupational contact with AN the hemoglobin adducts CEV [N-(cyanoethyl)valine], as biomonitoring parameter for AN, and HEV [N-(hydroxyethyl)valine], as parameter for ethylene oxide (EO), were assessed. Moreover, the genotypes of polymorphic GSTs were determined. Mean values (± S.D.) of the entire group were adduct levels of 59.8 ± 51.5 µg CEV/l blood and 17.0 ± 7.3 µg HEV/l blood. 25 persons carried at least one mutated allele at the GSTP1 A105G locus, 5 were homozygotes. The CEV and HEV adduct levels correlated significantly with the presence of this mutation (w/w< w/m<m/m). All subjects showing a mutated GSTP1 C114T locus were also carrying at least one A105G mutation; no homozygous mutants were found at the C114T locus. The hemoglobin HEV and CEV adduct levels were also significantly higher in the GSTP1 C114T heterozygotes. No influence of the GSTM1 or GSTT1 genotype was detected. The results suggest that AN is significantly metabolized by GSTP1, not by GSTM1 or GSTT1. Regarding of polymoprhism of GSTT1, there was a significant difference in the HEV adduct levels which are attributed to the endogenous ethylene oxide, indepent of the smoking habits. This known effect was confirmed in the present study; in addition, there was a similar effect of GSTP1 polymorphism on the background HEV levels. The differences in disposition of endogenous ethylene oxide induced by polymorphic GSTs are viewed on the background of differences in sister chromatid exchange levels.

S4/7

FISH ANALYSIS OF MICRONUCLEI IN NASAL EPITHELIUM OF STAINLESS STEEL WORKERS EXPOSED TO CHROMIUM: INDIVIDUAL SUSCEPTIBILITY FACTORS

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Workers in stainless steel production may be exposed to hexavalent chromium considered to be a nasal carcinogen. The present study aimed at investigating whether this exposure is high enough to induce an increase in micronucleated nasal epithelial cells. The study population comprised 29 men exposed to hexavalent chromium, 14 men exposed to trivalent chromium, 5 men exposed to chromite ore, and 39 industrial referents not exposed to known genotoxins. All the subjects were nonsmokers. To distinguish between micronuclei (MN) harboring whole chromosomes and chromosomal fragments, the contents of the MN were examined by fluorescence in situ hybridization (FISH) using a pancentromeric DNA probe. The subjects were genotyped for glutathione S-transferases (GST) M1, M3, P1 and T1, and N-acetyltransferases (NAT) 1 and 2, to control for possible differences in individual susceptibility due to genetic polymorphisms of xenobiotic-metabolizing enzymes. Occupational exposure did not significantly affect the frequencies of centromere-positive (C+) or -negative (C-) MN. In general, about 1/3 of all MN were C+. Homozygous deletion of GSTM1 (null genotype) together with the presence of erythrocytes in the cell specimens was associated with an increased baseline frequency of C- MN. Baseline levels of C+ MN were increased in subjects who had erythrocytes in their samples, a combination of the GSTM1 null and the NAT2 slow acetylation genotype, or variant GSTM3 genotype. In conclusion, occupational exposure did not influence the frequency of MN in nasal epithelial cells of stainless steel production workers. The baseline level of MN was affected by polymorphisms of GSTM1, GSTM3, and NAT2, and by the presence of erythrocytes in the samples; the reasons for these findings are presently unknown.

S4/8

GST POLYMORPHISM IN RELATION TO SMOKING, OXIDATIVE STRESS AND ANTIOXIDANT PROTECTION

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Glutathione S-transferase genotypes GSTT1, GSTM1, GSTP1 were characterised in 155 middle-aged men and compared with parameters of oxidative stress at the level of DNA and lipids, with antioxidant enzymes, and with plasma antioxidants in smokers and non-smokers. Persons with normal genotype GSTM1(+) had significantly higher levels of glutathione (GSH) than those with the GSTM1(-) genotype. The homoyzgous GSTP1a-1a genotype was associated with significantly higher levels of GST activity measured in lymphocytes, in comparison with homozygous GSTP1b-1b genotype. Sensitivity of lymphocyte DNA to H_2O_2 -induced damage was slightly higher in GSTT1(-) subjects compared with GSTT1(+) genotype. Using multifactorial statistical analysis we found significant associations between smoking, GSTP1 genotype, and level of oxidised purines, vitamin C, GSH, and GST. Smokers with the GSTP1b-b genotype had greatly increased levels of oxidised purines. Smokers with the GSTP1b-1b genotype had significantly higher levels of glutathione in comparison with GSTP1a-1b or GSTP1a-1a genotype. Smokers had lower levels of vitamin C than non-smokers and this was accentuated in those with GSTP1b-1b genotype. Oxidative DNA damage (endo III sites) was associated with smoking and GSTT1. The highest levels of net endo III sites were found in smokers with GSTT1(-) genotype. We found an association between smoking, GSTT1(-) and levels of total antioxidant capacity (FRAP). High alcohol consumption with GSTM1 genotype was associated with lower levels of vitamin C and Zn. An association between smoking, GSTM1 genotype and glutathione peroxidase (GPX) was also found.

S4/9

IMPACT OF METABOLIC GENOTYPES ON LEVELS OF BIOMARKERS OF EXPOSURE

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Phase I and Phase II enzyme families, including cytochrome P-450s (CYP), glutathione S-transferases (GST), N-acetyltransferases (NAT) and NAD(P)H:quinone oxidoreductase (NQO1) are involved in the metabolic activation and detoxification of various classes of environmental carcinogens. Particular genetic polymorphisms of these enzymes have been hypothesized to influence the levels of biomarkers of exposure to genotoxic agents in tobacco smoke and in occupational settings. The presentation gives an overview of the current knowledge from the literature of the relationship between metabolic genotypes and internal dose, biologically effective dose and cytogenetic effects of complex and specific genotoxic exposures of human study populations, and we report results from our recent studies. To explore the role of possible pharmacogenetic risk factors in the formation of direct DNA damage, we investigated the associations of various combinations of CYP1A1, CYP2C9, NQO1, GSTM1 and GSTP1 genotypes with the levels of aromatic carcinogen-DNA adducts, determined by $_{32}$P-postlabelling, in bronchial tissue from lung patients (Schoket et al, 1998, Ozawa et al, 1999) and in peripheral blood lymphocytes, as determined by 32P-postlabelling and benzo(a)pyrene-dG-DNA ELISA, from workers occupationally exposed to polycyclic aromatic hydrocarbons in aluminium foundries. Some relationships between levels of DNA adducts and metabolic genotypes have been found statistically significant, however, further research is needed to clarify controversies and the molecular mechanisms.

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S4/10

DIFFERENCES IN INDIVIDUAL SUSCEPTIBILITY LINEARIZE THE DOSE-RESPONSE RELATIONSHIP IN CHEMICAL CARCINOGENESIS AND RENDER THRESHOLDS IMPLAUSIBLE FOR A HETEROGENEOUS POPULATION

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Two types of dose-response relationships have to be distinguished in toxicology. The first type describes a dose-effect intensity relationship in one cell, organ, animal, or human individual. The second type describes a dose-incidence relationship in a population and is about the fraction of the population that shows a defined effect at a given dose within a specified period of observation. For cancer incidence, the probability for an individual to be affected can only be 0 or 1, depending on whether it is before or after tumor diagnosis. The individual that manifests cancer at the lowest dose could be considered to be the most susceptible in the given population. The dose-response curve therefore reflects the tolerance distribution. This understanding has two main consequences for cancer risk assessment: (i) a threshold dose can be defined for each individual. It is the dose required to induce the tumor at exactly the end of the period of observation. For a population, no single threshold dose can exist. (ii) Population heterogeneity linearizes the dose-response relationship. Both genetic and life-style factors modulate the rate of the process of carcinogenesis. Each modulating factor splits the population up into subpopulations of different susceptibility so that nonlinearities which could be present in a homogeneous population are flattened out. A linear extrapolation of a human cancer risk to dose zero might therefore be appropriate under certain conditions even if the dose-response curve in animals showed a strongly sigmoidal shape. For cancer prevention, the elimination of risk factors in the most susceptible subpopulations or individuals might be more effective than a minor general reduction of an exposure limit.

Reference: W.K. Lutz (1999) Hum Exp Toxicol 18: 707-712

S4/11

GENETIC SUSCEPTIBILITY AND ENVIRONMENTAL OESTROGEN-LIKE COMPOUNDS

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Polymorphisms in CYP17, CYP11a, CYP19, hydroxysteroid hydrogenase, steroid sulphatase as well as enzymes further hydroxylating oestradiol such as CYP1A1, CYP3A4, CYP1B1 may have a possible role in the link between environmental estrogens and hormone-like substances and the interindividual risk of breast cancer [rev1].It has been hypothesised that high 17a -hydroxylase/17,20-lyase activity may be at least one of the biochemical determinants of increased breast cancer risk. A polymorphism (a T-C substitution) in the 5' untranslated region, at +27 relative to the start of transcription, has been found more frequent in postmenopausal patients with advanced breast cancer than in controls and was associated with higher serum hormone levels in young healthy individuals. The substitution has several times been discussed to create a putative Sp-1 binding site (CCACT-CCACC) thus providing a mechanism for higher expression of the variant allele. The ability of motifs of CYP17 5'UTR, containing the T-C polymorphic site to bind to the human transcription factor Sp-1 in vitro has been investigated in our laboratory [2]. Local conversions of estrogens as well as the conversion of androstenedione to oestrone are catalysed by the aromatase cytochrome P450 complex (CYP19). An alternative switch from the usual adipose tissue promoter to an apparently stronger "ovary" promoter correlates with the level of CYP19 mRNA expression ($p<0,001$) [3]. Using a convenient method for detection of AT-rich repeats [4] we analysed 336 breast cancer patients and 172 control individuals. The allele, containing the largest number of repeats $(TTTA)_{12}$, was found to be more frequent in patients than in controls ($p=0.046$), indicating that individuals carrying the long allele of CYP19 may have a higher risk of developing breast cancer [5]. Another, C-T substitution + 19 bp in the 3'UTR was investigated. We modified the automated DNA sequencing method for mutation detection [6] and could demonstrate that this much more frequent polymorphism is in strong linkage disequilibrium with the (TTTA)n polymorphism in intron 5 ($p<0,0001$). Accordingly, we found a significant difference in distribution of genotypes between breast cancer patients and controls ($p=0,007$), particularly among those presenting with high stage disease ($p=0,004$) and with tumours larger than 5cm($p=0,001$) [3].

S4/12

RISK ASSESSMENT. THE IMPORTANCE OF GENETIC POLYMORPHISMS IN MAN

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In risk assessment the safety factor of 10 is generally accepted to allow for variation in individual susceptibility. Reviewing the literature justifies the factor of 10 when considering single polymorphisms.
Many genetic polymorphism in metabolism enzymes are important for the risk of cancer as shown in a large number of case-control studies. These polymorphisms are effect modifiers, i.e. without exposure they have no consequence and the effect can appear independent of the genotype.The relative risk estimates have shown large variations between such population studies. However, in meta-analyses the relative risk estimates are usually below 2. Similarly genetic polymorphisms in metabolism of environmental toxicants plays a significant role in exposures to traffic generated air pollution in Copenhagen, revealing higher levels of chromosomal aberrations and DNA-adducts in non smoking bus drivers with GSTm0 and NAT2 slow genotypes. Such results indicates an increased genotype dependent cancer risk. Actual calculations indicate the relative risk being below 2.
Accordingly, the safety factor should cover single genetic polymorphisms. However in an individual with several susceptible metabolism genotypes as well as other determinants of suceptibility, e.g. defective DNA repair, poor nutritional state etc. the risk may increase far above a safety of 10.
Historically genetic polymorphisms have been taken into consideration in employment and currently the application in insurance situations is critizised.

S4/13

ASSOCIATION BETWEEN GENETIC POLYMORPHISMS AND BIOMARKERS IN STYRENE-EXPOSED WORKERS

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A comprehensive approach to evaluate DNA damage caused by styrene exposure was employed in 44 hand-lamination workers. The acquired data on single-strand breaks in DNA, frequency of chromosomal aberrations and HPRT mutant frequency were compared to the results of genotyping some xenobiotic-metabolizing enzymes (CYP 2E1, 1A1, epoxide hydrolase-EPHX and GST M1, P1 and T1). Statistical analyses revealed that single-strand breaks in DNA (SSB) are significantly influenced by genotype CYP 2E1Rsa, multifactorial regression analysis (stepwise selection) indicate that SSB are associated with styrene exposure and CYP 2E1Rsa, R^2=0.614. The frequency of chromosomal aberrations (CA), as analysed by multiple regression analysis, significantly correlated with years of employment (P=0.004) and with EPHX genotype (P=0.044), where individuals with low activity EPHX genotype exhibited higher frequencies of CA. R^2 for this model was 0.563. ANOVA revealed that mutant frequencies at HPRT gene were significantly associated with years of employment (F=6.9, P=0.0001), styrene in blood (F=10.1, P=0.0001), CYP 2E1Dra (F=13.5, P=0.0008) and with GSTP1 (F=3.6, P=0.038). Our results suggest that analysed biomarkers are mainly modulated by polymorphic EPHX and CYP 2E1. In our study styrene-specific DNA and haemoglobin adducts are under investigation. Completing these datra with the whole set of genotypes on metabolizing enzymes may provide a useful tool for individual risk assessment.

S4/14

GENETIC POLYMORPHISMS IN MICROSOMAL EPOXIDE HYDROLASE AND CYP2E1 AND SUSCEPTIBILITY TO ADULT ACUTE MYELOID LEUKAEMIA WITH RESPECT TO DEFINED CYTOGENETIC ABNORMALITIES

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Non-occupational exposure to benzene, a risk factor for acute myeloid leukaemia (AML), comes from various origins including smoking. It was suggested that defined chromosomal abnormalities could be aetiologic indicators for AML. Our purpose was to test the hypothesis that smoking as well as genetic polymorphisms in enzymes involved in benzene metabolism could be risk factors for AML with del(7q) or t(8;21) but not for AML with normal karyotype. Twenty six AML patients with del(7q) and 24 with t(8;21) as well as 43 with normal karyotype and their 155 individually matched-controls were selected from a large population-based case-control study on incident adult acute leukaemia. Genetic polymorphisms in the 5'flanking region of CYP2E1 and in exons 3 and 4 of microsomal epoxide hydrolase (mEPXH) were determined through PCR-RFLP technics. Current smoking was significantly associated with AML with t(8;21) (OR=8.4; 95%CI=1.7-42.0) or del(7q) (OR=3.3; 95%CI=1.1-9.9) but not with AML with normal karyotype, relative to persons who were not current smokers. Genetic polymorphism in CYP2E1 was not associated with AML whatever the subset of cases and the gender. Putative high activity phenotype of mEPXH was associated with a significant increase in risk for AML in males with del(7q) or t(8;21) (OR=7.1; 95%CI=1.3-38.8) but not for AML with normal karyotype. Defined chromosomal aberrations in AML patients could be relevant fingerprints of exposure to carcinogens. Smoking and genetic polymorphisms in mEPHX, associated with toxification of benzene, could be risk factors for AML with del(7q) or t(8;21).

S4/15

ETHICAL IMPLICATIONS OF GENETIC ANALYSIS OF INDIVIDUAL SUSCEPTIBILITY

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The task of ethics is to question the validity of existing moral codes time and time again, always against the most current stage of knowledge. One cannot relax and consider the work done within the ethics of science any more than within the science itself. Genetic data is increasing exponentially or quicker. Several burning questions can be identified within the science of genetic analysis in general and genetic analysis for individual susceptibility especially. In addition to the safety of the persons studied, long-term implications on science, individuals and society exist. Is the purpose of the study ethically sound? Are we aware of the forces that keep us going? Unconscious motives and myopia of science are not generally recognized (1,2). Accuracy of the data is not self-evident, either. It is important to ask whether the methods used have been validated for the purpose and the work carried out thoroughly and well-controlled (3). Furthermore, one should also ask how relevant is the data. Does the analysis answer to the questions raised and what is the stage of development of the field (4)? Who owns the tissues studied and the data created using them: society, organisation, scientist or the person him/herself? Body tissues have also social and cultural meaning (1,5). Further implications include communication and the use of data (3,6). Protection or discrimination may be the end result even if such aspects are not originally pursued.

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Symposium 5

Germ cell cytogenetics and transgenerational mutagenesis

S5/1 - S5/5

S5/1

IN VITRO MATURATION OF OOCYTES AND PREANTRAL FOLLICLE CULTURE IN DETECTION OF ANEUGENS AND CHEMICALS REDUCING DEVELOPMENTAL CAPACITY AND FERTILITY

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Oocytes are extremely longlived cells and oogenesis in mammals is characterized by distinct periods of endowment of oocytes and primordial follicles, apoptosis and atresia of follicles and germ cells, and resumption of maturation of oocytes preceded by rapid proliferation of granu-losa cells prior to ovulation. Oocytes are unique in possessing components for self-assembly of spindles in absence of centrosomes and metaphase II arrest until fertilization. These features and the high risk of trisomic conceptions due to errors in chromosome segregation during oogenesis suggest that mammalian oocytes may be uniquely suceptible to certain types of aneugens. We provide evidence that chemicals interfering with chromosome congression like the pesticide trichlorfon have the potential to severely interfere with fidelity of chromosome segregation during oocyte maturation. Oocytes appear to lack a cell cycle checkpoint sensing such conditions. In vitro maturing mouse oocytes present a good model to detect the mode of action, metabolite and sensitive stage of chemical exposures while oocytes form a spindle during the last stages of oogenesis before ovulation and fertilization. To detect chemicals which may mainly exert their effect by interfering with folliculogenesis earlier in oocyte development thus reducing oocyte quality and increasing the risk for nondisjunction and random segregation of susceptible chromosomal configurations we have extended the in vitro model. In preantral follicle culture oocytes grow inside of a follicle during a 12 day culture. Follicle cells rapidly di-vide and an antrum is formed. After hormonal stimulation oocytes inside of follicles are competent to mature to metaphase II. Initial experiences with this new in vitro test will be presented. Supported by EU (ENV-CT97-0471).

S5/2

MECHANISMS OF CLASTOGENIC DAMAGE IN MALE AND FEMALE GERM CELLS: INSIGHTS FROM THE ANALYSIS OF CHROMOSOME ABERRATIONS IN FIRST CLEAVAGE MOUSE EMBRYOS

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Mutagenic DNA lesions can be induced by many chemical and physical agents in male and female gametes that are transmitted after fertilization, when become converted into chromosome aberrations through mechanisms related with DNA repair and chromatin structure remodelling. Balanced chromosome rearrangements compatible with cell survival and proliferation may be tolerated up to the completion of embryonic and fetal development; nevertheless they might subsequently cause impairment of fertility and congenital mental and physical retardation. Unbalanced aberrations will likely behave as dominant lethal mutations, producing especially preimplantation losses. The fate of the initial DNA damage is conditioned by a number of variables: 1) the chromatin structure and chromosome topology of the targeted cell, which varies between sperm and oocytes as well as during sperm maturation; 2) the initial molecular change in DNA, which is dependent, among other factors, upon the physical and chemical properties of the mutagenic agent; 3) the post-fertilization processing of the primary lesions which is mainly influenced by the genetic and environmental condition of the fertilized oocyte. The cytogenetic analysis of first cleavage metaphases of mouse one-cell embryos has been exploited to test hypotheses based on the above scenario and to obtain quantitative data for genetic risk assessment of chemical exposures. Chromosomes of paternal and maternal origin are separately analyzed and aberrations classified as chromosome- or chromatid-type, breaks or rearrangements, showing patterns specifically related to treatment protocols. The recent technical development in the field of molecular cytogenetics for mouse chromosomes has extended the power of analysis of the approach to the independent quantitation of stable and unstable aberrations, as well as to the analysis of specific chromosomes and chromosome regions. This, coupled with the new powerful models of mice genetically modified for DNA repair and other genes, makes very exciting the future of cytogenetics of embryonic cells.

S5/3

ESTIMATING THE GENETIC RISK ON THE BASIS OF HERITABLE TRANSLOCATION INDUCTION WITH CHEMICAL MUTAGENS

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Chemical exposure of humans can cause genetic disease in the offspring through mutations of chromosomal aberrations induced in the germ cells. Genetic risk can be determined on the basis of results from animal experiments that measure transmission of mutations or chrommosomal aberrations from germ cells to the first generation progeny. Most germ cell mutagenicity studies are performed with male mice. Male germ cells develop through a series of stages which are similar in all mammals including man (Adler, 1996). The timing of the male germ cell development determines the test protocols. Chemical mutagens are characterized by their differential spermatogenic response (Ehling et al., 1972). Sensitive post-meiotic germ cell stages imply a transient genetic risk for the time of exposure duration while sensitive pre-meiotic stages (stem cell spermatogonia) imply a permanent genetic risk throughout reproductive life. The progeny tests, such as the heritable translocation test (HTT) provide data for risk quantification by the indirect method, i.e. by determing a doubling dose, or by the direct method, i.e. by determining the number of induced heritable tanslocations at a given exposure dose. Data from the HTT with the occupationally important chemicals 1,3-butadiene and acrylamide as well as with the chemotherapeutic agent dacarbazine will be used to demonstrate the quantification of genetic risk, ie the risk for the exposed male parent to sire a chromosomally abnormal child, using the parallelogram approach proposed by F.H. Sobels (Sobels, 1989). The genetic risk for all three chemicals is transient, i.e. only conerns post-meiotic germ cell stages and ranges from 30% of the background for butadiene (Pacchierotti et al., 1998) to 100% of the background for dacarbazine.
Adler, 1996, Mutat. Res. 352, 169-172; Ehling et al., 1972, Mutat. Res. 15,175-184; Pacchierotti et a l., 1998, Mutat. Res. 397, 93-115; Sobels, 1989, Mutat. Res. 212, 77-89.

S5/4

INTRINSIC PROPERTIES AND GENOMIC POSITIONS OF MINISATELLITES - FACTORS OF SIGNIFICANCE TO MINISATELLITE GERMLINE MUTATION

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Studies in humans show that minisatellite length-mutations occur by germline-specific recombination-based mechanisms. Hypervariable minisatellites are different with respect to mutation rate and mutation spectrum, and data from humans and mice show that effects on mutation frequency following exposure to an agent may vary between minisatellite loci. To dissect the molecular basis of spontaneous and induced minisatellite length-mutation we have developed a model system in the yeast Saccharomyces cerevisiae in which the human minisatellites MS1, MS32, MS205 and CEB1 have been integrated into chromosome III in the vicinity of a hotspot for meiotic double-strand breaks. At their respective positions in the human genome the mutation rates at these loci range from 0.4 to 13%, while the mutation rates at the same position in yeast vary between 6 and 10%. Tetrad analyses of mutant allele structures demonstrated that inter-allelic transfer of repeats was always a result of conversion. Studies with MS32 showed that tetrads in which one spore disclosed the occurrence of an inter allelic event frequently contained one or two additional spores with mutant alleles, in most cases resulting from intra-allelic events. This indicates that several chromatids can be involved in the meiotic processes resulting in length mutation. In corresponding studies of mutation at MS205, tetrads with more than one mutant allele were rare, but interestingly, tetrads with three viable spores were enriched for length mutants resulting from inter-allelic events. Our results show that minisatellite mutation is a process that is even more complex than previously anticipated, and suggest that recombination events at certain minisatellites can reach a degree of complexity that results in lethality. We believe that properties of a minisatellite also modify the length-mutagenic effect of chemicals by yet unknown interactions with mutational pathways.

ANEUPLOIDY INDUCTION WITH CARBENDAZIM, TAXOL AND TRICHLORFON DETERMINED IN MOUSE SPERM BY MULTI-COLOR FISH

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The induction of aneuploidy was studied in young adult mice by the fluorescence in situ hybrization (FISH) assay. Chemicals chosen for the EU sponsored aneuploidy project were: carbendazim, a fungicide and the active metabolite of benomyl, taxol, an antineoplastic agent and trichlorfon, an insecticide. The aim of the study was to determine if these chemicals are germ cell aneugens. Male (102/ElxCH3H/El)F1 mice were treated once and 22 days later, sperm were collected from the Cauda epididymis. Using coded slides, 10,000 sperm were scored from each of five animals per chemical dose and control groups under a Zeiss Axiophot fluorescence microscope. With 500 mg/kg carbendazim (p.o.) no aneuploidy induction was observed, but a significant increase (10 fold higher) of diploidies was found in the treated group (0.020% vs. 0.002% in the control, $p<0.01$). With 50 mg/kg taxol (i.p.) only a marginal doubling of the disomy frequency (0.064% in the treated vs. 0.038% in the control group, $p=0.06$) was found. Administration of trichlorfon at doses of 200, 300 and 405 mg/kg (p.o.) caused a dose-dependent significant increase of the frequencies of disomic sperm (0.068%, 0.074% and 0.134% vs. 0.046%, 0.042% and 0.056% in the control, respectively). The prevalence of X-X-8 and Y-Y-8 sperm suggests that trichlorfon affected chromosome segregation predominantly during the second meiotic division. In conclusion, these findings indicate that trichlorfon induces aneuploidy in mouse spermatocytes during meiosis, which can be examined by multicolor FISH in sperm of mice. These results provide experimental support that trichlorfon exposure was causally related to the occurrence of congenital abnormality clusters in a Hungarian village.

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Symposium 6

Apoptosis

S6/1 - S6/4

S6/1

APOPTOSIS: MOLECULAR MECHANISMS LINKED TO CLINICAL FINDINGS

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The intense genetic, biochemical and cellular studies of recent years have revealed the existence of an evolutionarily conserved physiological form of cell death. The main molecular elements of the apoptotic program has been described including several signalling pathways (initiated either through cell surface receptors such as fas and TNF or through the mitochondria such as Bax), execution enzymes (e.g. caspases) and natural inhibitors (e.g. members of the bcl-2 family). The molecular findings have soon gained medical significance. In the process of carcinogenesis both the lack of apoptosis inducing elements, such as p53, and the gain of function mutations of anti-apoptosis genes have crucial roles. The "pathology" of apoptosis includes the pathogenetic role of too much apoptosis in degenerative diseases, the link between the lack of apoptosis and autoimmune disease or inappropriate apoptosis in AIDS.
Necrotic death in myocardial infarction or stroke is accompanied by apoptosis and it sometimes appears as the result of shifting the apoptotic program to necrosis. Several factors may be responsible for the latter including the low level of ATP, blocking of caspases or the lack of the action of protein cross-linker transglutaminases. These enzymes have been linked to the molecular program of apoptosis in my laboratory. We have shown that one form of this enzyme is usually induced and activated in dying cells, revealed several apoptotic regulatory elements of the tissue transglutaminase, demonstrated that the cross-links formed by transglutaminases in the apoptotic cells are resistant to proteolytic degradation and the e(g-glutamyl)lysine isodipeptide is released into the extracellular space. The isodipeptide accumulates in blood plasma or cerebrospinal fluid when the apoptosis rate is accelerated under normal and pathologic conditions. Furthermore, we could shift necrotic death to apoptosis transfecting cells by transglutaminase and identified several of its substrate proteins (actin, neuroblastoma protein) cross-linked during apoptosis.

S6/2

ACTIVATION OF APOPTOSIS SIGNALING PATHWAYS BY MICROTUBULE-INTERFERING AGENTS

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Microtubule-inhibitors are known to induce delay of the metaphase/anaphase transition and to trigger apoptosis. We recently demonstrated that inhibition of the microtubules results in a p53-independent and p53-dependent induction of apoptosis, and a p53-dependent control of the G1/S-transition after mitotic slippage. (Casenghi et al., 1999; Verdoodt et al., 1999). Our results indicate that not only the spindle microtubules but also the interphase microtubules are sensitive to nocodazole treatment. To confirm this isolated primary lymphocytes were treated with nocodazole after stimulation. At the time of mitosis (46,5h-48h) the viable and apoptotic cells were separated by magnetically labeled annexin-V microbeads, followed by labeling with mpm-2, an antibody which recognizes mitotic cells. The major population of mitotic cells was found in the viable fraction and only a small part of mitotic cells in the apoptotic fraction. This suggests that after exposure to nocodazole the lymphocytes undergo apoptosis in interphase before mitosis, only a small fraction can escape from that apoptotic induction. Since apoptosis can also occur before the first in vitro mitosis, our data strongly suggest that besides the interphase dependent apoptosis in the G1/S-transition after mitotic slippage, apoptosis can also be triggered by damage to the interphase cytoskeletal tubulins. Experiments with specific caspase-inhibitors show already inhibition of apoptosis by nocodazole after 1h, which confirms our results of the interphase dependent apoptosis by microtubule-inhibitors.

Casenghi et al. (1999) Exp. Cell Res., 250, 339-350.
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S6/3

APOPTOSIS INDUCIBILITY IN PERIPHERAL BLOOD LYMPHOCYTES AS A POSSIBLE BIOMARKER OF HUMAN CANCER RISK ASSESSMENT

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In order to test the apoptosis inducibility as a possible approach of cancer risk assessment were studied in vitro in phytohemagglutinin stimulated normal and leukemic human lymphocytes (PBLs) in the presence of growth and apoptosis modifiers (somatostatin analogue TT-232 or the wheat germ fermentation product Avemar™ and anti-metabolite 6-thioguanine (6-TG). Altogether 196 donors (56 controls, 16 industrial controls, 93 nurses exposed to cytostatics, 4 PCP exposed donors, 22 CLL patients, 1 patient with AML, 1 patient with CML, 1 patient with aplastic anemia and 1 patient with mamma carcinoma) were involved in the study. Proliferating cells were detected with scintillometry by measuring the incorporated ^{3}H-TdR and with autoradiography. The incorporated BrdU to determine the cell kinetics of S-phase and the apoptotic activity was measured by flow-cytomety. In vitro culture of CLL cells the S-phase decreased parallel to the increased apoptotic rate beside lower rate of spontaneous apoptosis after TT-232 treatment in a dose-response manner (detected both with FACS and ApopTag). 6-TG treatment stopped the S-phase, but had no effect on apoptosis. At low level of PHA stimulation index Avemar increased S-phase after low doses (=<1000 mg/ml) of treatment, although Avemar in higher doses stopped cell cycle in S-phase in PBLs and in hepatoma cell in vitro. In general the apoptosis inducibility of tumor cells was significantly higher than in normal cells after both treatment. In conclusion these data suggest that a comparison a spontaneous and induced apoptosis capability after TT-232 and Avemar treatment of different PBLs of investigated donors may serve as a possible biomarker of increased cancer risk.

S6/4

RESVERATROL IS A PROMISING ANTICARCINOGENIC MOLECULE, INDUCING APOPTOSIS AND CELL CYCLE DELAY IN A HUMAN LYMPHOBLASTOID CELL LINE

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Epidemiologic studies have shown that diet may play an important role in preventing carcinogenesis through an appropriate presence of natural antimutagenic/anticarcinogenic molecules. Resveratrol (RES), a polyphenol present in red wine and fruits, has been reported to exhibit preventive properties both acting as an antioxidant and causing cancer cells to undergo apoptosis.
We investigated RES activity in a human lymphoblastoid cell line (AHH1) growing in exponential phase. We have studied the relationship between the cellular response to combined treatment with X ray irradiation (5 Gy: at 6, 14, 19 and 24 hrs after irradiation) and RES (50 and 100 mM at the same sampling times) in terms of DNA damage, cell cycle delay and apoptosis with the expression of proteins, assessed by Western blotting, involved in cell cycle progression and apoptosis. The Comet assay was performed in order to study the ability of RES to act as a scavenger of free radicals modulating X-rays induced DNA damages Furthermore to analyse RES ability to cause cells to undergo apoptosis Comet assay and cytofluorimetric analyses were performed at successive sampling times after treatments.
Our results suggest that: 1) RES influences cell cycle triggering partial arrest in early S, also in combined treatment with X rays; 2) RES induces apoptosis per se in our cell line; 3) Comet assay reveals to be an useful test in studying this phenomenon because of the possibility to detect DNA fragmentation in cells committed to apoptosis. Moreover standard DNA cytofluorimetric analyses enabled us to detect later stages of apoptosis namely: cell condensation and apoptotic bodies formation.
RES antimutagenic/antioxidant activity appears still unclear and need successive deeper investigations.

Symposium 7

Regulatory aspects of mutagenesis and carcinogenesis - Directives and guidelines

S7/1 - S7/4

S7/1

REVISION OF THE UK GUIDANCE DOCUMENT ON STRATEGIES FOR THE TESTING OF CHEMICALS FOR THEIR MUTAGENIC ACTIVITIES

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The Committee on the Mutagenicity of Chemicals (COM) is an advisory committee of the UK Department of Health whose function is to provide advice to all UK Government Departments and Agencies on matters relating to the testing of chemicals for their mutagenic potential and the interpretation of complex data sets. COM has provided advice on strategies for the testing of chemicals at regular intervals over the past 20 years with the aim of ensuring that testing procedures make use of new developments in the field of mutagenicity research.
The revised guidance document from the COM became available for public consultation in the spring of 2000. Major changes to recommended strategies include the requirement for evaluation of the 3 genetic endpoints of induced point mutation, chromosome structural damage and numerical chromosome changes. To measure the induction of the chromosomal endpoints in vitro the COM has recommended the use of the in vitro micronucleus assay. Following the demonstration of in vitro mutagenic activity the COM has recommended a flexible approach to the selection of in vivo methods particularly for the analysis of activity in tissues other than the bone marrow.
Amongst the comments received concerning the proposed strategy, were reservations concerning the validation status of the in vitro micronucleus assay. We have undertaken a comparative review of published data comparing the detection rates of clastogenic chemicals in the micronucleus assay and in metaphase studies. The currently available data indicates that differences in detection rate between the two assay methods are little different from those seen when different cells are used in metaphase analysis.

S7/2

OECD HARMONISED SYSTEMS FOR HAZARD CLASSIFICATION OF MUTAGENS AND CARCINOGENS

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Hazard identification, first step in the process of Risk Assessment, is the basis for classifying chemical carcinogens and mutagens. Various classification schemes and criteria are used; the best known schemes for carcinogens are those of IARC, US.EPA and EU.
The classification systems are based on the so called “weight of evidence approach”, and not or barely on considerations on potency, human exposure and ultimately risk. This essentially qualitative approach is based on the evaluation of the intrinsic properties of the substances. It is reassuring that in the case of carcinogens there now exists a strong consensus on most of the concepts that underline the classification schemes and criteria as well as on the relevance given to information on the mechanisms of action. In this context, a harmonized hazard classification system was endorsed by OECD in November 1998.
Within this system chemical carcinogens and germ cell mutagens are classified in two broad classes. In short, in the case of carcinogens Class 1 comprises “chemicals known to have carcinogenic potential for humans”, largely based on human evidence (Class 1a) or “chemicals presumed to have carcinogenic potential for humans”, largely based on animal evidence (Class 1b). Class 2 comprises “suspected human carcinogens”, for which the overall evidence is not sufficient to place the Chemical in Class 1. In the case of mutagens Class 1 is for “chemicals known to induce heritable mutations in the germ cells of humans (Class 1a) or “to be regarded as if they induce heritable mutations in the germ cells of humans” (Class 1b). Class 2 is for “chemicals which cause concern for man owing to the possibility that they may induce heritable mutations in the germ cells of humans”.
The rationale and the criteria of the OECD systems will be presented and compared with those used by IARC and EU.

S7/3

NEW CATEGORIES OF THE GERMAN MAK COMISSION FOR MUTAGENS AND CARCINOGENS CONSIDERING MECHANISTIC AND QUANTITATIVE ASPECTS

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Chemical carcinogens were previously classified in the German list of MAK and BAT values based on qualitative criteria. This (and other classifications) reflected essentially the weight of evidence available to characterize the carcinogenic potential. On the basis of advancing knowledge of the mode of action and the potency of chemical carcinogens the three existing categories - corresponding with category 1,2,and 3 of European Union regulations - were supplemented in 1998 with two additional categories, 4 and 5. Chemicals known to act typically or primarily by non-genotoxic mechanisms and for which information is available about low dose expsures are classified in category 4. Genotoxic chemicals for which low carcinogenic potency follows from dose-response relationships and toxicokinetics are classified in categoriy 5. The essential feature for both categories is that a limit value is defined on the basis that exposure to a particular chemical does not contribute significantly to cancer risk in man, provided that the MAK or BAT value given is observed. The emphasis is on “contribution” to risk rather than on an untestable absolute risk or the definition of a tolerable risk. Various biochemical and biological endpoints, like biochemical exposure and effect marker in humans can be used to characterize the contribution to risk . The contribution is considered to be not significant for instance if an external exposure results in an internal exposure, or an internal biochemical effect, which is not significantly greater than the corresponding background in a population not specifically exposed to that chemical. The main difference between category 4 an 5 lies in the biochemical and biological endpoints which determine the generation of the limit value, since a clear differentiation between genotoxic and non-genotoxic chemicals is often not possible. “True” or “practical” thresholds are not part of the concept, but non-linear dose-response or dose-effect relationships help to define the limit value in both categories. The kind of arguments will be exemplified with TCDD (4), chloroform (4), ethanol (5).

S7/4

REVISION OF THE STRATEGY FOR GENOTOXICITY TESTING OF CHEMICALS IN THE EU

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In 1995 the European Competent Authorities for regulation of chemicals (new and existing substances) adopted a technical guidance document (TGD) in order to harmonize criteria for risk assessment of chemicals. In early 2000 a revision of the TGD was initiated including discussion on a modification of the genotoxicity testing strategy. Up to now discussion focuses on the following aspects:
(1) alternatives for the in vitro chromosomal aberration test: can it be replaced by the in vitro micronucleus test or by the mouse-lymphoma assay ?
(2) local genotoxicity: are new methodologies, such as the in vivo comet assay, useful for investigation of non-systemic genotoxic effects ?
(3) bacterial mutagens: is immediate in vivo testing needed if mammalian cell culture assays were negative ?
(4) genotoxic carcinogens: can more guidance be given for definition, identification and risk estimation (including the threshold problem) ?
From the viewpoint of the German Competent Authority main goals of the revision process are to allow for more flexibility in the choice of test systems, to establish the need for investigation of local genotoxicity (esp. at the site of contact) and to re-consider the risk characterisation process for carcinogens with genotoxic potentials.

Symposium 8

CANCER RISK

S8/1 - S8/6

S8/1

CHROMOSOMAL MUTATIONS IN SOLID TUMORS

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Most current efforts to detect the somatic mutations that drive tumorigenesis utilize molecular genetic methods. Cytogenetics gives no direct imformation about DNA-level changes, but instead offers other advantages. First, chromosome banding is a screening method; all mutations detectable by microscopy of metaphase chromosomes are seen, making the results unbiased by initial guesses as to which the important changes might be. Second, cytogenetics gives information about the genetic constitution of real cells, not of the theoretical means that chemical investigative methods depict; this is unimportant only if no cell-to-cell genetic heterogeneity exists within the tumor. Recent chromosome banding studies have shown that most solid tumors have characteristic aberrations. Besides thus improving diagnostic accuracy, the tumor karyotype also provides information about pathogenetic mechanisms, with different balanced rearrangements as well as consistent gains and losses of whole chromosomes or chromosome segments being found in both benign and malignant tumors. Examples of both increasing and decreasing genetic complexity during tumor progression have been registered. A main difference between epithelial and other tumors has been the frequent detection of karyotypically unrelated clones in the former, emphasizing that profound genomic heterogeneity is a feature of carcinoma cells.

S8/2

GENETIC SUSCEPTIBILITY TO MELANOMA AND SOFT TISSUE SARCOMA: SIMILARITIES AND DIFFERENCES

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Cutaneous melanoma and soft tissue sarcoma present an excellent paradigm for understanding gene-environment interactions because major environmental factors, sun exposure and herbicide exposure, and inherited predisposing genes have been identified, INK4A and p53. To date, we have found that subjects with multiple primary melanoma are four times more likely to have an INK4A mutation or polymorphism than single primary controls. Multiple primary subjects are twelve times more likely to have a mutation in the DNA repair genes than general population controls and four times as likely to have a polymorphism in MC1R. Stratified analyses indicate potential interactions. When intermittent sun exposure throughout life was high, both multiple and single primary melanoma cases who had polymorphisms in DNA repair genes had a 4-5 fold increased risk for developing melanoma. Among those who experienced low intermittent sun exposure, polymorphisms in DNA repair genes in multiple melanoma cases had an almost nine-fold increased risk compared to the general population controls whereas the same polymorphisms in single primary melanoma cases had a smaller effect of approximately two-fold. There is a strong suggestion that subjects carrying the GST-theta and mu null genotypes are at a six-fold increased risk for developing soft tissue sarcoma. We have sequenced exon 7 of the AH receptor in 30 cases and 30 controls obtained from the general population and find a four-fold increased risk for sarcoma with the A/G polymorphism.Taken together, our data for both tumors show new trends for susceptibility with more genetic variability. Large studies will be necessary to test for interactions with environmental agents.

S8/3

ENVIRONMENTAL OR HEREDITARY RISK OF CANCER IN TESTICULAR TUMOR PATIENTS AND THEIR FIRST DEGREE RELATIVES?

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The common environment of the relatives, i.e. of brothers and offspring, and the doubled worldwide incidence of testicular cancer over the past 25 years suggest the strong involvement of the environmental factors in the formation of testicular cancer. Familial cancer-aggregations and the occurrence of bilateral tumors in the patients seem to be arguments in favor of the major influence of genetic factors. The aim of this study was to sort out the role of hereditary components in testicular tumor patients (TTPs) by determining cancer occurrences in their families, versus occupation- and fertility-rate matched normal controls. Familial aggregation of testicular- and other cancers were investigated in first degree relatives of 293 TTPs and 600 age matched controls, under the same socioeconomic and environmental circumstances. The incidence of cancers was significantly higher in TTP families than in the controls (10 % vs. 7.9 %), but this result could be accounted for almost entirely by the finding of more cancers in brothers (11.2% vs. 3%) and offspring (3% vs. 0% in controls, and/or 15.3 /100 000 prevalence of childhood tumors in Hungary). There was no association with other cancers except testicular malignancy in 5 brother-brother pairs and one father-son case. Two percent of patients reported familial, and 1.7% had bilateral testicular cancers. Significant shift was found in the sex-ratio of the descendants: Testicular cancer patients fathered more girls than boys (58 % : 42% vs. 47 % : 53% in controls). Six cancers occurred in 200 offspring of 153 TTP families (bilateral Wilms' tumor, bilateral neuroblastoma, brain tumor, acute lymphoid leukemia, testicular tumor and histiocytosis-X), while no cancer was found in 423 offspring of 600 normal controls. As a form of genetic instability increased yield of spontaneous chromosomal aberration was detected in both index patients and their offspring (2% and 0.90 % vs. 0.87 % and 0.62 % in controls).
The familial aggregation of testicular malignancy in brothers, the altered sex ratio in the offspring, the dramatically increased incidence of childhood tumors, and the elevated frequency of chromosomal aberrations in index patients and their offspring under the same socioeconomic conditions indicate more significant role of hereditary factors in the predisposition to testicular- malignancy than that of environmental factors.

S8/4

THE TP53 TUMOR SUPPRESSOR GENE AS A SENSOR OF CARCINOGENIC EXPOSURE: LESSONS FROM MUTATION ANALYSIS

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The T53 gene is located on chromosome 17p13 and is the most frequently mutated gene in human cancer. To date, about 14 000 p53 mutations have been detected in human tumors and reported in the literature. These mutations are compiled in a standardized electronic database (Hernandez et al., 1999)[1]. This gene encodes a multi-functional transcription factor which controls cell cycle progression, DNA integrity and cell survival in cells exposed to several forms of stress. Some mutagens and carcinogens damage TP53 DNA genome in characteristics ways, and mutations do not form at an equal rate at all base positions. In many cancers, mutation patterns are consistent with fingerprints of DNA damage induced by defined exogenous carcinogens or by endogenous mutation mechanisms. The most spectacular examples are (1) G to T transversion at codon 249 (AGG to AGT, Arginine to Serine), in hepatocellular carcinoma of individuals with high exposure to AFB1 and HBV; (2) tandem CC to TT transitions resulting from UV exposure in non-melanoma skin cancer, (3) G to T transversions in lung cancers in relation with tobacco smoke. The analysis of mutations in other types of cancer reveals more complex mutation patterns with interesting geographic and ethnic variations. These variations are useful to generate hypotheses for further experimental or epidemiological studies, which may contribute to improve our knowledge of the etiopathogenesis of human cancers.
[1]http://www.iarc.fr./p53/homepage.html, Hernandez - Boussard et al., Hum. Mutat., 14, 1 - 8, 1999.

S8/5

A NEW MODEL FOR CANCER RISK ASSESSMENT OF CHEMICAL CARCINOGENS

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A linear multiplicative model is at present used for assessment and projection of cancer risks of ionizing radiation (BEIR, 1990). This model has been shown to be valid for chemical carcinogenesis in experimental animals (Granath et al., 1999). This model predicts that the incremental cancer risk D(P) from exposure to chemical carcinogens is proportional to the target dose (D) of genotoxic compounds or metabolites, and to the background risk (P^0), according to $DP = bD P^0$. The risk coefficients b reflect the genotoxic potency but are independent of species and tumour site.
Assessment of cancer risk by the multiplicative model has one basic requirement, viz., measurement of dose in humans of studied reactive compounds or metabolites. This dosimetry can be achieved by measurement of doses via determination of adducts to proteins (e.g. hemoglobin) or DNA. The risk coefficient b (per in vivo dose unit) may be obtained from animal cancer tests or mutation frequency per dose unit in in vitro tests. The measurement of dose facilitates inter-species extrapolation. The default procedures recommended by USEPA for inter-species extrapolation in cancer risk assessment may lead to large under- or overestimates of risks (USEPA, 1996).

References:
BEIR V (1990) National Research Council, Committee on the Biological Effects of Ionizing Radiations, Health Effects of Exposure to Low Levels of Ionizing Radiation, National Academy Press, Washington DC.
Granath, F., Vaca, C., Ehrenberg, L. and Törnqvist M. (1999) Cancer risk estimation of genotoxic chemicals based on target dose and a multiplicative model. Risk Analysis 19, 309-319.
USEPA (1996) Proposed Guidelines for Carcinogen Risk Assessment, EPA/600/P-92/003C, Fed. Reg. 61, pp. 17960 - 18011.

S8/6

TUMOR PROFILE DISTRIBUTION IN EUROPE AND ITALY: PATTERNS AND SOCIOECONOMICAL CORRELATES

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The geographical distribution of tumor incidences and tumor types in Europe was investigated. A number of stable associations among tumor types was highlighted: most of them, except the association between head and neck tumors, were unrecognized before. Since these associations are area-based (in the cancer registries each patient is attributed only one tumor), they provide a reliable probe for investigating the environmental component of cancer without confounding, and can be considered as proxies of underlying macro-causes of cancer. The above associations were shown to correlate with a range of socioeconomical indicators. Data from the periods 1982-1988 and 1988-1992 were fairly comparable. In addition, a minor - but statistically significant- correlation between pathology and AB0 blood groups system was found for the female population, whereas the correlation was weak or insignificant for the male population. This difference between sexes, together with the evidence of the higher tumor incidence in males, suggests a greater relative importance of non-genetic (environmental) factors in cancer incidence compared to the genetic ones. Finally, the between-sexes differential induction of cancer was shown to correlate with indicators of the female condition, with different patterns in Europe and Italy.

Poster session 1

MAJOR TOPICS:

GENOTOXICOLOGY, BIOMONITORING, METHODOLOGICAL DEVELOPMENTS

P/1 - P/93

P/1

A NEW DOSIMETRIC METHOD FOR 1,3-BUTADIENE

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Butadiene is a common chemical in the polymer industry and has been shown experimentally to be carcinogenic. Butadiene forms three different genotoxic metabolites in vivo: diepoxybutane, epoxybutene and epoxybutanediol. Diepoxybutane, which is bifunctional and crosslinking, is 100 times more genotoxic than the monofunctional metabolites. In work aiming at cancer risk assessment it is therefore important to know the in vivo dose of diepoxybutane. This can be achieved by determination of hemoglobin-adducts in exposed humans or animals.

Diepoxybutane forms a pyrrolidine ring when reacting with N-terminal hemoglobin and therefore the modified Edman degradation, used for measurement of adducts from the monofunctional metabolites, is not applicable (Rydberg et al. 1996). A new method permitting analysis of the specific adduct from diepoxubutane has been developed. This method comprises analysis of modified peptides by LC/MS.

Rydberg, P, Magnusson, A.-L., Zorcec, V., Granath, F. and Törnqvist, M. Adducts to N-terminal valines in hemoglobin from butadiene metabolites Chem. Biol. Interact. 101 (1995) 193-205.

P/2

EVALUATION OF THE VITOTOX® TEST IN COMPARISON WITH THE AMES TEST AND THE COMET ASSAY FOR THE SCREENING OF INDUSTRIAL EFFLUENTS

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The Vitotox(test is a very rapid and sensitive bacterial genotoxicity and toxicity test for the screening of chemicals. Previously the use of the Vitotox® test for the analysis of concentrated watersamples was limited. This problem was mainly due to high toxicity of the elution solvents used in the concentration method for extracting genotoxins, which made further analysis impossible. Therefore, evaporation to dryness of the extracts under a stream of nitrogen and reuptake of the concentrate in DMSO (10%) is necessary for evaluation of complex samples with the Vitotox®. Effluents from petroleum, metal, organic chemistry and waste treatment industries were studied for their mutagenicity. The samples were concentrated either on silica C18 cartridges or SDB (styrene-divinylbenzene) cartridges. The genotoxic activity of the extracts was assessed with the Ames test, the Vitotox® test and the Comet assay. The results suggest that the genotoxicity of extracts is related to the type of industry.The effluents from organic chemistry production plants were highly genotoxic towards all three tests, with low toxicity. Effluents from the petroleum and metal industry were not significantly genotoxic nor toxic. Effluents from waste treatment industries were not significantly genotoxic, but highly toxic. The Vitotox® test gives results that correlate well with the Ames test, although with higher sensitivity for detection of toxicity.

P/3

APPLICATION OF THE IN VITRO SHE CELL ASSAY TO THE PREDICTION OF RODENT CARCINOGENESIS

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Syrian hamster embryo (SHE) cell cultures have been demonstrated to be perhaps the most useful short-term test system for identifying rodent carcinogens (Yamasaki, 1996). The morphological transformation (MT) phenotype for colony growth pattern is the marker used, although this marker does not actually represent the conversion of the cells to the malignant state. From studies beginning in the early 1960's, this assay system has evolved, particularly with the discovery that a slightly acidic culture environment (pH 6.70) greatly improves assay repeatability and predictability. Approximately 96 chemicals (including both genotoxic and nongenotoxic substances) have been tested to date (using the reduced pH method) for which there is also rodent carcinogenesis data. The results relevant to screening predictivity show 78% concordance, with 83% positive predictivity, and 69% negative predictivity (Gibson et al., 2000). This predictivity is substantially higher than for the Ames test and other routine genotoxicity assays. A problem in the wide-spread use of the assay, however, is the standardization of scoring of the MT phenotype and the intensive labor (high cost) of scoring. These impediments are being removed with the introduction of an automated image analysis scoring system. This system uses a library of MT phenotypes to rapidly score fixed and stained colonies with better than 90% concordance with highly trained human scorers. This innovation should allow the SHE assay to become an economical and very useful predictive method for choosing and developing new pharmaceuticals least likely to cause tumors.

Gibson, DP, Aardema, MJ, Custer, L, Isfort, RJ, LeBouef, RA, Env. Mol. Mutagen., 35: 25, 2000.
Yamasaki, H., editor, Special Issue, Mutation Res., 356: 1-128, 1996.

P/4

COMPARATIVE MICRONUCLEUS ANALYSIS IN MOUSE BONE MARROW BY MANUAL AND FLOW CYTOMETRIC METHODS

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In the present investigation the flow cytometric analysis of the micronucleus test was compared with the analysis by means of manual assessment. The data were obtained from negative control animals, cyclophosphamide (CPA) and methyl methanesulfonate (MMS) treated animals. Single doses of the different formulations were administered by oral gavage. The mice were killed 24 hours after administration. From each animal, bone marrow was taken for manual analysis and flow cytometry. For manual scoring the ratio of polychromatic to all erythrocytes was assessed by examination of the first 1000 erythrocytes observed. Altogether 2000 polychromatic erythrocytes from each animal were examined for the presence of micronuclei. The corresponding number of analysed cells using the flow cytometry was increased to 15000-250000 and 42000-88000, respectively. The incidence of micronucleated polychromatic erythrocytes were similar in the negative controls analysed by the two methods. Both scoring methods revealed an elevation of micronucleated cells after treatment with MMS and CPA but the elevation was higher in data from manual scoring. Occasionally, very low frequencies of polychromatic erythrocytes were recorded by flow cytometry. Very high reproducibility was shown for the flow cytometry in the present study. Inter animal variation of the incidence of micronucleated polychromatic erythrocytes was much lower in data from flow cytometric analysis than from manual scoring. It is concluded that the flow-cytometry is a very fast method having many advantages and with some additional developmental work it has high potential of being used in routine testing.

P/5

MUTAGENIC ACTIVITY OF AIRBORNE PARTICULATE MATTER TESTED BY SALMONELLA ASSAY AS AN INDICATOR OF AIR POLLUTION

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The objective of the study was to evaluate the mutagenic effect induced by complex mixtures of atmospheric air pollutants in Silesia province - Poland. The investigations were carried out in 21 measurement points in winter and summer with the use of the plate incorporation Salmonella/mammalian-microsome mutagenicity assay with and without metabolic activation. Salmonella typhimurium strains with different levels of nitroreductase and O-acetylotransferase enzymes: TA98 with its derivatives YG1021, YG1024 and YG1041 were applied in order to determine the class of mutagenic compounds in airborne particulate matter.
The results of the study revealed high exposure of the inhabitants of Silesia province to mutagenic substances. The presence of polycyclic aromatic hydrocarbons (PAHs) was confirmed by the results of chemical analyses and the Salmonella/microsome assay with metabolic activation. The nitro aromatic compounds were detected by YG derivatives what resulted in the higher response compared to their parent strain. Moreover in summer the response of YG1024 and YG1041 (with higher levels of O-acetylotransferase) after metabolic activation was much lower compared to the variant without S9 mix. In winter increased values of mutagenic activity detected by YG1024 and YG1041 after addition of S9 mix suggested the presence of aromatic amines and hydroksyloamines in airborne particulate matter.
In conclusion, the results of the study confirmed that derivatives with higher levels of enzymes involved in intracellular metabolism of nitro aromatics, aromatic amines and hydroksyloamines are very useful in environmental monitoring of air pollutants based on the Salmonella assay. In winter the mutagenicity of air samples was caused by PAHs, nitro aromatics, aromatic amines and hydroksyloamines, and in summer mainly by nitroaromatic compounds.

P/6

APPLICATION OF THE PLANT-BASED TEST-SYSTEMS FOR THE ESTIMATION OF THE MUTAGENIC BACKGROUND INTENSIVENESS

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Aimed at the assessment of the mutagenic factors influence upon living organisms we have studied cytotoxicity and genotoxicity of drinking water and soil samples from various regions of Precarpathia: the territory near the plant of fine organic synthesis, on the territories of increased radiological control - zone 4 (Snyatin District) and the ecologically clean mountainous area of Verkhovina District. The standard method of the ana-telophase analysis of Allium cepa apical meristem primary rootlets cells have been utilized. We have also worked out methodological approaches to the application of transgenic plants Arabidopsis thaliana, containing in the genome, a visual-marker gene of b-glucoronidase as a recombined substraction for the indication of intensivity antropogenic pollution. We have established that mitotic activity in all the investigated objects is reduced in comparison with the control. The mitotic index of the seedlings grown on the water and soils from the zone 4 has turned out to be 40.5+4.8%, 39.5+3.8% respectively, in the chemically polluted region 44.8+5.3%, 30.5+4.1%. The correlation between the frequency of chromosomal aberrations within the cells of primary meristem Allium cepa and the degree of water and soil pollutedness has been revealed. Fragments, C-mitosis, vagrant chromosomes refer to the radiation mutagenesis, while chromatides aberrations - to the chemical one. As a result of 32 series of the experiments held, we have received 50 plant transformation and 14 transgenic lines Arabidopsis thaliana with the necessary constructions including the following genes: RPD3, SIR2 - like and SU (VAR)3 - 7. A selection of the most sensitive transgenic lines for the purpose of bioindication of antropogenic pollution is being conducted. A combined application of Allium cepa-test and transgenic plants Arabidopsis thaliana can be used as complex plant - based test-systems for the estimation of the mutagenic background intensiveness.

P/7

AN ESTIMATION OF THE URBAN SYSTEM MUTAGENIC BACKGROUND ON THE BASIS OF CYTOGENETIC PARAMETERS OF POPULUS SIMONII CARR. AND POPULUS BEROLINENSIS DIPP.

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Urban terrains are characterized by the availability of a great deal of contamination sources, their irregular distribution, and composite distributional pattern of pollutants. Many of them are cytogenetics fissile. Toxic and nontoxic mixtures are capable to interact among themselves and to be transformed into new products, both in environment, and in alive organisms. Practically, it is impossible to make a real prognosis of such chemical transformations.
For an estimation of the mutagenic intensity in different districts of city Ivano-Frankivsk used an anaphase method and mitotic activity characteristics in rudimentary leaf meristem cells of P. simonii Carr. and P. berolinensis Dipp. These species are represented by the genetically homogeneous populations. The plants from an ecologically pure territory were control.
Especially large percent of aberrant anaphases was in plants of central region and industrial north-east suburb, it was, respectively, 1.3 and 7.8 times in Populus simonii Carr. and 3.0 and 4.2 times in Populus berolinensis Dipp. more then in the control. The research of a chromosome reorganization spectrum has shown, that the increase of a level aberrations occurred basically at the expense of chromatide bridges and fragments.
The reduction of the mitotic index is observed in northern and north-east part of city and in industrial suburb (in 1.5 - 2.2 times concerning the control) mainly at the expense of decrease of prophase cells number. In the central area of city the mitotic index authentically doesn't differ from the control level, though the prophase index was lower. However, the number of cells in metaphase and telophase is considerably increased. It specifies presence in environment potentially genotoxic and sytotoxic substances.
At the finish, we determined the state of the investigated areas on the mutagenic background: north-east suburb - "dangerous", central - "critical", northern - "alarming", north-east - "satisfactory", south-east and southern - "favorable".

P/8

BIOMARKERS FOR ORGANIC POLLUTION IN THE ENVIRONMENT

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The main goal of this project is to evaluate biomarkers for organic pollution. Organisms are exposed to complex mixtures of xenobiotics in the environment. Organic compounds represent a major challenge because they can lead to cancer, enhance the aging process and reduce reproduction in exposed individuals. The damage can also be transmitted to the offspring. High genotoxic burden in the environment can eventually result in large-scale changes in biological communities and even ecological systems. Better methods are needed to predict the probability of future ecological problems before damage can be seen. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are the major persistent organic compounds in the environment. Long-term exposure to these compounds may cause different effects, but there may be certain common features. The methods that are used in this project are based on these features. In this paper we are going to present results from the first step in the project (in vitro study). DNA adduct formation and CYP1A1 induction were evaluated in a rat hepatoma cell line (Fao), as biomarkers of exposure to organic compounds. B(a)P was used as an indicator for genotoxic PAHs, and 3,3´,4,4´-tetrachlorobiphenyl (TCB) as a representative for PCBs. Both compounds are environmental contaminants of concern. Fao cells were exposed to B(a)P or TCB to study the possible association between CYP1A induction and B(a)P-DNA adduction. Cells were also exposed to different combinations of TCB and B(a)P. DNA-adducts were analyzed by the ^{32}P-postlabeling assay. CYP1A induction was measured in terms of CYP1A immunopositive protein (Western blotting). The results show an dose-dependent increase of CYP1A1/2B content after exposure to B(a)P. DNA-adducts are found at the highest concentrations of B(a)P. Exposure to TCB also show an dose-dependent increase in CYP1A/2B content, but no DNA-adducts are seen. The cotreatment of TCB together with B(a)P may indicate an increase in DNA adduct formation in vitro.

P/9

DIMETHYL SULFOXIDE (DMSO) IS A CLASTOGEN IN THE TRADESCANTIA-MICRONUCLEUS ASSAY

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Plant bioassays are uniquely suitable for biohazard assessment of environmental pollutants in terrestrial and aquatic ecosystems. Beside high sensitivity a further major advantage is their versatile applicability for in situ monitoring. In recent years the Tradescantia-micronucleus assay ("Trad-MCN assay") has gained increasing use in ecogenotoxicity monitoring. In this test system micronuclei (MN) formed in pollen mother cells of young inflorescences from Tradescantia plants serve as indicators of mutagenicity. Mutagenic compounds need to be dissolved in water thus enabling an efficient uptake through the stem of the plant cuttings. Unfortunately many important environmental pollutants such as PAHs or pesticides show a very limited solubility in water. In consequence, organic cosolvents are applied among which DMSO represents the most often used. According to various reports published in recent years, it is generally applied in final concentrations of up to 5%. Nevertheless, Ma et al (1984) noted that higher concentrations of DMSO were positive in the Trad-MCN assay.
Therefore, our objective was to establish a dose-response relationship for DMSO in the Trad-MCN assay as rationale basis for its further use in monitoring applications. Treatment with DMSO alone was found to be clearly clastogenic and led to a dose-dependent increase in MN frequencies. The highest concentration tested was 8% and resulted in a 5.3-fold increase over tap water controls after 6 hrs treatment. Lowest observed effect concentrations were 4% after 6 hrs and 2% after 24 hrs treatment. Correspondingly, additive effects on overall MN frequencies were found after using DMSO as solvent for two MN-inducing hydrophobic pesticides. Taken together, these results not only emphasize the need for concurrent solvent controls but also demand for the use of adequately low DMSO concentrations in the Trad-MCN assay in order not to overload this test system.

P/10

GENOTOXICITY TESTING OF DANUBE RIVER WATERS AFTER INCIDENTAL POLLUTION

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After the Pancevo rafinery (18km NE from Belgrade, Serbia) was bombed in Macrh-June 1999., the tanks were destroyed and petrol spilled into environment including river Danube.The samples of river Danube water and mud from water station waste channel and upstream from station, were tested for genotoxicity by several procedures, on different organisms.
In the present contribution, the results of Drosophila melanogaster sex linked recessive lethal (SLRL) test, are given. The sterility of males was significantly increased in both mud samples and frequency of SLRL mutations were increased in upstream water and both mud samples. However, the obtained results reveal the importance of the exposure method and chemical concentration most likely to be genetically effective according to the organism used in a test system.The results differ significantly if extracted organic compounds from mud samples are administered to flies, than those when water is given directly to Drosophila. The biological meaning should be highly considered in evaluation of genotoxicity results obtained in monitoring the ecological pollution.

P/11

DETECTION OF DNA DAMAGE IN HAEMOCYTES OF ZEBRA MUSSEL USING COMET ASSAY

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The aim of this study was to evaluate the potential application of the comet assay on haemocytes of zebra mussel, Dreissena polymorpha Pall. for genotoxicity monitoring of freshwater environment. Zebra mussels were exposed for seven days to different concentrations (10, 80, 100 and 150 (g/l) of pentachlorophenol (PCP) and for one month in the River Sava, downstream from the municipal waste discharge of Zagreb town. The comet assay was conducted as described by Singh et al (1988) with slight modifications. Statisticaly significant increase in DNA single strand breakes was observed after exposure to 80, 100 and 150 (g/l of PCP and after exposure in the River Sava as well. Our study confirmed that the comet assay applied on zebra mussel haemocytes is very valuable in biomonitoring studies for determining the potential genotoxicity of water pollutants.

Singh N.P., M.T. McCoy, R.R. Tice, E.L. Schneider (1988) A simple technique for the quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175:184-191.

P/12

POTENTIATING EFFECTS OF UV RADIATION ON PAH-INDUCED GENOTOXICITY IN MYTILUS EDULIS

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Ultraviolet radiation (UVR) is known to alter the toxicity of certain chemical compounds, in particular polycyclic aromatic hydrocarbons (PAHs). In the current study, micronuclei (MN) induction was assessed in haemocytes from adult M.edulis exposed to (a) B(a)P in the absence of UVR; (b) B(a)P which had received previous UV photoactivation, or (c) B(a)P with simultaneous UV irradiation. Samples were collected on days three and six of continuous exposure.
Spontaneous levels of micronuclei induction were within the range of 0-6 per 1000 cells (mean frequency of 1.7 ± 1.6) . Following three days of exposure, the mean frequency of micronuclei was (a) 7.00 ± 6.05 and 10.5 ± 4.04 in mussels exposed to 1µg/L B(a)P and 10µg/L B(a)P respectively; (b) haemolymph sampled from mussels exposed to previously photoactivated B(a)P showed a micronuclei frequency of 13.17 ±4.07 and 15.83 ± 4.9 (1µg/L B(a)P and 10µg/L B(a)P respectively); and (c) mussels exposed to B(a)P with simultaneous UV irradiation, exhibited a mean micronuclei frequency of 16.7 ± 4.5, in animals which had been exposed to a concentration of 10µg/L B(a)P.
These highly significant ($p<0.000$) increases in genotoxicity clearly indicate that there is an interaction of UVR with B(a)P in the marine environment. Observations from this study suggest that the acute phototoxic effects of B(a)P and other PAHs should taken into consideration when assessing the impact of physical and chemical agents on aquatic biota.

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EVALUATION OF DNA DAMAGE INDUCED BY THREE GENOTOXINS WITH DIFFERENT MODES OF ACTION IN THE HAEMOCYTES OF THE MARINE MUSSEL, MYTILUS EDULIS

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Induction of DNA damage was evaluated at the molecular and cellular levels using the comet and micronucleus (MN) assays, from haemocytes collected from the bivalve mollusc, Mytilus edulis, at intervals over a period of time. Three genotoxins, with different modes of action, Ethyl methane sulphonate (EMS) (a direct acting alkylating agent), Hydrogen peroxide (H_2O_2) (which produces free radicals and possibly triggers the activation of nucleases) and the beta emitting radionuclide tritium, (in the form of tritiated water) were used in this study.

The comet assay detected the maximum DNA damage after 1 hour for all three genotoxins. After 24hrs the H_2O_2 exposed mussels showed no significant difference in comparison to the controls, although the EMS and tritium exposed mussels still showed evidence of DNA damage. After 48 hours there was no difference between the tritium exposed mussels and the controls, however the EMS exposed mussels still showed a significant difference in the number of normal haeomcytes compared to controls. On the other hand, the induction of MN was only observed following 24 hours in all of the genotoxic exposed mussels with a slight increase at 48 hours.

The studies suggested that the induction of MN is generally observed following 24 hours exposure irrespective of the mode of action of the genotoxin used over a range of concentrations. In comparison, the comet assay showed an increased sensitivity for detection of DNA damage induced by various genotoxins. The H_2O_2 produced short-term effects, followed by tritium and finally EMS, which produced a continuous level of DNA damage.

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DNA FINGERPRINT ALTERATIONS OF RAINBOW TROUT AFTER B(A)P EXPOSURE

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Consequences on biodiversity has been observed after exposure of natural populations to antropogenic chemicals. Ecogenotoxicology assessment could be addressed prior or after environmental release of both pure chemical and/or complex mixtures but sensitive and reliable assays on exposed populations are required to detect and prove this deleterious effects. Random Amplified Polymorphic DNA (RAPDs) is a modification of the polymerase chain reaction that can be used for the detection of genomic alterations, once the constant and specific band pattern has been established. Here we show the results on DNA fingerprint of Rainbow trout individuals exposed to a single intraperitoneal injection of 65 (g/g b/w of b(a)p for 90 days. Blood samples was taken before, during and at the end of the experiment.
Differences both qualitative (presence/absence of bands) and quantitative (bands intensity) on the DNA fingerprint obtained with different primers were studied between control and exposed individuals.
Keywords: mutagenicity, in vitro, RAPDs.
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EFFECT OF CYANOBACTERIAL EXTRACT ON HUMAN LYMPHOCYTES

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Cyanobacterial (blue-green algal) blooms are becoming an important water quality problem in many countries of the world including Poland. The production of cyanobacterial toxins and their presence in drinking and recreation waters represent a growing danger to human and animal health. In humans the frequently observed symptoms of microcystin toxicity are skin irritation, cutaneous rash, fever, vomiting, diarrhea, and acute liver damage.
The aim of this work was investigation dose the cyanobacterial extract containing MC-LR from Polish bloom samples could cause permanent changes in human lymphocytes and DNA, in vitro.
The comet assay was used to detection of single-strand DNA damage. Mainly comets of the 3 class was observed after incubation with cyanobacterial extract (MC-LR>= 250 nM, 18h). The morphological changes as condensation and margination of chromatin was observed after incubation with cyanobacterial extract (MC-LR >= 750 nM, 24h). Flow cytometry demonstrated loss of plasma membrane asymmetry with the externalization of phosphatidylserine (PS). Nearly 50% of lymphocytes exhibited the disturbed membrane asymmetry after exposure to cyanobacterial extract (MC-LR >= 750 nM, 24h). Typical apoptotic hallmark “ladder pattern” indicative of uniform DNA fragmentation was demonstrated after agarose gel electrophoresis of DNA exposed to the action of cyanobacterial extract (MC-LR >= 750 nM, 48h).
The results confirm high cytotoxicity and genotoxicity of cyanobacterial blooms collected in reservoirs of drinking water in Poland. It was showed that MC-LR may be not only clastogenic for human lymphocytes but the cyanobacterial extract containing MC-LR can causes changes, which can be interpreted as an apoptotic effect.

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DNA-ALKYLATING INTERMEDIATES ARE FORMED BY REACTION OF NITRIC OXIDE WITH GLYCINE UNDER SIMULATED PHYSIOLOGICAL CONDITIONS

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Nitrosation of amino groups in amino acids, peptides and bile salts react with DNA to form alkyl DNA adducts, which are thought to play a role in GI tract cancers (1). The adducts formed include N^7-methylguanine (N^7MeG), N^7-carboxymethylguanine (N^7CMeG), O^6MeG and O^6CMeG adducts; of these the O^6MeG adduct is known to be mutagenic (2). The nitrosated intermediate thought to be formed from glycine is diazoacetate (DA). DA has been shown to react with DNA in vitro to form higher levels of O^6CMeG than O^6MeG adducts (3), where the formation of carboxymethyl adducts been shown to be mutagenic and carcinogenic in other tissues (4). In vitro studies on the nitrosation of glycine and subsequent alkylation of DNA were performed. Nitrosation of glycine with nitric oxide has shown a dose-dependent formation of diazoacetate measured by HPLC at physiologically relevant levels of glycine and nitrite. Incubation of this nitrosated glycine intermediate with calf thymus DNA has shown detectable levels of O^6CMeG and O^6MeG adducts. These studies add further evidence that nitrosated amino acids and N-nitroso compounds are risk factors for GI tract cancers.

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DAMAGE AT PURINE RESIDUES IN OLIGONUCLEOTIDES AND CALF THYMUS DNA INDUCED BY THE MUTAGEN, 1-NITROSOINDOLE-3-ACETONITRILE

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The endogenous formation of N-nitrosoindoles is of concern since humans are exposed to a variety of naturally occurring and synthetic indolic compounds. N-nitrosoindoles modify isolated nucleotides resulting in deamination, depurination and the formation of a novel product oxanine, via a transnitrosation mechanism (Lucas et al, 1999). In this study we examine the relevance of these pathways for the mutagen 1-nitrosoindole-3-acetonitrile (NIAN) at the macromolecular level.
Reaction of NIAN with oligonucleotides containing various guanine motifs at physiological pH, produced single strand break products at guanine sites due to the formation of alkali-labile lesions. High performance liquid chromatography analysis resulted in the detection of guanine, xanthine and oxanine. Analysis of NIAN treated calf thymus DNA afforded two additional products, hypoxanthine and adenine and all reaction products exhibited a dose-response relationship. Cytosine and thymine residues were inactive toward NIAN. Calf thymus DNA treated with NIAN (12.5 to 1600µM) was also analysed using a ^{32}P-postlabelling assay for apurinic sites. Abasic sites were detected at levels from 98 to 2278 femtomoles per µg of DNA. Incubation of NIAN treated DNA with exonuclease III, a class II apurinic endonuclease, resulted in the disappearance of postlabelled apurinic residues.
All of these modification processes are potentially mutagenic events (Loeb et al, 1986) and these results indicate that the transnitrosating ability of NIAN to modify purine residues is preserved at the macromolecular level.
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EVALUATION OF THE MUTAGENIC POTENTIAL OF 27 NEW SYNTHESIZED ACRIDINE DERIVATIVES AND RELATED COMPOUNDS IN THE SALMONELLA MUTAGENICITY TEST

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Twenty-seven new synthesized acridine derivatives and related compounds were tested for their mutagenicity in the Salmonella mutagenicity test. Using a modified technique of the liquid incubation assay, the mutagenic activity was evaluated in S. typhimurium TA 97a with rat liver S9 metabolic activation (S9 mix) and in S. typhimurium TA 98 after photoirradiation. Twenty-six molecules were found mutagenic in TA98+S9 mix. The mutagenic activity ranged from 0 revertant/nmole to 6,402 revertants/nmole. Following photoirradiation, the mutagenicity was limited to 17 molecules in TA 98. The mutagenic activity ranged from 0 revertant/nmole to 98,712 revertants/nmole. In TA97a+S9 mix, the mutagenicity was modulated by substitutions on the 9-position. After photoirradiation, the mutagenicity increased by aliphatic and chloro substituents on 1 and 2 positions.

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GENOTOXICITY OF DIFFERENT SIZE FRACTIONS OF ATMOSPHERIC PARTICULATE MATTER: A LONG-TERM STUDY

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Some human diseases, as cancer, are suspected to result from multiple, comulative exposures to environmental contaminants. U.S. EPA define "comulative risk" as the risk from all routes of exposure to a group of substances: airborne particulate matter (PM) is a typical example of a multiple contaminant. Association between PM concentrations and human health effects are well studied; particles with different size, deriving from different sources and different formation mechanisms, reach defined district of the pulmonary system.
We evaluated the genotoxicity effects of three fraction of PM: TSP (total), PM_{10} (aerodynamic diameter < 10µm),and $PM_{2.5}$ (aerodynamic diameter < 2.5µm). The monthly samples were collected continuously (24/24h) during the period September 1998 - December 1999, and tested on Salmonella typhimurium TA98 and TA100 strains, with and without metabolic activation. $PM_{2.5}$ mutagenicity data, when compared with PM_{10} and TSP, suggest that $PM_{2.5}$ finest particles are not well represented in the two other fractions. This hypothesis is confirmed by electronic microscopy preliminary observations that indicate that while the $PM_{2.5}$ is mainly composed by particles with aerodynamic diameter < 1µm, this ultrafine particulate is represented in a lesser extent in PM_{10} and TSP. Our data confirm the $PM_{2.5}$ as a better predictor of health effects than the other two fractions: together with its ability to reach the inner pulmonary districts, the fine fraction, generally, shows the highest specific (revertants/µg) and absolute (revertants/ Nm^3) mutagenic activity. Furthermore, the findings prove that a long-term period monitoring by mutagenicity tests can be helpful for health risk assessment since these biological assays appear more sensitive on such an environmental mixture than the chemical-physical parameters alone.

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MUTAGENICITY MONITORING OF CHLORINATED DRINKING WATER

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The monitoring of chlorinated drinking water mutagenicity is carried out with YG1041 indicator strain. C18 cartridges are used for the separation of mutagenic components from 1 to 2 litre of acidified tap water. The recovery of potential mutagens with the C18 columns proved with water samples spiked with reference mutagens benzo/a/pyrene, 2-anthramine, and dimethylbenzanthracene was almost 100%. Tap water samples (n = 150) were collected in the years 1999 - 2000 in four cities including Prague. For calculating maximum mutagenic potencies in number of revertants per litre water sample we use Bernstein linear regression model (GeneTox Manager). The significant dose-related responses are repeatedly found with YG1041 strain in the absence of S9 mix, whereas the mutagenicity detected with TA98 and TA100 is low or even negligible. Significant local differences in mutagenicity are observed with the highest values in samples obtained in Prague water samples with a mean level of 961 rev/l (range 189 to 3800). In each water extract, chemical analysis of trihalomethanes (with the mean value of chloroform 8.3 mg/l), haloacetonitriles (values mostly under the detection limit) and benzo/a/pyrene (mean value 1.1 ng/l), together with chromatographic profiles were done, but no correlation between the chemical analytical results and the bacterial mutagenicity were observed. We did not succeeded in proving the presence of MX in our water samples. So the relationship between the chemical composition of the drinking water samples and their mutagenic potencies remains unclear.

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MODULATION OF GENOTOXIC EFFECTS BY TERPENOIDS FROM SAGE (S. OFFICINALIS L.)

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The possibility to modulate the response of cell to environmental and endogenous mutagens has opened a new frontier for cancer control. The concept of preventing cancer by delay, i.e. by decreasing the rate of mutation accumulation, promoted the study of plant antimutagens as possible dietary anticarcinogens. With appropriate screening and fractionating methodologies it was possible to identify a wide variety of structural types in higher plants (tannins, ethereal oils, flavonoids, etc.) possessing inhibiting or modulating effect on environmental genotoxic agents.
In last 10 years, in our laboratory, over 25 differently prepared extracts of wild and cultivated sage, as well as pure constituents, were screened for antimutagenic potential against UV- and EtBr-induced mutations. These included terpenoid fractions obtained from ethanolic extracts at different CO_2 pressure; ethereal oils and their fractions; camphor, a+b thujone, 1,8-cineole, limonene, myrcene, linalool, rosmanol 9-ethyl ether, carnosic acid 12-methyl ether, carnosic acid 12-methyl ether g-lactone and oleanic acid.
A set of newly constructed E.coli strains, aimed at the detection of spontaneous and induced mutations and at the estimation of the mechanisms of antimutagenic action, was used along with S.typhimurium (Ames) strains. Moreover, S.cerevisiae D7 strain was used as eukaryotic model system. The obtained results have shown that monoterpenoids from sage inhibit UV-induced mutagenesis by modulating DNA repair pathways. On the other hand, terpenoids of high molecular weight inhibit EtBr-induced mutations by interfering with metabolic activation of theutagen. Preliminary experiments indicate that dietary exposure to ethereal oil of sage suppressed in vivo mytomicine C induced chromosome aberration in mice.

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GENOTOXIC EFFECTS OF VEGETABLE EXTRACTS TREATED WITH PESTICIDES USING ALLIUM CEPA ABERRATION AND MICRONUCLEUS TEST AND SALMONELLA MUTAGENICITY TEST

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The presence of chemical residues in vegetable foods is a main source of human exposure to toxic and genotoxic chemicals. The mutagenic and cancerogenic action of herbicides, insecticides and fungicides on experimental animals is already known (Grover et al.,1990). Several studies have linked the possible damage caused by chronic exposure to low levels of pesticides to birth defects from prenatal exposure and to interference with the immune system. However, there is still scarse information about exposure to small amounts after food contamination and its association with carcinogenicity. Here, we monitored the potential genotoxic effects of chemicals extracted from treated vegetable. Pesticides in the samples were measured by gas-chromatography or HPLC. A dichloromethane extraction, followed by concentration of the samples was performed to evaluate genotoxic effects. The screening methods consisted of the Allium cepa aberration and micronucleus test (Grant et al, 1994) as well as the reversion test on plates using Salmonella typhimurium TA 98 and TA 100 with and without S9. The findings show that chromosomal aberration test on Allium cepa and Salmonella test are less sensitive than micronucleus test and a positive correlation between micronucleus frequency and sample concentration was found.
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GENOTOXICITY OF INDIUM CHLORIDE IN FOUR SHORT-TERM ASSAYS

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Indium have been used for producing of metal alloys for many years. Application of indium in semiconductory industry is increasing. Radioisotopes of indium in compounds such as indium trichloride and colloidal indium hydroxide are used for organ scintigraphy and for tumor therapy. Concerning genetic and related effects, very few studies have been published. In Bacillus subtilis rec assay, negative results for DNA damage were obtained. Induction of apoptosis in rat thymocytes and inhibition of intercellular communication in vitro have also been reported. In order to asses the genotoxic potential, indium(III)chloride was tested for mutagenicity in Salmonella typhimurium strains and in Drosophila melanogaster. Chromosomal damages were assayed in vitro in CHO cells and in vivo in mouse bone marrow cells. Indium chloride did not induce reverse mutations in four histidine-requiring strains of S. typhimurium with or without metabolic activation, and it was negative in the Drosophila SMAR (wing spot) test. In CHO cells it produced structural and numerical chromosome aberrations both in the presence and absence of an exogenous metabolic activation system. In mouse bone marrow, exposed to indium chloride by intragastric intubation, the number of micronucleated polychromatic erythrocytes was elevated, but only at highly toxic dose, which caused lethality as well. In summary indium chloride did not cause point mutations in Salmonella or somatic recombination in Drosophila, but it proved to be clastogenic in mammalian cells both in vitro and in vivo.

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IN VITRO GENOTOXICITY OF MICROCYSTIN-RR

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Microcystins are hepatotoxic cyclic heptapeptides produced by different species of bloom forming cyanobacteria (Microcystis, Anabaena, Nostoc, Oscillatoria). They are inhibitors of serine/threonine protein phosphatases 1 and 2A and act as tumor-promoters. Recent studies indicate that they may also act as tumor initiators. However, data regarding to their genotoxicity are still relatively limited.
In vitro genotoxicity of microcystin-RR (MCYST-RR) was evaluated with the SOS/umu test with S. typhimurium TA1535/pSK1002 and with Comet assay on primary cultured rat hepatocytes and HepG2 cell line. MCYST-RR induced weak but significant dose dependent SOS response only in the presence of exogenous metabolic activation (S9-mix). A 2-fold increase of b-galactosidase activity was induced by 0,28 + 0,04 µg/ml MCYST-RR.
DNA damage induced by MCYST-RR in primary cultured rat hepatocytes and HepG2 cell line was examined after the short-term (3h) and long-term (13h) treatment with 0,01, 0,1 and 1µg/ml of MCYST-RR. After the short-term treatment no increase in DNA damage was detected as compared to the non treated control. After the long-term treatment a clear dose dependent increase of DNA damage was observed in treated cells in comparison to the control.
These results indicate that MCYST-RR is genotoxic and may act also as tumor initiator.

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EVALUATION OF MIGRATION OF MUTAGENS/CARCINOGENS FROM PET BOTTLES INTO MINERAL WATER BY TRADESCANTIA/MN TEST, COMET TEST ON LEUKOCYTES AND GC/MS

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In recent years increasing importance has been given to the distribution of safe drinking water. However, soft drinks packed in plastic containers, especially PET (polyethylene terephthalate) bottles, are now widely used and migration of mutagenic compounds, from PET into water has previously been found. It is therefore necessary to develop a protocol for the routine control of this phenomenon. The aim of this study was to monitor the release of mutagenic/carcinogenic compounds into natural and carbonated mineral waters from PET bottles during a period of 24 months after bottling, by a combined approach using analytical methods (gas chromatography-mass spectrometry analysis, GC-MS), a Comet test on leukocytes and an in situ plant test which reveal micronuclei formation in pollen (Tradescantia/MCN test). The water samples were freeze-dried and processed as follows: the powders were extracted with a solvent and then analyzed by GC/MS and by the Comet test, or reconstituted with distilled water to obtain concentrates (50X and 10X) for the exposure of Tradescantia inflorescences. DNA-damaging activity in leukocytes and micronuclei formation in pollen were found in different samples stored for different numbers of months. GC/MS analysis showed after nine months of storage the presence of di(2-ethylexyl)phthalate (DEHP), a nongenotoxic hepatocarcinogenic plasticizer. No correlation was found among the data obtained from these chemical and biological tests.

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GENOTOXICITY OF OLD AND NEW DRINKING WATER DISINFECTANTS IN PLANT TESTS

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Disinfection is one of the most important and problematic steps in the treatment of drinking water. Since 1974 water chlorination has been shown to produce mutagenic/carcinogenic disinfection by-products (DBPs). For this reason, many researchers are experimenting with disinfectants alternative to chlorine. Millions of humans are exposed daily to residues of different disinfectants (chlorine, chlorine dioxide or ozone) and their DBPs. However, few studies have been carried out on the potential mutagenicity of pure disinfectants. The aim of this research wos to study the clastogenicity of two widely used disinfectants, i.e. sodium hypochlorite (NaClO) and chloride dioxide (ClO_2) and of a new disinfectant, peracetic acid (PAA), never used before in drinking water.The disinfectants were tested at different concentrations and for different exposure times using three plant tests: the Tradescantia-micronucleus (Trad-MCN) assay, the Allium cepa root anaphase aberration (AL-RAA) test and the Vicia faba root micronucleus test (VF-MCN). The inflorescences of Tradescantia # 4430 or the Allium cepa and Vicia faba roots were dipped into bidistilled water containing different doses of disinfectants for different periods of time. Prior to the AL-RAA test toxicity of disinfectants on Allium cepa roots was studied and then the genotoxicity test was carried out with EC50 as the highest concentration. The results show that all the disinfectants , particularly NaClO and PAA, were toxic at the higher concentrations (2 or 10 mg/l) in both Trad-MCN and AL-RAA assays. Some clastogenic effects in the plant tests were found for the disinfectants at different doses.

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GENOTOXICITY OF CHROMIUM AND ITS MODULATION BY SELENIUM IN DROSOPHILA, BY USING THE WING SMART ASSAY

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The Somatic Mutation and Recombination Test (SMART) has been used in Drosophila melanogaster wings to verify the genotoxic activity of two hexavalent chromium compounds (potassium chromate and potassium dichromate) and one tetravalent chromium compound (chromium chloride) as well as the mutagenicity of a selenium compound (sodium selenite). This assay allows to detect the mitotic recombination effects as well as those from different mutational phenomena. The genotoxic effects have been evaluated from the incidence of mutational sectors in the wings of trans-heterozygotic flies from the recessive markers from the third multiple wing hairs (mwh) and flare-3 (flr3), as well as in heterozygotic flies for mwh and the multiple inversion TM3. The genetic changes induced in somatic imaginal disc cells of the wings lead to the formation of mutant clones in both surfaces of the wings. The simple sectors can be produced by different genotoxic mechanisms; while the double sectors can only be due to the mitotic recombination. The antigenotoxicity evaluation has been carried out by using pre- and co-treatments and sodium selenite and potassium dichromate as test compounds.
From the results obtained in the current work, both chromium compounds (VI) evaluated clearly increase the incidence of mutant clones, inducing high levels in the frequency of all the types of clones analysed. On the other hand, chromium chloride (III) does not increase the frequency of mutation clones. A high proportion of the induced clones is due to somatic recombination, which confirms the recombinogenic capacity of chromium compounds (VI).
The results obtained in the antigenotoxicity experiment show that both pre- and co-treatment with selenium reduce the effects of chromium (VI) to control levels. Thus, selenium shows to be highly anti mutagenic in front of the chromium (VI) effect.These results would confirm the useful role that the Drosophila tests, as in vitro systems, have in the genotoxic and antigenotoxic evaluation.

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CHROMATIN CONDENSATION, DNA-ADDUCTS AND MUTAGENESIS

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Chromatin condensation and its role in modification of mutagenic processes is the field which practically does not research although this phenomena is observed in vivo under several important influences such as immunomodulation or adaptive alteration in common. Moreover, chromatin condensation answers for access of DNA-target for mutagens and reparative processes regulation. For this reason we undertook the next study. As modifier of chromatin condensation was used ethidium bromide (EB) - intercalating agent. We demonstrated that mice cells nucleoid condensation depends of EB concentration in neutral sugar gradient. Using sedimentative profile we determined diapason of EB concentrations in which chromatin condensation was altered, and studied [3H]-benz(a)pyren metabolites ([^{3}H]BPM) incorporation into DNA of rat's hepatocytes chromatin in vitro. In these experiments we demonstrated that level of ([3H]BPM) altered in EB diapason tested, but it was almost 10 times more than in control at 5 mkg/ml, significantly lower than one at 10 mkg/ml and equal with control at 50 mkg/ml. It showed that access of DNA for [^{3}H]BPM depended of chromatin condensation. In experiments with drosophila fly we tested 5 carcinogens - N-nitro-N-nitrozoguanidin, diethylnitozamine, 3-methylcholantrene, benz(a)pyrene and aflatoxin B1 in complex with EB. Somatic mosaicism (F1 yellow x white singed^3) test results demonstrated that level of mutations similarly and significantly altered for all of the compounds tested. Moreover, locus-specificity of an answer was expressed reflectively for sections eu- and heterochromatin of drosophila' X-chromosome. All of the results demonstrates that: 1. regulation of chromatin condensation play an important role in modification of mutagenesis; 2. EB using allow to decrease doses detected as mutagenic on drosophila to 100 - 1000 times in comparison with GAP-99.

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APPLICATIONS OF MICRONUCLEUS TEST "IN VITRO" FOR GENOTOXICITY ESTIMATION

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This paper proposes to describe:a) The clastogen effect evaluation of cyclophosphamide (CP) related with isonicotinic acid hydraside (INH) through technique of micronucleus test (MT) (Zorica-Ileana Hertzog, 1988) in secundary and tertiary kidney cultures of Rattus norvegicus (Wistar). Cells were cultivated on lamella in Barski tubes. A secondary culture was subcutivated in 10 tubes (10,356 cells/ml), each one containing 5ml of culture medium. At time 0, cultures were treated and after 16 hours since treatment, lamella were fixed in metanol:glacial acetic acid (3:1) mixture for 30 minutes, were included in Canada balsam on microscopical slides. In CP treatment, the frecquency of cells with micronuclei increases directly proportional to the concentration. The frequency of cells with micronuclei, induced by INH, was unsignifiantly. When the two substances were administrated together, the frequency of cells with micronuclei increased significantly. Hence it might deduce that INH not induces mutations, but intensifies the breaks production by CP. b) The potential genotoxic effect evaluation of some biomaterial samples based on colagen (reticulated colagen with plants extract; shark colagen; colagen + plants extract; colagen + plants triturate; colagen + plants macerate; in concentration of 0,5mg/ml culture, 24 houre before the culture sacrifice), through MT by Lindhom et. al (1991), using the human lymphocites culture from peripheral blood. Mitomycine C (MMC-6mM) is used as positive control.The 5 samples of biomaterials based on colagen determine the apparition of micronucleated cells, between the value estimated in the control sample (10,94‰) and the one estimated in the sample treated with MMC (38,97‰).

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CYTOGENETIC ACTIVITIES OF WATER POLLUTANTS PBTAs AND THEIR PRESUMED MOTHER COMPOUNDS AZO DYEs

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2-[2-(Acetylamino)-4-[bis(2-methoxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotraizole (PBTA-1) and 2-[2-(acetylamino)-4-[N-(2-cyanoethyl)ethylamino]-5-methoxy- phenyl]-5-amino-7-bromo-4-chloro-2H-benzotraizole (PBTA-2) are newly identified potent mutagenic water pollutants from the Nishitakase River in Kyoto, Japan. They are postulated to be derived from AZO DYE-1 and AZO DYE-2, respectively, which are used in the dyeing industry. In the present study, cytogenetic activities of them were studied in the in vitro micronucleus (MN) test in a Chinese hamster cell line CHL. PBTA-1 and AZO DYE-1 were negative but PBTA-2 and AZO DYE-2 were positive. Interestingly the latter two chemicals induced polynuclear (PN) cells strongly and more than half of them were equal-sized binucleate cells. Rhodamine phalloidin staining showed that PBTA-2 causes actin filament abnormalities similar to those caused by cytochalasin B. The results suggest that PBTA-2 has cytochalasin B-mimetic activity (Matsuoka et al. 2000). The difference in chemical structure between AZO DYE-1 and AZO-DYE-2, and PBTA-1 and PBTA-2 is the same side chain. It was suggested that the side chain may strongly contribute to the cytogenetic acitivity. PBTA-2 induced polyploidy only at a highest dose tested and did PN cells significantly at three higher doses. It may be easier to detect chemicals which disturb cell division by using a parameter of PN cells in the MN test than in the chromosome aberration test. Matsuoka, A., H. Sakamoto, S. Tadokoro, A. Tada, Y. Terao, H. Nukaya and K. Wakabayashi, The 2-phenylbenzotriazole-type water pollutant PBTA-2 has cytochalasin B-mimetic activity, Mutat. Res., 464 (2000) 161-167.

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CHROMOSOME ABERRATIONS, SISTER-CHROMATID EXCHANGES AND DNA DAMAGE INDUCED BY CHLOROHYDROXYFURANONES IN MAMMALIAN CELLS

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Several epidemiological studies suggest a cancer risk associated with chlorinated drinking water. The compounds which cause the risk are not known, but chlorohydroxyfuranones (CHFs) seem to be one class of candidate chemicals. We studied the genotoxicity of four different CHFs, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), 3,4-dichloro-5-hydroxy-2(5H)-furanone (MCA), 3-chloro-4-(chloromethyl)-5-hydroxy-2(5H)-furanone (CMCF) and 3-chloro-4-methyl-5-hydroxy-2(5H)-furanone (MCF) in mammalian cells. MX is a known mutagen and rat carcinogen, causing a wide range of genotoxic effects in vitro and in vivo. There are much less data on the genotoxicity of other CHFs. MX, MCA, CMCF and MCF were tested in the chromosome aberration (CA), sister-chromatid exchange (SCE) and single cell gel electrophoresis (SCGE) assay using Chinese hamster ovary (CHO) cells. In all cases, the exposure was in PBS for one hour. All of the compounds induced CAs, mainly chromatid-type breaks and exchanges, dose-dependently. MX caused severe chromosome damage at >=1 µg/ml, MCA at about >=2 µg/ml, CMCF at >=6 µg/ml, and MCF at about >=150 µg/ml. MX, CMCF and MCF increased also the frequency of SCEs significantly, MX at 0.25-0.75 µg/ml, CMCF at 1-4 µg/ml, and MCF at 22-66 µg/ml. MCA was a weak inducer of SCEs. Preliminary studies with the SCGE assay showed that the compounds induce also DNA strand breaks in mammalian cells. The data indicate that MX, MCA, CMCF and MCF all are genotoxic in mammalian cells. The are some differences, however, in their potency of genetic activity.

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DIMETHYLAMINOAZOBENZENE AND 1.2-DIMETHYLHYDRAZINE: IN VITRO AND IN VIVO RESULTS OF TWO POTENT GENOTOXINS IN THE ALKALINE COMET ASSAY

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Two potent genotoxins were tested by the in vitro and in vivo comet assay as a tool for validation Dimethylaminoazobenzene (DAB) and 1.2-dimethylhydrazine (DMH) were chosen because of their organ specificity. In vitro effects on the cell line CHO-K1 were first determined after one hour exposure with and without metabolic activation. For in vivo experiments, the compounds were administered to rats by gavage. Three and 24 hours after administration, various organs (blood, thymus, bone marrow, liver, kidney and intestine) were collected and cells were isolated.
On CHO cells, strand breaks were observed at 0.5 µg/ml DAB with metabolic activation. In vivo, DAB induced DNA damages in liver after 3 hours but they disappeared after 24 hours. Effects with DMH were obtained mainly in liver and intestine as soon as 3 hours after administration with an increase of the damages after one day.
This work showed that in vivo comet assay is able to detect the target organs of these two compounds. However, a clear difference of response was obtained between DAB and DMH and could be explained by the different type of lesion produced on DNA : whereas DMH is mainly an alkylating agent, DAB induces preferentially bulky adducts. The ability of in vivo comet assay for detecting bulky adduct compound inducers is discussed.

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INFLUENCE OF ASCORBIC ACID ON OXIDATIVE DNA DAMAGE INDUCED BY LIGHT IN CHINESE OVARY CELLS

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Antioxidants are supposed to prevent cells from oxidative DNA damage and thus might reduce spontaneous mutagenesis and cancer development. In our studies we have examined the effect of ascorbic acid (VitC) on steady state levels, induction of oxidative DNA damage, toxicity and induction of micronuclei.
Single-strand breaks and oxidative DNA modifications recognized by the Fpg protein (a repair endonuclease) - mostly 8-hydroxyguanine residues - were determined by means of an alkaline elution assay. Incubation of AS52 (CHO) cells with VitC in concentrations below 10mM did not change the steady state (background) levels of oxidative base modifications in the cells. At higher concentrations prooxidative effects were observed. After an incubation of AS52 with 50 and 100 µM VitC for 2h, we did not find any reduction of the oxidative damage induced by exposure to UVB light or the photosensitizer Ro 19-8022 in the presence of visible light, while the oxidative DNA damage by exposure to visible light alone was decreased. Furthermore, we have analysed the influence of VitC on the toxicity and induction of micronuclei caused by the above treatments. After preincubation with 50µM VitC these treatments appeared to be less toxic and the induction of micronuclei seems to be slightly reduced.
Our experiments indicate that VitC inhibits photosensitized DNA damage when applied at an appropriate concentration.

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ASSESSMENT OF DNA DAMAGE IN VARIOUS MAMMALIAN CELLS USING THE ALKALINE COMET ASSAY

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Lung, Liver and immune systeme are target organs for lot of substances and chemical mixtures. As a consequence toxic or genotoxic effects may be released in the affected cells.
The main subject of the study was to analyze the ability of a selected complex mixture of perchlorinated hydrocarbons generated as a waste product in plasma etching processes to produce DNA single strand breaks in various mammalian cells. The degree of DNA damage was determined indirectly by measuring the comet tail length in primary cultured human lymphocytes, human bronchial epithelial cells and rat hepatocytes in the comet assay. Even following a 2h-treatment we could show a direct concentration-dependent increase of comet tail length (0.01 - 0.08 mg/ml complex mixture) in all tested cell types. Significant effects were registered after gavage of lowest test concentration in comparison to control values in lymphocytes (1.1 µm vs. 0.1 µm), in bronchial epithelial cells (1.4 µm vs. 0.1 µm) and in rat hepatocytes (4.5 µm vs. 1.2 µm). Considerable DNA damages were observed after treatment with the highest test concentration.
In order to identify the toxicological key compounds (so called causative agents) the mixture was fractionated. By genotoxicological investigations of these three fractions the group of possible key compounds could be delimited: We were able to exclude about 8 from 26 identified compounds. From the remaining 18 compounds modell-mixtures are prepared and investigated for genotoxic effects in the comet assay.

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CYTOTOXICITY AND INDUCTION OF THE DNA CROSSLINKING OF PLATINUM AND PALLADIUM COMPLEXES IN MOUSE LYMPHOMA CELL LINES

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DNA crosslinks are induced by various chemical and physical agents, some of which (e.g., platinum compounds) are used as cytostatic drugs in chemotherapy for malignant cancers and many of which are known or suspected carcinogens. In our studies we compared the platinum complex with methyl 3,4-diamino-2,3,4,6-tetradeoxy-alfa-L-lyxo-hexopyranoside with identical palladium complex in terms of their cytotoxicity and genotoxicity in two mouse lymphoma L5178Y cell lines (LY) differing in the ability to repair DNA strand breaks (dsb) and their ability to crosslink DNA. Toxicity was evaluated by dye-exclusion and comet assays. The ability to induce DNA cross-links was evaluated by measuring and comparing the retardation of DNA fragment migration in cells treated first with the compounds and then with 2 Gy of X-radiation. Both compounds were markedly toxic to both cell lines; however, the platinum compound was equally toxic to both cell lines while the palladium compound was much more toxic to the repair-defective cell line. The platinum compound retarded migration similarly in both cell lines and in a dose-dependent manner. In contrast, the palladium compound retarded migration more in the repair-defective cell line, though this ability did not correlate with the compound's cytotoxicity. This lack of a correlation between cytotoxicity and DNA crosslinking ability led us to conclude that the palladium compound had a different mode of action than the platinum compound, thus confirming that the cyto- and genotoxicity of metal complexes are influenced by the nature of metal, structure of the stable ligand, and the number of leaving groups on the metal.

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MEASUREMENT OF CYTOTOXICITY IN THE L5178Y MOUSE LYMPHOMA MUTATION ASSAY

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There are two commonly used measures of the cytotoxicity of a test substance in the L5178Y $TK^{+/-}$ mouse lymphoma assay (MOLY). Day 0 relative survival (RS) measures the cloning efficiency of the cell cultures, relative to the control cultures, immediately after the treatment period whereas relative total growth (RTG) measures the growth of the cell cultures, relative to the control cultures, throughout the expression period and including the post expression cloning efficiency. Recent debate over the assay has raised the question of whether one measurement is preferable and whether using one measurement over the other could qualitatively affect the outcome of an assay. We have reviewed data from 94 studies performed in this laboratory covering a wide range of chemical structures. Of these studies, 16 had given a positive response, and the survival data were examined in more detail. Most (12/16) of the positive studies showed no significant difference between the RS and RTG profiles. Of the remaining studies showing significant and reproducible differences between RS and RTG, all gave lower values for RTG than for RS for any given concentration. Selecting the maximum concentration based on the RTG values, rather than the RS values, would not however, have affected the qualitative outcome of these studies. This targeted review of our database suggests that both measures of cytotoxicity are comparable for the identification of positive materials in the L5178Y assay.

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THE ETHYL METHANESULFONATE ACTION IN THE MOUSE MICRONUCLEUS TEST

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The effects of the monofunctional alkylating agent ethyl methanesulfonate (EMS) were studied in the micronucleus (MN) test in mice CBA×C57Bl/6j. Adult males and pregnant females were administrated with the mutagen by intraperitoneal injections. The frequencies of micronucleated polychromatic erythrocytes (PCEs) were analyzed in bone marrow of adults and in fetal liver 6, 12, 18, 24, 30, 36, 48 or 24, 36, 48 and 72 h after the mutagen injection.
In adults, when applying the most mutagen dose, such as 300 mg/kg, the yield of EMS-induced micronuclei increased within the interval from 18 to 48 h, with a maximum of their production (21,3‰ in males and 20,4‰ in females) being observed 36 h after the mutagen exposure. When applying EMS in the dose of 200 mg/kg, dynamics of MN frequencies in male bone marrow was characterized by a peak at 24 h. In fetuses, that were treated in a maternal organism, the greatest number of micronucleated cells (15,2‰) was found 24 h after EMS (300 mg/kg) injection to pregnant females.
Thus, the alkylating agent EMS induced MNs in mouse bone marrow and fetal liver cells; the greatest number of micronucleated PCEs was observed at 24 or 36 h for adults and at 24 h for fetuses. The peak of MN production 36 h after this alkylating agent exposure is a new result compared with earlier one referred by Kondo et al. (1989).
Kondo K., Suzuki H., Hoshi K., Yasui H. Micronucleus test with ethyl methanesulfonate administrated by intraperitoneal injection and oral gavage // Mutat. Res.-1989.- Vol.223, No.4.- P.373-375.

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MUTAGENIC POTENTIAL OF OXIBENDAZOLE AND GRISEOFULVIN

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Oxibendazole and Griseofulvin are widely used as pharmaceutical drugs and are suspected to be aneugenic substances. Both Oxibendazole and Griseofulvin were reported negative in the in vivo bone-marrow micronucleus assay in mice and rats, respectively. To study micronuclei (MN) induction in bone marrow, Oxibendazole was orally administered once to male NMRJ mice at 200, 1000, 2000 and 5000 mg/kg bw. whereas the dose of 2000 mg/kg bw. was also administered twice at 24 hours interval. Griseofulvin was orally administered once to male NMRJ mice at 2000 mg/kg as well as for 5 days to rats (Wistar and Fisher). The results obtained showed that Oxibendazole increased the incidence of MN in mice both after a single administration of 5000 mg/kg bw. and after two administrations at 2000 mg/kg bw. On the opposite, Griseofulvin did not induce MN neither in mice nor in rats, even after five 2000 mg/kg bw. administrations. As an in vitro aneugenic potential of Griseofulvin is usually suggested in the literature, further studies are therefore needed (in particular pharmacokinetics) to elucidate these different results.

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AN INVESTIGATION OF THE EFFECTS OF DRUG DOSE, SCHEDULE AND MDR-1 EXPRESSION ON ETOPOSIDE-INDUCED GENOTOXIC DAMAGE

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Etoposide is commonly used to treat a variety of cancers and exposure is often associated with severe myelotoxicity and in some cases the development of secondary leukaemia that is both cumulative dose and schedule dependent. Transduction of the human MDR1 (multi-drug resistance) gene into mouse bone marrow confers protection to the haemopoietic compartment against myelotoxicity. However, the effects of such an approach on the chronic toxicity have not been previously investigated. This study details the clastogenic effects of dose, route of exposure and schedule of etoposide in vivo, as well as the gene therapy strategy, retroviral transfer of MDR1. Using the mouse bone marrow micronucleus assay (BMMN) a dose dependent rise in frequency of micronucleated polychromatic erythrocytes was observed over a range of doses which were not acutely marrow toxic (0.1-1.0mg/kg). Moreover, the model discriminated between route of administration (oral dosing being less clastogenic than intraperitoneal) and between schedules (5 different schedules examined, total dose 10mg/kg, fractionated over 1-10 days). This assay was therefore employed to determine if the frequency of clastogenic events could be reduced in mice reconstituted with MDR1 transduced bone marrow. Mice in whom 15% of marrow colony forming cells were positive for exogenously expressed MDR1 were significantly protected against MN induction. It was possible to select for the transduced cells such that they represented 35% and 64% of the marrow following a single or double dose of 50mg/kg of etoposide respectively. However, there was no increase in protection from further MN inducing doses of etoposide in these mice, indicating that there is an upper limit to the protection possible, using the BMMN assay.

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THE IN VIVO GUT MICRONUCLEUS TEST AS A SENSITIVE GENOTOXICITY TEST FOR THE DETECTION OF ORALLY ADMINISTERED CLASTOGENS AND ANEUGENS

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A testing battery for chemicals and pharmaceuticals includes besides a bacterial gene mutation assay, an in vitro chromosomal aberration test on mammalian cells, and an in vivo test for chromosome/genome mutations to be performed (EU legislation) in the bone marrow. In this study the sensitivity for detection of clastogens and/or aneugens administered orally by gavage of the in vivo mouse gut micronucleus assay in comparison to the in vivo bone marrow micronucleus assay was assessed. Both bone marrow and gut cells prepared from the same animals were analysed. The reference substances tested were colchicine (COL), carbendazim (CAR), tubulazole (TUB), and griseofulvin (GRI), all known aneugens, and 1,2-dimethylhydrazine (DMH) which is a colon carcinogen with clastogenic activity (both intraperitoneally and orally as shown by Wargovich et al., 1983; and by Goldberg et al., 1983). COL and TUB induced micronuclei in both gut and bone marrow cells, DMH, CAR and GRI only in gut cells. In our experimental conditions the in vivo gut micronucleus assay is more sensitive than the in vivo bone marrow micronucleus assay for the detection of aneugens and clastogens given orally by gavage. Therefore, in the absence of proof of bone marrow exposure, another tissue should be considered. The gut may be a good alternative, since in this study it has proven to be more sensitive, and since for compounds intended to be administered orally to man, the cells of the gastrointestinal tract are the first to come in contact with the compounds.

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Wargovich, M.J., Goldberg, M.T., Newmark, H.L., and Bruce, W.R. (1983) JNCI, 71(1), 133-137.

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GENOTOXIC EFFECTS OF CISPLATIN IN LACZ PLASMID-BASED TRANSGENIC MICE

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Cis-diamminedichloroplatinum (cisplatin) is widely used as a cytostatic drug in chemotherapy for the treatment of various types of cancer. Since it is known to induce secondary malignancies, additional information about its genotoxic effects is relevant. In the present study, the frequency and spectrum of mutations induced by cisplatin in vivo in lacZ plasmid-based transgenic mice were analysed. Additionally, cytogenetic effects were studied in the same mouse model, using sister chromatid exchange (SCE) analysis in splenocytes and the micronucleus (MN) assay in bone marrow polychromatic erythrocytes (PCE). Mutant frequencies in livers of animals injected with 6 mg/kg of body weight of cisplatin were 2-fold significantly increased above the background. Restriction analysis of mutants showed that cisplatin can induce both point mutations and deletions. The clastogenic effect was consistent with the results of MN analysis, showing a 16-fold increase in the frequency of micronucleated PCE in treated animals. A significant increase in SCE was also observed in spleen lymphocytes. Taken together, the present results indicate that cisplatin has clastogenic, recombinogenic and mutagenic activities in vivo. These genotoxic effects may be responsible for the induction of secondary tumours.

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DNA ADDUCTS IN LIVER, LUNG, AND ISOLATED LUNG CELLS OF RATS AND MICE EXPOSED TO 160 PPM [ring-U-^{14}C]-STYRENE BY NOSE-ONLY INHALATION FOR 6 HOURS

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Clara cells are predominant in mouse but less numerous in rat and human lung and contain various CYPs, which may oxidise styrene to the rodent carcinogen styrene-7,8-oxide (SO). SO or other reactive metabolites might form DNA adducts and cause the bronchioalveolar tumours seen in mice exposed to 160 ppm styrene. In rats no tumours were seen at 1000 ppm. 12 Rats and 60 mice were exposed to 160 ppm ^{14}C-styrene (52 mCi/mmol) for 6 h by nose-only inhalation. Liver and lungs were isolated 0 and 42 h after exposure. Fractions enriched in Type II cells and Clara cells were isolated from rat and mouse lung, respectively. DNA adduct profiles in mice and rats differed quantitatively and qualitatively in liver, total lung and enriched lung cell fractions. At 0 and 42 h after exposure, the 2 isomeric N7-guanine adducts of SO (GS) were present in liver at 3.0± 0.2 and 1.9±0.3 (rat) and 1.2±0.2 and 3.2±0.5 (mouse) per 10^8 bases. Several unidentified adducts were present at 2-3 times higher concentrations than GS in mouse, but not rat liver. In both rat and mouse lung GS was the major adduct at (1 per 10^8 bases at 0 h and these levels halved at 42 h. In both rat Type II and non-Type II cells, GS was the major adduct and was 3 times higher in Type II cells than in total lung. For mice DNA adduct levels in Clara cells and non-Clara cells were similar to total lung. The hepatic covalent binding indices (CBIs) were 0.17±0.05 (rat) and 0.35±0.20 (mouse). The pulmonary CBIs were 0.17±0.04 (rat) and 0.24±0.04 (mouse). Compared to CBIs for other genotoxicants, these values indicate that styrene has only very weak genotoxic potency. The overall results of this study suggest that an epigenetic mechanism, possibly caused by a cytotoxic metabolite, is involved in the tumorigenicity in chronically exposed mice.
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DNA ADDUCT FORMATION BY THE UBIQUITOUS ENVIRONMENTAL CONTAMINANT 3-NITROBENZANTHRONE IN RATS DETERMINED BY 32P-POSTLABELING

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Diesel exhaust is known to induce tumors in animals and is suspected of being carcinogenic in humans. Of the compounds found in diesel exhaust and in airborne particulate matter 3-nitrobenzanthrone (3-NBA) is a particular powerful mutagen. We investigated the capacity of 3-NBA of forming DNA adducts which can be used as biomarkers for exposure to diesel emissions. Female Sprague-Dawley rats were treated orally with 2 mg/kg and 20 mg/kg of 3-NBA and DNA was isolated from various organs and analysed by ^{32}P-postlabeling. High levels of 3-NBA-specific adducts were detectable in all organs at both doses. When butanol enrichment was used to analyse DNA from rats treated with the high dose a pattern consisting of four major and one minor adduct was observed in all tissues examined. For the low dose the highest levels of DNA adducts were found in the small intestine (38 adducts per 10^8 nucleotides) followed by forestomach, glandular stomach, kidney, liver, lung and bladder. In order to provide information on the nature of the adducts formed rat forestomach DNA adducts were cochromatographed in two independent systems (TLC and HPLC) with standardized dG adducts and dA adducts produced by reaction of 3-NBA in the presence of xanthine oxidase with deoxynucleoside 3'-monophosphates, in vitro. In both systems each of the rat forestomach adducts comigrated either with dG or dA derived adducts. In addition to reaction with dG (60%) substantial binding to dA (40%) also occurs. On TLC-plates all adducts observed migrated primarily along a diagonal zone, typical for DNA adducts derived from extracts of airborne particulate matter. Our results demonstrate that 3-NBA binds covalently to DNA after metabolic activation, forming multiple DNA adducts in vivo all of which are products derived from reductive metabolites bound to purine bases. They further suggest that the specific adduct pattern found in various organs will provide a basis to monitor human exposure to environmental sources containing 3-NBA.

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TIME- AND DOSE-DEPENDENT INDUCTION OF MUTATIONS AND MITOSES BY CYPROTERONE ACETATE IN LIVERS OF FEMALE TRANSGENIC BIG BLUETM RATS

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Cyproterone acetate (CPA), an active component of several antiandrogenic drugs, has been shown to induce mutations at high doses (>50mg/kg b.w.) and DNA adducts at doses 0.1 mg/kg b.w. in the liver of female transgenic Big BlueTM rats, 6 weeks after CPA administration [Krebs et al., Carcinogenesis 19, 241 (1998)]. To study the impact of the expression time on mutation frequencies (MF), mitotic activity and DNA adduct levels, animals were treated with 100 mg CPA/kg b.w. and sacrified 1, 2, 3 days and 1, 2, 4, 6 and 8 weeks after dosage. MF showed a strong increase on day 2 and 3, to a maximum 2 weeks after CPA administration. However, 4, 6 and 8 weeks after dosage, MF had decreased to about 30% of the maximum value. DNA adduct levels only decreased moderately during the experimental period. The number of mitotic figures was highest 2 days after dosage and decreased on day 3 to the control level. The high MF within the first days after CPA administration indicated that mitogenic activity, which is highest between day 1 and 2, is a crucial determinant for the expression of mutations. To demonstrate an association between mutations and mitogenic activity, transgenic rats were treated with single doses of 5, 10, 20, 40, 80 and 160 mg CPA/kg b.w. MF and DNA adducts were determined 2 weeks later, at the expression time supposed to provide the largest mutagenic response. Our data reveal that already a dose of 10 mg CPA/kg b.w. caused a significant increase ($p=0.027$) of the MF, indicating that the No Observable Effect Level (NOEL) is 5 mg CPA /kg b.w., i.e. 10-fold lower than reported by Krebs et al. Likewise, an increase in the incidence of mitotic figures was already observed at CPA doses of 5-10 mg/kg b.w. Our data support our previous observation that the genotoxic agent CPA induces gene mutations above a particular threshold dose. The magnitude of the threshold dose depended on the expression time.
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MUTAGENICITY AND MUTATION SPECTRA OF VARIOUS ANTITUMOR AGENTS IN THE MUTA™ MOUSE

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Antitumor agents generally show strong clastgenicity, which is important in considering secondary carcinogenesis. We used the transgenic MutaMouse to reveal their potential to induce gene mutations in vivo. Male mice were treated with mitomycin C, cisplatin, etoposide, bleomycin, Ara-C, 6-thioguanine, procarbazine, and monocrotaline by single or subchronic ip injections. Mutant frequencies of lacZ and cII genes were analyzed in bone marrow and some additional organs after an appropriate expression period. Micronucleus induction was simultaneously monitored by using peripheral blood. All chemicals showed strong induction of micronucleated reticulocytes. In contrast, no or only a slight increase in lacZ mutant frequency was observed after single treatment of these chemicals. Increased dosages by subcronic treatment resulted in more than two-fold increase of lacZ and cII MF in bone marrow for mitomycin C, cisplatin, procarbazine, and monocrotaline. Positive response was also observed in lung for cisplatin and procarbazine. Sequencing of cII mutants revealed chemical-specific mutation spectra. Although the incidence is not so high, tandem base mutations were recovered from mitomycin C-, cisplatin-, and monocrotaline-treated samples, suggesting an involvement of intrastrand-crosslink adducts in their mutagenesis.

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EFFECT OF CADMIUM ON IQ AND B(a)P INDUCED DNA DAMAGE IN HepG2 CELLS

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Cadmium is an important heavy metal environmental toxicant, which is classified as human carcinogen. It is genotoxic in mammalian cells in vitro only at high cytotoxic concentrations. Cadmium also enhances genotoxicy of several DNA damaging agents by interfering with DNA damage recognition and ligation step of nucleotide excision repair.
In this study, we used Comet assay to evaluate the levels of DNA damage in metabolically competent HepG2 cell line after treatment with low (10nM, 100nMand 1000nM) non-cytotoxic concentrations of cadmium alone and in combination with indirect mutagens 2-amino-3-methyl-imidazo(4,5-f)quinoline (IQ) and benzo(a)pyrene (B(a)P). No increase of DNA damage was detected after exposure to 10 nM, 100 nM and 1000 nM $CdCl_2$ alone. Co-treatment of the cells with IQ (300mM) and $CdCl_2$ (10 nM, 100 nM and 1000 nM) induced dose dependent increase of DNA damage compared to the treatment with IQ alone. In cells co-treated with 25 mM B(a)P and $CdCl_2$ (10 nM, 100 nM and 1000 nM) increased DNA damage was detected only at the highest concentration (1000 nM) of $CdCl_2$ compared with that caused by B(a)P alone. In conclusion, $CdCl_2$ at low, biologically relevant concentrations showed co-genotoxic effect with the model dietary mutagen IQ and with the carcinogenic polyaromatic hydrocarbon B(a)P. This data support the hypothesis that Cd is carcinogenic through indirect genotoxic effects.

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ACTIVATION OF DIESEL PARTICULATE POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) TO DNA ADDUCTS IN HUMAN MAMMARY CARCINOMA CELL LINE (MCF-7) AND SKIN CULTURE

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It is believed that the genotoxicity of diesel emissions (DE) is caused both by the particles and known carcinogenic chemicals, such as PAHs. This study investigates the activation of particulate PAHs derived from three diesel emissions to DNA binding metabolites in the MCF-7 cells and human skin tissue culture. The soluble organic fraction (SOF) from particulate exhausts was Soxhlet extracted with dichloromethane. 14 PAHs were determined from the extracts by HPLC/FLD. The time and dose dependence of adduct formation in MCF-7 cells was studied using benzo[a]pyrene (B[a]P) and 5-methylchrysene (5-MeCHR) as model compounds of single PAH activation. Extract of SRM 1650 standard reference material was used as a representative of complex mixtures. Adducts were measured by the 32P-postlabeling method. Adducts were formed dose dependently by B[a]P and the SRM 1650 extract. The highest adduct level by B[a]P was formed with 5 µM concentration. 2,5 µM B[a]P formed maximum adduct levels at 12 h, whereas, adducts formed by 2,5 µM 5-MeCHR were 2-fold higher at this time point, increasing to 6-fold at 48 h. Time dependent formation of DNA adducts by three diesel particulate extracts was compared to those formed by single PAH compounds. Qualitative and quantitative differences in adduct formation were detected between DE extracts in MCF-7 cells. Individual differences in PAH metabolism were analyzed by treating skin explants from different individuals with DE particulate extracts. (Supported by Neste Oy's Foundation and TDC of Finland; MCF-7 cells from ABL-Basic Research Program, MD, USA).

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HYDROGEN PEROXIDE: ARE TISSUES LESS SUSCEPTIBLE TO MUTATION?

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There are various reasons why chemicals that are mutagenic in vitro do not cause mutation in vivo, including altered metabolism, distribution and detoxification. Another possible reason is that cells may be less sensitive to chemicals when they are part of a tissue than as a single cell. This would contribute to the oversensitivity of many in vitro assays.
The objective of this investigation was to examine the relative contribution of cell line and tissue structure in susceptibility to the genotoxic effects of a model mutagen, hydrogen peroxide (H_2O_2), in two types of human cell and a 3D tissue equivalent model. The mutagenic potential of H_2O_2 was examined in human peripheral blood lymphocytes (HPBL) using the standard in vitro chromosome aberration assay. The Comet assay is a simple electrophoretic technique for measuring DNA damage in individual cells and was used to assess DNA damage in the buccal mucosa squamous cell carcinoma cell line, TR146 and a 3D buccal mucosa tissue equivalent, constructed using TR146 cells (SkinEthic, France).
A significant increase in H_2O_2-induced chromosome aberrations was seen in HPBL, at concentrations of 5.0µg/ml and above. DNA damage, in the form of single strand breaks, was detected in the comet assay at 6.8µg/ml and above for the TR146 cell line and at 43µg/ml and above for the 3D buccal mucosa tissue equivalent. These results indicate that there was little difference in the concentration at which chromosome damage and DNA damage was observed between the two cell lines, despite one being an epithelial cell and the other a blood cell. In contrast, there was a six-fold difference between the cell lines and the 3D tissue equivalent. This was not related to cell type since the cells used to construct the 3D tissue equivalent were the same as one of the cell lines. These results suggest that tissues may be less susceptible than single cells to damage by genotoxins.

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EFFECT OF A BRUSSELS SPROUTS EXTRACT ON OXIDATIVE DNA DAMAGE TO HUMAN LYMPHOCYTES IN VITRO

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An aqueous extract of Brussels sprouts has shown potent protective effect against guanine oxidative in isolated DNA in vitro. In order to study such effect in intact cells, human lymphocytes were pre-incubated with the extract (0 -100 µg/ml) and afterwhere were treatment with hydrogen peroxide (0-25 µM). DNA damage was examined by comet assay in term by using the endonuclease III and FPG to determine the level of oxidized pyrimidines and purine respectively.
The extract produced a concentration-dependent reduction oxidative DNA damage in the range 10 - 100 µg/ml. When concentration above 100 µg/ml DNA damage was not decreased. At concentration 100 µg/ml of Brussels sprouts extract strand breaks were reduced by 25-42% ($P<0.05$), endonuclease III sensitive sites by 2-7% and FPG sensitive sites by 5-21% ($P<0.05$). The effect in an intact cell system was less in isolates DNA in vitro.

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ANTICLASTOGENIC EFFECT OF GARLIC DERIVATIVES ON HUMAN PERIPHERAL LYMPHOCYTES IN VITRO

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The objective of study was to identify anticlastogenic potentials of:
1) Synthetically prepared and pyrolyzed derivatives of garlic (S-methyl-L-cysteinsulphoxide [MCSO], S-propyl-L-cysteinsulphoxide [PCSO], S-allyl-L-cysteinsulphoxide [ACSO].
2) Allicin (Diallylthiosulphinate; C6-H10-O-S2; CAS: 539-86-6).
3) Dimethylthiosulphinate; C2-H6-O-S2,
using cytogenetic analysis of human peripheral lymphocytes, exogenous metabolic activation system (MAS) and reference clastogenes Thiotepa (TT) and Cyclophosphamide (CP).
* Remarkable anticlastogenic activity of garlic organosulphur derivatives (MCSO, PCSO, ACSO) was found when exogenous metabolic activation system was used. Rate of their anticlastogenic activities could be expressed: PCSO > MCSO > ACSO, resp. MCSO> ACSO > PCSO when combined action of tested derivatives and reference clastogenes (TT resp. CP) were used. Dose response effect was not observed.
* There was found high level of Allicin anticlastogenic effect when the combined action with CP was used. The anticlastogenic effect was not detected when direct clastogen TT was used.
* Anticlastogenic effect of Dimethylthiosulphinate and direct clastogen TT was found unsignificant.

Abbreviations:
MCSO : S-methyl-L-cysteinsulphoxide; PCSO : S-propyl-L-cysteinsulphoxide; ACSO : S-allyl-L-cysteinsulphoxide; TT : Thiotepa; CP : Cyclophosphamide;

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INTERFERON a CAN CHANGE GENOTOXICITY OF ALKYLATING ANTICANCER DRUGS

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In modern cancer therapy conventional chemotherapeutic drugs are frequently used in combination with immunomodulatory agents such as interferon a (IFNa). Despite promising results of clinical studies the degree of therapy-related toxicities is high and seems to be cumulative for cytokine and chemical drugs used in the treatment schedules. This stimulated us to study the possible changes in genotoxicity of alkylating anticancer drugs in interaction with IFNa. For this purposes peripheral blood lymphocytes from three healthy donors were grown 24 hours with 50 U/ml of IFNa and then exposed to increasing concentrations of alkylating chemotherapeutic drugs cisplatin, phopurine and pharanox. The rate of induced genetic damage was evaluated by the test of sister chromatid exchanges (SCE), cell proliferation rate was measured as replication index (RI).
All three alkylating drugs analyzed in our study increased the number of SCE in dose-dependent manner. Meanwhile cell preincubation with 50 U/ml of IFNa statistically significantly ($P < 0.05$) decreased the genotoxic effect of phopurine in two independent studies. The highest antigenotoxic effect of IFNa (up to 70%) was observed when cytokine was used in combination with 5 µM of phopurine. However, no protective effect of IFNa was detected when the interactions of cytokine with pharanox and cisplatin were studied. In contrary, the number of SCE induced by 10 and 25 µM of pharanox and by 0.1µg/ml of cisplatin were increased significantly (up to 40%) when lymphocytes were preincubated with 50 U/ml of IFNa.

Different effects of IFNa on genotoxicity of alkylating anticancer drugs are mainly dependent on the proliferation rate of cells. IFNa did not change cytostatic effect of phopurine but significantly increased cell proliferation rates suppressed by cisplatin and pharanox. The rise in SCE rate may be due to a reduced duration or a decreased efficiency of DNA reparation processes.

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CYTOGENETIC EFFECTS OF VINCRISTINE ON HUMAN LYMPHOCYTES IN VITRO

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Vincristine belongs to vinca alkaloids, antineoplastic agents, and has been used in various chemotherapeutic regimens. The genotoxic potential of vincristine is assessed on human peripheral blood lymphocytes following administration of the drug at doses within the therapeutic range (0.05 (g/ml) by use of cytokinesis-blocked micronucleus assay combined with Giemsa and DAPI staining techniques. In vitro treatment of human lymphocytes with vincristine was performed on cells in G0 phase and lasted for 24 h. Afterwards, lymphocytes were cultured in vitro for 72 h. By use of Giemsa staining in vincristine-treated sample 1000 cells were analysed and 68 micronuclei, 77 multimicronucleated cells as well as 136 C-mitoses were recorded. In control sample, neither C-mitoses nor multimicronuclear cells were observed. On 1000 analysed cells only 5 micronuclei were recorded. By use of DAPI staining in vincristine-treated sample 1000 cells were analysed and 77 micronuclei, 81 multimicronucleated cells as well as 153 C-mitoses were recorded. In control sample, on 1000 analysed cells only 7 micronuclei were recorded. On the basis of DAPI flourescence signals, micronuclei were divided in signal-positive and signal-negative. In vincristine-treated sample 66.2% micronuclei were signal-positive and 33.8% signal-negative, while in control there were 42.9% signal-positive and 57.1% signal-negative micronuclei. Observed differences were tested by means of X^2 test, and it was found out that they are statistically significant. Results obtained in this study by use of micronucleus assay combined with Giemsa and DAPI staining techniques confirmed that vincristine exhibit predominantly aneugenic effects on human lymphocytes.

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THE GENOTOXICITY OF CARBADOX IN VITRO

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The antimicrobial preparation Carbadox, a product of Phizer Animal Health Pharmaceutical company (USA), is a member of a synthetic organic compound classified as metil-3(2 quinoksalmetilen) karbozat-N1-N4 dioksi. The genotoxic effects of Carbadox has been investigated in vitro on human peripheral blood lymphocytes by following the capability of Carbadox to induce numerical and structural chromosome changes. To test a possible effect of the investigated substance on DNA we used the sister chromatid exchange (SCE) assay in vitro. The investigated substance Carbadox was tested through three experimental concentrations: 10 µM; 20µM and 40µM. Negative control groups were treated with physiological saline.
Chromosomes for karyotipic analysis were prepared by the procedure of Evans and Riordan (1976), modified by Zimonjic et al (1990). The procedure of Perry and Evans (1976) , modified by Zimonjic et al (1990) was used for differential staining of sister chromatid detection in human peripheral blood lymphocytes.
Our results show that all experimental doses of the investigated substance Carbadox shows the potential for transformation of the karyotype of human lymphocytes by inducing numerical aberrations type aneuploidy and polyploidy and structural aberrations type gaps and lesions. The overall cytogenetic changes induced by Carbadox in all three doses clearly shows a statistical high significant increase in respect to the control group. We have also estimated a correlation between a increase of dosage and cytogenetic changes. Cytogentic changes and a dose-effect dependency clearly shows a genotoxic potential of Carbadox on lymphocytes of human peripheral blood.
All three doses of Carbadox induced a highly statistically increase in the frequency of SCE in respect to the untreated control groups. High SCE frequencies shows a possibility of the investigated substance to generate changes in the DNA structure.
Our results classify Carbadox as a clastogenic agent.

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GENOTOXIC EFFECTS AND SURVIVAL OF LYMPHOCYTES AFTER IN VITRO EXPOSURE TO COBALT/HARD METAL COMPOUNDS

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The evaluation of the in vitro genotoxic effects and survival of lymphocytes exposed to varying cobalt species may help to explain their in vivo human carcinogenicity. Cobalt metal (Co), tungsten carbide (WC), cobalt-tungsten carbide mixture (WC-Co) and cobalt chloride ($CoCl_2$) were compared for several endpoints: 1) Solubilisation of cobalt ions in the culture medium by AAS. Ionic cobalt concentration after 24h: WC-Co<Co<$CoCl_2$. 2) Apoptosis by annexin-V. Apoptosis triggering by WC, which was not inducing DNA damage in the comet assay, displayed a bimodal pattern. Co and $CoCl_2$ showed a gradually increasing frequency of apoptosis. When lymphocytes were exposed to WC-Co, only the WC-characteristic short response was maintained. Moreover WC-Co showed a lower frequency of apoptotic cells than Co or WC alone. These data are consistent with enhanced production of oxygen free radicals (no apoptosis) during the solubilisation of cobalt in the presence of WC. They also may suggest a counteracting effect of both transition metals on apoptosis. Inhibition of the ceramide-apoptosis pathway by fumonisin was almost complete for WC. 3) Inhibition of repair of UV-induced DNA lesions by comet assay. Mainly the incision step was affected. 4) Induction of chromosome breakage/loss by combination of the in vitro micronucleus test with FISH (pancentromeric probe). This analysis complements previous in vitro genotoxicity studies demonstrating an elevated effect of WC-Co as compared to Co. Cobalt compounds are thus able to induce in vitro several types of (geno)toxic damage in human lymphocytes: apoptosis, DNA damage, DNA repair inhibition and chromosome/genome mutations. Their relevance for cancer induction will depend on the capacity to cause cell death and subsequent repair proliferation.

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EXPOSURE AND EFFECT MONITORING OF WORKERS IN THE COBALT/HARD METAL INDUSTRIES: ABSENCE OF SIGNIFICANT GENOTOXIC EFFECTS

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An increased lung cancer risk is associated with occupational exposure to mixtures of cobalt metal and metallic carbide particles, but when exposure is to cobalt metal alone. The current TLV-TWA was established without consideration of carcinogenicity data. The present study was designed to assess whether an increased cancer risk can be detected in workers currently exposed on average to the TLV-TWA (20 µg/m^3). Therefore, different complementary genotoxicity endpoints were integrated to assess biomarkers of effects which represent both initial DNA damage (8-hydroxydeoxyguanosine in urine and comet assay on lymphocytes) and definitive chromosome breakage/loss (micronuclei in lymphocytes). As measures for cobalt exposure and recent smoking, cobalt and cotinine were determined in urine, respectively. Serum concentrations of selenium and vitamin E were determined as indicators of protection against oxidative damage. No significant increase in any genotoxicity biomarker was detected in workers exposed to cobalt-containing dust as compared to their matched controls. No significant difference in genotoxic effects was observed between cobalt and hard metal exposed workers. Multivariate analysis indicated that 8-hydroxydeoxyguanosine and micronuclei levels were significantly increased in smoking workers exposed to hard metal dust. This confirms a previous epidemiological study where an increased risk of lung cancer mortality was found in smoking workers from the hard metal industry. Thus, this specific group of workers deserves closer medical surveillance.

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CYTOGENETIC MONITORING OF WORKERS EXPOSED TO HEAVY METALS

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Exposure to heavy metals has been considered to be potentially genotoxic and related to risk of cancer and adverse reproduction outcomes. In order to assess genotoxic hazards from exposure to cadmium and lead, we evaluated two cytogenetic changes - the level of micronuclei (MN) and sister chromatid exchanges (SCE) in peripheral lymphocytes of workers. In our studies we selected 22 workers exposed to cadmium, 30 workers exposed to lead, employed in storage battery plant, and 43 unexposed persons. The blood samples from all subjects were used for determination of metal concentrations and preparation of 72 hours lymphocyte cultures for SCE and MN analysis, with BrdU or cytokinesis block respectively. Worker's occupational exposure to heavy metals was relatively high. The lead concentrations in worker's blood varied from 282 to 655 µg/l, and cadmium levels ranged from 2.4 and 30.8 µ/l. The concentrations of lead in control persons were from 17 to 180 µl/l and - of cadmium from 02 to 5.7µ /l.
The mean frequencies of sister chromatid exchanges in two exposed groups were similar to each other and slight higher ($p<0.05$) than in control group. The mean levels of micronuclei in Cd - and Pb - exposed workers were two times higher as compared to the control population.
Our findings from cytogenetic analysis demonstrate a higher sensitivity of micronuclei test compared to sister chromatid exchanges in detection of genotoxic effects of cadmium and lead exposure.

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THE ASSESSMENT OF DNA DAMAGE IN THE WHITE BLOOD CELLS OF WORKERS EXPOSED TO CADMIUM AND LEAD

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Among the carcinogenic metals, some evidence is available supporting an indirect mechanism of action for cadmium and lead. Reactive oxygen species (ROS) and free radicals are generated during this mechanism. It has been documented that free radicals and ROS may play an important role in the initiation and promotion if carcinogenesis.
The aim of this study was to assess the genotoxic effects in workers exposed to heavy metals particularly to cadmium and lead in the storage battery plant. The levels of DNA damage in the white blood cells of 22 and 43 workers exposed to cadmium and lead respectively and of 41 control subjects have been evaluated. The blood (about 100 ml) from each person was taken for the preparation of agarose suspension and the alkaline cell gel electrophoresis assay (comet assay) in individual cells was applied for detection of DNA single strand breaks (SSB). DNA damage was estimated in the cells before as well as after incubation of cells in RPMI 1640 medium at 37o C and 5% CO_2 for 3 hours to assess the repair of DNA SSB. The level of damaged DNA before and after repair process was expressed as a percentage of cells with 2,3 and 4 comet types. The percentage of cells with damaged DNA before repair was 9.5 and 9.8 respectively for cadmium and lead workers and was slight but significant higher than in control group (7.0). The effect was similar both in smokers and nonsmokers in each exposed groups and was higher compared to the respective groups in control population. After incubation cells in the culture medium, the DNA SSB in cadmium and lead workers were found at the level of control group. The study showed that the level of DNA damage observed in the workers exposed to cadmium and lead was not so serious and could be repaired.

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BIOMONITORING OF ENVIRONMENTAL EXPOSURE TO COMPLEX MIXTURES (HEAVY METALS AND PAHs) IN CHILDREN LIVING IN UPPER SILESIA, POLAND

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Katowice province is the region in south-western part of Poland. This province occupies 2% of the total area of Poland and has 11% of the population. In the past rich local mineral resources of coal, zinc and lead ores and other materials have stimulated the development of many industrial branches in this region, which caused intensive deterioration of the natural environment. The objective of the study was to assess genotoxic effects: chromosomal aberrations (CA), sister chromatid exchanges (SCE) and the level of micronuclei (MN) in peripheral blood lymphocytes in 74 children in relation to their exposure to complex environmental mixtures. The assessment of exposure was based on the following biomarkers: urinary mutagenicity tested by Salmonella assay (TA 98, YG: 1021, 1024, 1041), urinary 1-hydroxypyrene (1-Hp) determined by HPLC method, PAH-DNA adducts (^{32}P-postlabelling method), lead and cadmium in whole blood (AAS method). Mutagenic effect of urine and the level of urinary 1-Hp did not influence any of cytogenetic effects. We found higher numbers of SCE in children with higher level of PAH-DNA adducts. Exposure to lead had significant effect on the number of MN and exposure to cadmium on CA level in the peripheral blood lymphocytes. ETS determined as urinary cotinine concentration did not affect cytogenetic biomarkers.

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MICRONUCLEI, LIPID PEROXIDATION AND CHROMIUM CONCENTRATION IN TANNERY WORKERS

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The leather processing industry (tanning industry) is a major source of environmental contamination due to the number of potentially toxic substances involved, the heavy metal chromium (Cr) being one of the most relevant.
The aim of this study is to evaluate the health effects of this chemical burden on the directly exposed working population. For this purpose we collected blood and urine from 33 tannery workers, who were divided into 2 groups of estimated exposure to Cr: high (H) and low (L). The samples were analysed for Cr concentration (urine and plasma), lipid peroxidation (urine and plasma) and micronuclei frequency by the cytokinesis blocked micronuclei assay in peripheral blood lymphocytes as a measure of DNA damage.
The results for plasmatic chromium were 6,41±2,44 (H) and 1,78±0,24 (L) (µg/L ± SEM). The frequency of binucleated lymphocytes with micronuclei was 8.3±1.25 (H) and 5.43±0.39 (L) (average±(SEM).
No correlation was found between lipid peroxidation and urinary or plasmatic Cr concentration.
Additionally, genetic polymorphism of glutathione-S-transferase (GST) M1 and T1 were analyzed, but this polymorphism did not seem to influence the cytogenetic damage.

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CYTOGENETIC EFFECT OF CHROMIUM ON BULGARIAN OCCUPANTIONALLY EXPOSED CHROME PLATING WORKERS

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There is substantial evidence that chromium (Cr) is a widespread environmental pollutant and a known mutagen. Occupational exposure to hexavalent chromium (CrVI) compounds through inhalation is associated with lung carcinogenesis.
The aim of this study was to investigate the cytogenetic effect in peripheral blood lymphocytes (PBLs) of workers exposed to various Cr (VI) compounds. The study comprised 15 workers whose working conditions imply rather elevated chromium exposure. The exposed group was compared to a control group from the administration staff of the same plant. Three cytogenetic end-points were investigated: chromosome aberrations (CA), micronuclei (MN), and sister chromatid exchanges (SCEs) in PBLs.
No statistically significant differences were observed in the frequency of structural chromosome aberrations and sister chromatid exchanges. The chromium exposed workers showed a significant increase only in the frequencies of micronuclei. Additionally, micronucleus assay in exfoliate buccal cells was carried out. The frequency of the micronuclei in the buccal cells was significantly higher than the frequency of MN in PBLs.

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FLUORESCENCE IN SITU HYBRIDIZATION IN PERIPHERAL BLOOD LYMPHOCYTES AND BUCCAL CELLS FROM CHROMIUM EXPOSED WORKERS

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The aim of the present study was to investigate the genotoxic effects of chromium exposure under occupational conditions. The cytokinesis block micronucleus assay and fluorescence in situ hybridization technique was aaplied to peripheral blood lymphocytes (PBLs) and exfoliate buccal mucose cells from chromium plating workers, as well as from gender and age matched controls from the administration staff of the same factory. Pancentromeric DNA probe was used to detect the micronuclei with centromeric signal, indicating aneugenic effects. Our results demonstrate a significant increase in the frequency of both micronucleated PBLs and buccal mucose cells from chromium platers as compared to the matched controls. However, the distribution of centromere containing lymphocytes and buccal epithelium cells is similar to that in the controls.

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DETECTION OF DNA DAMAGE IN PERIPHERAL LYMPHOCYTES OF ALUMINIUM WORKERS: IMPROVEMENT OF THE SENSITIVITY OF THE ALKALINE SCGE ASSAY BY ARA-C

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A study was carried out to assess the occurrence of DNA damage in peripheral lymphocytes of alluminium plant workers exposed to low levels of PAHs (range:8.7-1.4µg/m^3). DNA damage was evaluated by the alkaline single cell gel electrophoresis (SCGE) assay in unstimulated lymphocytes of 93 workers, categorized in six groups according to different level of exposure to PAHs and 16 controls from a rural area. The level of DNA damage was parametrized by tail moment values .Moreover, in order to maximize the expression of bulky adducts formed by PAH as DNA strand breaks detectable by SCGE, lymphocyte from 37 subjects (24 workers and 13 controls) were cultured 16 h in complete medium with 2% of phytohemoagglutinin, with and without 1µg/ml of the DNA repair inhibitor Cytosine Arabinoside (Ara-C), before being analysed by SCGE.
The results obtained do not show significant differences in individual tail moment values (average from 100 cells) in unstimulated lymphocytes of subjects with different levels of exposure to PAHs. However, a significant excess of heavily damaged cells, with tail moment values greater than the 90° percentile of the overall distribution, is observed in some exposed groups compared to the controls.
No significant difference between exposed and control subjects is shown by the SCGE analysis of stimulated lymphocytes. On the other hand, a clearcut difference in individual tail moment values, with higher values in exposed workers, is highlighted by the SCGE analysis of lymphocytes cultured in the presence of Ara-C ($p<0.001$, Mann-Whitney U test). These results indicate that Ara-C may facilitate the SCGE detection of premutagenic lesions in human white blood cells, inhibiting gap filling in their processing after mitogen stimulation.
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GENOTOXIC STUDY OF NIMODIPINE IN HUMANS

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This work was done to study the genotoxic potential of chronic long-term therapy with nimodipine by measures of sister chromatid exchanges (SCE) and micronuclei (MN) in peripheral human lymphocytes of patients with long-term exposure to this drug. Besides, peripheral human lymphocytes of control individuals exposed in vitro to nimodipine were studied to assess the effect of the drug itself. The in vivo study was carried out on 5 patients under antihypertensive treatment with nimodipine. The in vitro study was performed on 5 control individuals by adding the drug to the culture medium at a final concentration similar to the levels found in plasma (controls/medium).
In vivo study showed no genotoxic effects of long-term therapy with nimodipine because the frequencies of SCE and MN in exposed patients did not show significant differences as compared with control individuals. A statistically significant increase in the frequency of MN was detected in controls/medium as compared with control individuals without the drug. Fluorescence in situ hybridization (FISH) analysis revealed statistically significant differences with respect to the frequency of centromeric signals in nimodipine-induced MN in vitro.
Different results in vivo and in vitro were due to the extensive metabolism of nimodipine, indicating that the cytogenetic effect observed was due to the drug itself rather than its metabolites. Attending to the in vivo results, chronic long-term therapy with nimodipine is not associated with increased genotoxicity.

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FOLLOW-UP GENOTOXICOLOGICAL MONITORING OF NURSES HANDLING ANTINEOPLASTIC DRUGS

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Hospital nurses are subject to possible occupational carcinogen exposure during handling and administration of cytostatic infusions. A multiple end-point follow-up genotoxicology monitor developed in our laboratory including determination of sister-chromatid exchange (SCE), high frequency SCE (HFC) and structural and numerical chromosome aberration (CA) frequencies and UV-light induced unscheduled DNA synthesis (UDS) was performed in peripheral blood lymphocytes (PBLs) among four groups of 95 nurses. In Hospital 1 without safety cabinet, mean CA was significantly increased (2.87±0.69%) compared to the control. In Hospital 2 with horizontal airflow significantly elevated means of CA (4.17±1.08%), SCE (8.01±0.47/mitoses), HFC (9.32±3.36%) and UDS (8.01±0.47 rel. unit) were detected. During the second study the mean CA yields remained high (3.50±1.06%), although safety conditions had been improved. In Hospitals 3 and 4 with safety cabinets, the mean CA yields were lower than those in the previous two groups. In Hospital 3 in the first study the mean CA (1.57±0.42%) was at the level of industrial controls (1.10±0.26%). A fluctuation in CA yields above the control level and an increase in HFC in the 4th and in the 6th year of the study (8.76±1.12 and 12.00(1.77%) were observed. In Hospital 4 both mean CA (2.29±0.45%) and mean HFC (9.64±2.40%) were also elevated. The fluctuation in CA and HFC yields in these groups suggests a cytostatic exposure with other possible ways even with the use of suitable safety cabinets. These data indicate a higher risk for nurses with inadequate protection and a certain expression time of the genotoxic changes leading to late somatic mutations and higher cancer risk.

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WORKING CONDITION-RELATED IMPROVE IN THE GENOTOXICOLOGY PARAMETERS OF HUNGARIAN ROAD PAVERS

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A multiple end-point follow-up genotoxicology monitor developed in our laboratory including determination of sister-chromatid exchange (SCE), high frequency SCE (HFC), structural and numerical chromosome aberration (CA) and HPRT mutation frequencies (VF) and UV-light induced unscheduled DNA synthesis (UDS) was performed in peripheral blood lymphocytes (PBLs) among 22 tar-free asphalt pavement finishers (8 pavers and 14 finishers, mean age: 37 years) between 1996 and 1999 in Hungary. The results were compared with those of 6 local controls (35 years), 101 historical (38 years) and 87 industrial controls (38 years). The most pregnant changes were found in the CA yields. The mean control CA was 1.82%. In the first study we found increased CA in donors either exposed to hot asphalt fumes or who cleaned the equipment with crude oil. The mean CA of the 14 finishers working in closed cabins was 3.67% in 1996. The increased CA was attributed to the high level of hot asphalt fumes in the closed cabins. By 1999 CA decreased to 1.23%. For the 8 pavers working in open air the mean CA in 1996 was 3.60%. Data suggested that increased CA was due to crude oil-exposure therefore it was later substituted by harmless detergents. By 1999 CA decreased to 1.00%. CA dropped down the control level one year earlier (1998) than in case of finishers. The observed individual variations were attributed to smoking and the inadequately used personal protection. The results suggest that use of tar-free asphalt and harmless detergents with adequate personal protection does not increase the genotoxicology parameters compared with controls and an improve of working conditions can prevent further exposures and thus decrease the cancer risk.

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DOSE-RELATED CHANGES IN CHROMOSOME ABERRATION YIELDS OF BENZENE-EXPOSED WORKERS DURING A 9-YEAR-LONG FOLLOW-UP STUDY?

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The changes in structural and numerical chromosome aberration (CA) frequencies were studied in peripheral blood lymphocytes (PBLs) among 43 benzene-exposed oil refinery workers (mean age: 35 years at the start) during a 9-year-long follow-up genotoxicology monitoring between 1990 and 1998. The results were compared with those of 101 historical (mean age: 38 years) and 87 industrial controls (38 years). The mean peak ambient benzene concentration was over 20 mg/m^3 in 1990, and then by 1992 it decreased gradually to 10 mg/m^3. However, in 1994 it reached again a maximum of 44 mg/m3, then due to the improved safety conditions in 1995-96 it dropped down below 5 mg/m^3 the MC in Hungary. In 1997 it was again over 15 mg/m^3, and remained at the same level until the end of the study. CA yields of the exposed donors were significantly higher than the industrial control (1.5%) in each year except 1992. The highest CA yield (5.2%) was observed in 1990. Parallel to the changes in ambient benzene concentrations the CA yields also decreased and reached a minimum (1.8%) in 1992 but in 1993 it increased again, and in 1994 (with the maximum benzene concentration) it was 3.1 %. However, it remained 4% and 2.8% in 1995-96 (respectively) with the minimum benzene exposure. In the last two years when benzene exposure increased again the CA yields remained unchanged compared with 1996. An ANOVA of the data supported dose-related changes in the CA yields (DF=6, F=0.838, significance of F=0.781) between 1990-94, and a not significant relationship for the rest. These data suggest that a follow-up study probably can reveal a dose-response in the CA yields.

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TOXICOKINETICS OF XENOBIOTICS AND THEIR METABOLITES-IMPORTANCE IN THE EVALUATION OF GENOTOXIC EFFECTS

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Styrene, an important and high volume industrial chemical, has been shown to be genotoxic in a number of studies. Daily intake of styrene via inhalation during hand lamination work may entail gram quantities daily. The data on styrene carcinogenicity are inconclusive. Styrene is in vivo metabolized to styrene 7,8-oxide (SO), which is probably responsible for styrene genotoxicity. These facts point out the need to measure parameters of internal exposure in styrene-exposed individuals, i.e. styrene concentrations in blood (SB), in tidal air (SEA) as well as SO in blood. Our recent study showed an excellent correlation between SB, styrene-specific O6-guanine adducts (r=0.810, P=0.001) and singlre-strand breaks (TM). Most recently, we found significantly enhanced (15-fold) SB and SEA in styrene-exposed individuals, SB and SEA correlated with styrene concentration at the workplace. Surprisingly, no obvious relationship was found between SB, SEA and single-strand breaks (SSB) in DNA. SB and SEA significantly correlated with chromosomal aberrations (r=0.408, P=0.001, r=0.497, P=0.001, resp.). The multiple regression analysis confirmed the close relationship between exposure parameters (styrene at the workplace, SB, SEA), duration of exposure and both SSB in DNA (r=0.633) and the frequency of CA (r=0.601). In the exposed group, significant correlation has been revealed between the mutant frequency at HPRT gene and SB. Moreover, ANOVA confirmed the relationship between SB and HPRT MF in the whole set. Additionally, an association was found between SB and 7-alkylguanine adducts in mice after three-weeks inhalation exposure to styrene.
Our study in progress attempts to assess internal exposure parameters (SB, SEA and SO in blood), their role in genotoxic effects, as well as parameters of individual susceptibility (genotyping CYP 2E1, epoxide hydrolase and GSTM1, P1 and T1).

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CHROMOSOMAL ABERRATIONS AND DNA REPAIR CAPACITY AS MEASURED BY THE CHALLENGE ASSAY IN BOATBUILDERS EXPOSED TO LOW LEVELS OF STYRENE

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Styrene is an organic solvent used in the production of plastics, resins and synthetic rubber. In 1994 it was classified as a possible human carcinogen (IARC, 1994). However, results concerning its mutagenicity are conflicting and the carcinogenic pathway is still unknown. Interference of styrene with DNA-repair processes was one hypothesis of the present study. 14 workers exposed to low levels of styrene (mean <100mg/m^3 styrene in air; 35 µg/l styrene in blood) and seven controls were investigated for structural chromosomal aberrations using FISH. The rate of exchange-type aberrations per metaphase was 0.001 (95%CI 0.0005-0.003) in controls and 0.002 (0.00127-0.004) in exposed. DNA-repair was measured in the challenge assay. The cohort was divided into two subgroups based on their lifelong exposure to styrene. Exchange-type aberration after X-ray challenge of 1.6 Gy was 0.133 (0.105-0.165), 0.155 (0.142-0.170) and 0.179 (0.156-0.204) in controls, low and high exposure group, respectively. The repair capacity of the short- and long-term exposure group was 85% and 74% of the control. Age and smoking habits were confounding factors of the challenge assay. Smokers were mainly present in the lower exposure group so that the styrene induced difference in aberration frequency between these groups was likely underestimated. Considering the low level of exposure in our cohort the findings are consistent with the hypothesis of interference of styrene with DNA-repair.

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BIOMONITORING OF A GROUP OF FEMALE WORKERS IN THE SHOE INDUSTRY: GENOTOXIC EFFECTS OF EXPOSURE TO ORGANIC SOLVENTS

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According to the IARC, there is an increase in cancer risk for workers from the shoe industry, especially for leukaemia, nasal and lung cancer. On the other hand, biomonitoring studies in populations exposed to organic solvents present inconclusive and variable results.
In order to add further information about the genotoxic potential of this exposure, a large biomonitoring study has been carried out in women working in the shoe industry in Bulgaria. For this aim, the COMET, micronuclei (MN) and sister-chromatid exchanges (SCE) tests were used in peripheral blood lymphocytes. The levels of toluene, gasoline, acetone, ethylacetate and diisocianate were measured at the workplace. Individual analyses of the concentration of haemoglobin (HG), hipuric acid (HA) in urine were conducted, and the possible influence of genetic polymorphism in the glutation-S-transferase enzymes (M1 and T1), related to the metabolism of different carcinogens was investigated.
The biomarkers studied have demonstrated the existence of biologic effects associated to the occupational exposure to organic solvents: an increase in the HA concentrations and a decrease in the HG levels. Nevertheless, when using the COMET and SCE assays, there were no significant effects. On the other hand, there was a substantial increase in the frequency of MN in the women exposed to organic solvents. These results lead us to suggest that the genotoxic activity of these compounds would be due, mainly, to an aneugenic mechanism. In addition, no relationship was observed between the level of genetic damage (MN) and the GST genotype of the donors.

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CYTOGENETIC BIOMONITORING OF WORKERS FROM LABORATORIES OF CLINICAL ANALYSES OCCUPATIONALLY EXPOSED TO CHEMICALS

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A cytogenetic investigation was carried out on a group of workers enrolled from 30 laboratories of clinical analyses. Analyses of chromosomal aberrations and micronuclei were performed on peripheral blood samples of 50 workers and 53 subjects of a control group (from healthy blood donors) matched for gender and age. All the subjects (age 25-49 years) were strictly selected according to the personal healthy questionnaire (proposed by the International Commission Protection against Environmental Mutagens and Carcinogens) in order to exclude bias due to the lifestyle confounding factors (smoke and alcohol consumption, Rx and medical treatment, etc.) affecting the Dna damage level that could influence the outcome of the study. The workers were also selected using a questionnaire concerning the individual occupational exposure to chemical substances (about 300 different chemicals have been identified). A total of 10.300 metaphases (100/subjects) and 103.000 binucleated cells (1.000/subjects) were analysed.
The chromosome and chromatid aberrations frequencies are significantly higher ($p<0.002$, $p<0.001$ respectively) in workers (chromosome aberration 1.12%,chromatid aberration 1.52%) than in controls (chromosome aberration 0.43%, chromatid aberration 0.73%). Similarly, micronucleus frequencies are significantly higher ($p< 0.001$) in workers (8.06‰) than in controls (3.94‰).

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OCCUPATIONAL EXPOSURE TO ACRYLONITRILE - FISH ANALYSIS

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The impact of occupational exposure to acrylonitrile (ACN) on chromosomal damage was studied in the group of workers (N=23) exposed to ACN in a petrochemical industry, in matched controls (N=26) from the same region (CON 1), as well as in another controls from non-ACN-polluted region (Prague; CON 2). Cultures of human lymphocytes were incubated for 72 h. Probes for chromosomes 1 and 4 (Cambio, UK) were used. Translocations were determinated in 1000 metaphases/subject. The total genomic translocation frequency (F_G) was calculated from the frequency of translocations detected by FISH with a few whole chromosome probes (Fp) using equation $F_G=F_p/2.05[f_r(1-f_r)+f_g(1-f_g)-f_rf_g]$ (f_r and f_g are the fractions of the genome covered by the composite probes). The genomic frequency of translocations per 100 cells calculated as $F_G/100$ for all the groups was as follows (medians): 2.61, 2.06 and 1.12 for ACN-exposed, CON 1 and CON 2, respectively. A significant differences were found only between ACN-exposed and controls from non-ACN-polluted region (CON 2; p=0.022). No effect of smoking was observed in any of the groups analysed. The frequency of translocations by FISH were significantly affected by age (r=0.357; p=0.002). Our results indicate that the both groups, workers and controls from the same region (CON1), are probably exposed to the mixture of various chemicals from this petrochemical plant. Cytogenetic data by FISH imply a higher chromosomal damage than in general population (CON 2). Supported by the EC grant IC 15-CT97-0302.

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EXPRESSION OF P53 AND P21^{WAF1} PROTEINS IN BLOOD SERUM OF ACRYLONITRILE-EXPOSED WORKERS

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Acrylonitrile (ACN) is an important compound used in the sysnthesis of a number of organic products, such as rubber, fibers and plastics. It has been shown that ACN induces brain neoplasms in rats following chronic treatment. However, regarding the carcinogenecity of ACN in humans, data are not consistent, some studies found an increase of some types of cancers, but the others did not. In laboratory animals ACN was found to be toxic for the mother and the fetus/embryo, and to be teratogenic.
The impact of occupational exposure to ACN on the expression of p53 a p21^{WAF1} proteins in blood serum was studied in the group of workers (N=50) exposed to ACN in a petrochemical industry.
(ACN exposure levels were 0,1-0,5mg/m^3), in matched controls (N=26) from the same region (CON 1), as well as in another controls from non-ACN-polluted region (Prague, CON 2, N=33). The expression of both the proteins was determined by ELISA immunoassays. The results showed the significant difference in the expression of both the p53 and p21^{WAF1} proteins only between the group of ACN-exposed workers and the control group from non-ACN-polluted region (CON 2, p< 0,001). There was no difference in the expression of respective proteins betwee nthe groups of ACN-exposed workers and CON 1. No effect of smoking was observed in any of the groups analyzed.
The results suggest that the wxpression of the respective proteins is induced by the complex mixture of various chemicals, rather thatn by ACN itself. To confirm the results, the changes of the expression of both the proteins during the time course should be monitored.
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THE NOR ACTIVITY IN COKE OVEN WORKERS

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The NOR activity is a stable inherited characteristic. It indirectly influences the rate of protein synthesis in the cell. The study of the NOR in tumor cells and in the cells exposed to carcinogenic is of greater importance. Since coke oven workers are in a direct contact with polycyclic aromatic hydrocarbons (including B[a]P) they were chosen as an object of our study.
The NOR activity has been studied on interphase nuclei (Ag-positiv zones) and metaphase chromosomes of leucocytes (51 coke oven workers and 54 control subjects). According to the mean group values all the cytogenetic markers were no changes in the exposed groups. However it has been stated that the frequency of carriers of the great NOR for coke oven workers (12%) is larger that that for control donors (5%). The level chromosomal aberrations for the carriers with the great NOR is much lower than their mean level for coke oven workers. Thus may be assumed that the present of highly active NORs somehow associated with human stability to the exposure by PAHs.

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CYTOGENIC MONITORING OF COCE-CHEMICAL WORKERS

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The frequencies of chromosomal aberration and sister-chromatid exchanges (SCEs) in the peripheral blood lymphocytes of 141 workers of the Coce-Chemical plant in Kemerovo which is the centre of chemical industry in Western Siberia were studied. The control group was represented by 143 healthy intact donors. The monitoring was carried out for 4 years (1995-1998): chromosomal aberrations - annually, while SCEs - in 1998 only.
The average value of chromosomal aberrations was:
In 1995 (40 workers) - 4,97+-0,57%, (30 controls) - 3,64+-0,37%
In 1996 (55 workers) - 5,28+-0,34%, (37 controls) - 3,51+-0,37%
In 1997 (12 workers) - 5,54+-0,74%, (47 controls) - 3,53+-0,64%
In 1998 (34 workers) - 7,98+-0,63%, (29 controls) - 3,29+-0,48%

There were no significant differences in SCEs between workers (6,27+-0,34) and controls (7,79+-0,58).

It was stated that chemicals cause stable clastogenesis in workers. Genotoxical risk for this group partly results in higher sickness rate as compared with intact donors.

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CHROMOSOMAL ABERRATIONS IN RAILWAY WORKERS EXPOSED TO TRANSIT CHEMICALS

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Complex chemical mixtures containing aromatic and aliphatic hydrocarbons are transported from Russia to Finland either for shipment to central Europe or for use by Finnish industry. Railway workers handling the tank wagons can be exposed to these chemicals. As the exposure occurs intermittently to a wide variety of chemicals, both by skin contact and inhalation in the open air, exposure assessment by traditional means is very difficult. Since the transported chemicals include genotoxic components (especially benzene and polyaromatic hydrocarbons), we studied whether the exposure was high enough to result in an increase in chromosomal aberrations (CAs) in peripheral lymphocytes. An initial survey among 48 railway workers and 39 age-matched unexposed referents (all men) showed an elevated level of CAs particularly in nonsmoking railway workers who were in close contact with the tank wagons. A campaign was started to reduce exposures through training and improved protection. Tank wagon inspection and control at the border was made more effective. About 5 years after the first study, a group of 39 railway workers in close contact with chemical tank wagons and 30 age-matched referents (all nonsmoking men) were investigated for lymphocyte CAs. No increase in CAs was observed in either of the exposed groups. The finding probably reflects the fact that chemical exposure has decreased as a result of improved condition of the wagons, enhanced protection, and awareness of the potential hazard.

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EVALUATION OF DNA DAMAGE AND REPAIR BY COMET ASSAY IN THE WHITE BLOOD CELLS OF WORKERS EMPLOYED IN THE RUBBER INDUSTRY

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The study was aimed at the assessment of genotoxic effects in workers exposed to raws dust and fumes emitted to the occupational environment during rubber manufacture and rubber shoes production. The levels of DNA damage and repair in the white blood cells of 37 workers and of 16 control subjects have been assessed. The blood (about 100 ml) from each person was collected by finger puncture and the alkaline cell gel electrophoresis assay (comet assay) in individual cells was applied for detecting the presence of DNA single strand breaks (SSB). Fragmentation of DNA strands was estimated in the cells before as well as after incubation of cells in RPMI 1640 medium at 37° C and 5% CO2 for 3 hours to assess the repair of DNA SSB. The level of DNA strand breakages was evaluated as a percentage of cells with 2,3 and 4 comet types. The percentages of cells with damaged DNA in the rubber workers were similar to the control subjects and amounted respectively 6.8±3.8 and 6.3±3.0 before repair and 6.6±4.2 and 5.3±1.9 after repair. The fragmentation of DNA strands among smokers both in the exposed and control groups was not higher than in the respective nonsmokers. Lack of genotoxic effects in the rubber workers was probably arised from the low exposure to genotoxic substances in the occupational environment.

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GENOTOXIC EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE IN SUBJECTS SUFFERING LOW EXPOSURE TO URBAN AIR POLLUTION

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The levels of bulky DNA adducts were measured by ^{32}P-postlabelling in lymphocytes of 194 students living in the city of Athens, Greece and the less polluted region of Halkida. All subjects, who were declared non-smokers, exhibited a similar pattern of DNA adducts which was characterised by a small number of discreet spots (usually 2), with no evidence of a diagonal radioactive zone. Adduct levels were low (below 1 adduct per 10^8 nucleotides for all individual adducts). Highest adduct levels were observed in a sub-group of subjects living in or near the Halkida institute campus residence, which was located in rural surroundings with minimal burden of urban air pollution, while Athens subjects showed the lowest adduct levels. This trend was observed over 2 winter and 2 summer monitoring seasons and consistently paralleled the variation of 3 markers of exposure to environmental tobacco smoke (ETS), namely a) declared times of exposure to ETS during the 24 hours prior to blood donation, b) plasma cotinine levels and c) the chrysene/benzo[g,h,i]perylene ratios in the profile of personal PAH exposure. Furthermore, positive correlations were observed among the Halkida campus area subjects (but not the remaining subjects) between DNA adducts and a) time of declared ETS exposure and b) the chrysene/benzo[g,h,i]perylene. These correlations suggested that, for a group suffering minimal exposure to urban air pollution, exposure to ETS was a major determinant of the observed DNA damage.

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A MULTI-BIOMARKER APPROACH TO STUDY SMOKING-INDUCED OXIDATIVE STRESS

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We studied smoking-induced oxidative stress in healthy smokers (n=20) (smoking status: 17 ± 8 cig/day) and non-smokers (n=24) using a multi-biomarker approach. Oxidative DNA damage, 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-OH-dG) level in lymphocytes quantified by high performance liquid chromatography with electrochemical detection (HPLC-ECD) in smokers was significantly lower than that in non-smokers (38.6 ± 5.2 vs 50.9 ± 4.6 / 10^6 dG, P=0.05). Also, the level of the oxidized pyrimidine bases in lymphocytic DNA quantified by endonuclease III comet assay was non-significantly lower in smokers as compared to non-smokers (tail length: 69 ± 13 vs 96 ± 10; % DNA in tail: 13 ± 3 vs 14 ± 2 and tail moment: 6416 ± 1220 vs 7545 ± 1234). Urinary excretion of the 8-OH-dG repair product measured by enzyme-linked immunosorbent assay (ELISA) did not differ between smokers and non-smokers (197 ± 31 vs 240 ± 33 ng/ body mass index, P=0.3). Overall DNA repair activity in lymphocytes measured by unscheduled DNA synthesis (UDS) assay was not different between smokers and non-smokers (2.9 ± 0.3 vs 3.3 ± 0.3, P=0.4). Further, plasma antioxidative capacity measured by Trolox equivalent antioxidant capacity (TEAC) assay was higher in smokers as compared to non-smokers (440 ± 16 vs 400 ± 15 µM Trolox, P=0.09). Genotyping of human 8-OH-dG glycosylase/apurinic lyase (hOGG1) and glutathione-S-transferase (GSTM1) by polymerase chain reaction (PCR) technique showed that polymorphism in either/both of the two genes does not affect any of the quantified biomarkers. We conclude that oxidative stress imposed by cigarette smoking has low potential to impact upon the biomarkers of exposure and effects.

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DIESEL PARTICULATE EXHAUST - DETERMINATION OF PAH CONCENTRATIONS AND ASSESSMENT OF EXPOSURE IN BUS GARAGE AND WASTE COLLECTION WORK

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Diesel-powered engines are used both in light- and heavy-duty vehicles. Particulate exhaust emissions containing carcinogenic polycyclic aromatic hydrocarbons (PAHs) are genotoxic and cause chronic effects in humans. Recently, interest has been focused on small particles and their effects, when inhaled through upper respiratory defensive mechanism. This is a part of the ongoing biomonitoring study on diesel exposure in the work environment. In Finland, about 150.000 workers are exposed to diesel exhaust emissions, however, no data of the exposure levels at different work places is available. In the present study we assess personal exposure to diesel particulate PAHs (15 EPA PAHs) by using short- and long-term biomarkers and personal air monitoring in bus garage and waste collection work. Air, blood and urine samples from 43 nonsmoking exposed and 48 control persons were collected in winter and summer. Diesel particles were collected on PTFE filters and volatile PAHs were adsorbed to XAD-2 material. These samples were analyzed by HPLC-FLD. Total particulate PAH analyzed from air samples of exposed workers and control persons varied between 1,0-117,4 ng/m^3 and 0-14,6 ng/m^3, respectively. Correspondingly, volatile PAH concentrations of exposed and control samples ranged between 93-5465 and 188-279 ng/m^3, respectively. Lymphocyte DNA adducts were analyzed by the ^{32}P-postlabeling method. The mean total DNA adduct levels for exposed workers were 0.7±0.46 (± SD) and for the control persons 0.4±0.33 (±SD).
Finnish Work Environment Fund is thanked for financial support.

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IN VITRO AND HUMAN EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) DERIVED FROM DIESEL PARTICULATE EMISSION

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Diesel exhaust contains several carcinogenic compounds in gas and vapor phase, including particles, having been suggested to increase lung cancer risk in humans. Here we summarize mutagenicity and DNA adduct studies analyzed from exposures of diesel particulate extracts to in vitro CT DNA and mammary carcinoma cell line (MCF-7). Furthermore, results of occupatio-nal diesel exposure measurements carried out at workplaces are discussed. Metabolic activat-ion of PAH and nitro-PAH to DNA-reactive metabolites was studied by treating CT DNA (+S9 and xanthine oxidase) and MCF-7 cells with soluble organic fraction (SOF) of diesel particulate. Ames test was used to detect the mutagenicity of diesel particulate. 15 PAH compounds from personal air samples of bus garage and waste collection workers and DNA adducts from lypho-cytes by the ^{32}P-postlabeling method were analyzed. Direct and metabolically activated PAHs and nitro-PAHs from the in vitro experiments showed higher nitro-PAHs than PAHs in the diesel extracts. Results from TA98 and YG1021 strains of Salmonella typhimurium confirmed also these findings. A clear difference in metabolic activation between two single PAHs (B[a]P and 5-MeCHR) and three diesel extracts in MCF-7 cells was detected. PAH-DNA adducts analyzed from exposed workers and control persons varied from 0.1 to 1.7 and from 0.1 to 0.9 adducts per 10^8 nucleotides, respectively. Mean total DNA adducts for exposed workers were 0.7±0.46 (± SD) and for the control persons 0.4±0.33 (±SD). Particulate and volatile PAH contents in per-sonal air samples of exposed workers ranged between 1.0-117 and 93-5465 ng/m^3, respectively. In control samples the range was between 0-279 ng/m^3. Biomarkers measuring external and internal exposure showed low levels of PAH exposure derived from diesel engine exhausts.
Supported by grants from Finnish Work Environment Fund, Neste Oy Foundation and TEKES.

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BIOMONITORING IN TRAFFIC POLICEMEN OF ROME CITY: PRELIMINARY RESULTS FROM CYTOGENETIC ANALYSES

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Air pollutants in urban area include many chemicals known to have high potential genotoxic effects. Relevant efforts are nedeed in order to establish any causative relationship between air pollutants exposure and human cancer. In this framework a biomonitoring study is ongoing in the city of Rome using traffic police workers as the referent population exposed to urban air. The sample includes 192 subjects, comprehending two different groups of exposed individuals working in the urban traffic and one group of control policemen employed in the offices. Individuals of the three groups were matched by age, gender and smoking habits. Such a study includes the analysis of markers of exposure (both external and internal), of genotoxic effects and of susceptibility. A preliminary assessment of baseline chromosome damage using classical cytogenetic end-point considers Proliferation index (PRI) (N=139), Sister chromatid exchange (SCE) (N=69) and Micronucleus (MN) (N=82) frequencies on a sample of the total population. The results so far obtained do not highlight significant differences among the three study groups for any of the end-point considered. Smoking habits was shown to significantly increase SCE rates ($p<0.05$) and, to a more extent, the High Frequency Cell (HFC) levels ($p<0.001$). Female subjects display a low but not significant increase in HFC, compared to age-matched males. No association between age, gender, smoking habits and micronucleus rates have been detected. These results suggest that conventional cytogenetic approach may lack the sensitivity required to disclose weak effects resulting from exposure to low levels of air pollutants. Further studies are in progress using FISH based methods.
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AIR POLLUTION AND OXIDATIVE DNA DAMAGE

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We are studying air pollution-induced oxidative DNA damage in populations and animal experiments with biomarkers, including urinary excretion of 8-oxodG, the level of 8-oxodG in DNA by HPLC-EC and the comet assay. We have shown increased excretion of 8-oxodG among Copenhagen bus drivers from the City centre compared with drivers from suburban/rural areas.
As a model for the effects of air pollution we have studied 3 groups of 60 heavy smokers before, 3 and 7 days after quitting without nicotine substitution or with dermally applied nicotine or continued smoking. There was no effect of smoking cessation on the lymphocyte level of 8-oxodG or the comet assay. The urinary 8-oxodG excretion decreased significantly after smoking cessation with dermal nicotine but not in the other two groups. There were no correlations between the three biomarkers.
In ongoing population studies individual personal, indoor and outdoor exposure to aromatic hydrocarbons, NOx and particulate matter ($PM_{2.5}$) are monitored and correlated with biomarkers. So far, a significant correlation between exposure to toluene and FPG sensitive sites in the comet assay has been shown. The excretion of the benzene metabolite from a toxic pathway trans,trans-muconic acid was correlated with the personal exposure to benzene and was dependent on GST genotypes. The 8-oxodG level in DNA measured by sodium iodine based extraction correlated with single strand breaks ($r=0.65$) and FPG sites ($r=0.43$) in the comet assay, whereas there was no correlation with ENDOIII sites or urinary excretion of 8-oxodG.
In Guinea pigs intratracheal administration of diesel particles induced DNA damage assessed by 8-oxodG levels and strand breaks by the comet assay in the lungs. Benzene interacted with inflammation in generation of 8-oxodG and strand breaks in the bone marrow of mice.
Biomarkers of oxidative DNA damage are valuable tools in the study of air pollution effects.

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EVALUATION OF DNA DAMAGE IN PERIPHERAL LYMPHOCYTES OF TRAFFIC WARDENS IN RELATION TO AIR POLLUTANTS EXPOSURE AND METABOLIC GENOTYPE

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In an on-going biomonitoring study on traffic wardens of a high traffic Italian city, a few biomarkers of DNA damage are being evaluated to investigate the biological effects of exposure to airborne pollutants. Two hundred subjects have been enrolled in the study and categorized in three groups of exposure: A and B, with occupational exposure to airborne pollutants, and C as control group. DNA damage in unstimulated lymphocytes have been detected by alkaline single cell gel electrophoresis (SCGE) and parametrized by tail moment values. In order to investigate the influence of metabolic genotype on individual levels of DNA damage, subjects have been characterized for a few polymorphic loci involved in xenobiotic metabolism (CYP1A1, CYP2E1, DT-diaforase and GSTM1) using a PCR-RFLP method.
Preliminary results on 109 subjects do not highlight significant differences in individual average tail moment values in exposed and control subjects. However, a significant prevalence of heavily damaged cells, with tail moment value greater than the 90° (A vs C, $p<0.001$; c^2 test) and 95° percentile (A vs C $p<0.01$, B vs C $p<0.05$; c^2 test) of the overall distribution, was observed.
Preliminary results on 99 subjects show a significant prevalence of higher tail moment values in subjects with GSTM1 null genotype compared to wild-types ($p=0.03$, Mann-Whitney U test). A similar trend, close to statistical significance, was observed in the subset of exposed subjects ($p=0.07$, Mann-Whitney U test), whereas no difference was observed between GSTM1 null and wild type controls. These preliminary results suggest a role for GSTM1 in the modulation of DNA damage induced by atmospheric pollutants.
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THE EFFECTS OF AIR POLLUTION ON DNA DAMAGE OF HUMAN LYMPHOCYTES OF EXPOSED POPULATIONS

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Athens, like other European places, appear to have over-increased levels of air-pollutants (sulphur dioxide; particulate matter; nitrogen dioxide; ozon etc.) in the atmosphere (Touloumi et al 1994). Several of these compounds are carcinogenic in animals and are suspected to be carcinogenic to people.
In this study, using the comet assay technique (Piperakis et al 1998, 1999), we investigated the basal DNA damage and repair of peripheral blood lymphocytes and the different degree of response to hydrogen peroxide and UV irradiation between urban (Athens) and rural (outside Athens) healthy individuals. Endonuclease III treatment was also used to reveal the level of oxydised pyrimidine formation between these groups. The results have shown that there is a relationship between leaving area and DNA damage of the human lymphocytes.

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SEASONAL VARIATION OF DNA ADDUCT PATTERNS IN FARMERS AND NON-EXPOSED POPULATION

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DNA-adducts are one of the biomarkers selected in a molecular epidemiology study aiming to estimate the genotoxicity associated with agricultural activities, especially pesticide exposure. We are currently performing a DNA-adduct pattern analysis of farmers in the county of Calvados (France) by the ^{32}P-postlabeling method using NP1 enrichment. The aims of this study were firstly to evaluate effects of a one-day pesticide exposure period and then attempt to draw-up a seasonal pattern for the quantitative and qualitative fullness of adducts.
In the first step, blood samples were collected on two consecutive mornings between which there was pesticide exposure. Two sub-groups (n=14 and 8, respectively) were constituted at the full spraying season (G2) and a few weeks later, when spraying operations were less intensive (G3). No intra-individual difference was found in adduct level but DNA adduct levels were higher in G2 compared to G3.
In the second step, seasonal effects were investigated more accurately. Unique samples were collected for farmers (n=31) and for non-exposed population (n=35) in a period free of exposure for several weeks (P0) and in intensive exposure season (P4). For farmers, mean level of adduct was higher for P4 compared to P0, whereas no difference was observed between P0 et P4 for non-exposed subjects. This increase seems to be due to an intensification of pre-existent adducts rather than appearance of new ones. For both populations, adducts patterns will be completed by butanol-enrichment procedure.

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MOLECULAR EPIDEMIOLOGY STUDY ON GENOTOXIC EFFECTS IN A POPULATION OF FARMERS

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In order to estimate the genotoxicity associated with agricultural activities (especially pesticide exposure), blood and urine samples were collected from farmers and various bio-markers of genotoxicity were applied : urine mutagenicity, DNA adduct level, DNA damage on lymphocytes and cytogenetic alterations. We have investigated first of all the genotoxic effects of a one-day exposure to selected pesticides by collecting 2 blood samples at a 24h interval (S1 and S3) and 3 urine samples (S1, S2 and S3). Four groups of farmers (11 to 16 farmers/group) were constituted along the spraying season: G1 at the beginning, G2 during the full spraying season, G3 and G4 at the end. The second step consisted in studying the seasonal bio-marker variations by collecting samples for farmers (n=31) and for non-exposed population (n=35) outside the spraying season (samples S0), and in full spraying season (S4). We have considered that samples S0 allow the evaluation of the basal level for the different bio-markers.
As opposed to other groups, one-day exposure period to chlorothalonil+insecticides (G3) induced a significant increase of DNA damage and a weak (but non-significant) increase of DNA adducts and of urine mutagenicity. For seasonal variation, significant increase of the level of DNA adducts and DNA damage were observed at full spraying season compared to their level outside the season. No significant variation of CA was observed, but surprisingly, SCE appeared lower in S4 compared to S0. Together, these results tend to show that agricultural activities could be associated with genotoxic effects, with an increase of positive responses together with progression in the spraying season, which was not observed on non-exposed population.

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CHROMOSOMAL ABERRATIONS AND SISTER CHROMATID EXCHANGES IN LYMPHOCYTES OF PESTICIDE USERS IN FOUR EUROPEAN COUNTRIES

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Pesticides are widely used in agriculture, however, the impact of pesticides on health of agricultural workers is still largely unknown. Previous studies have demonstrated that the potential genotoxic effects of pesticides on humans cannot be ignored.
One of the main goals of a large European project was to study in vivo the genotoxic effects of pesticides in human cells. In Greece, Hungary, Poland and Spain an epidemiological survey was done before taking blood from agricultural workers exposed to pesticides and reference groups. Cytogenetic analysis of human blood lymphocytes was performed by culturing and screening the lymphocytes from the two groups (455 samples) of the four European countries. The rate of chromatid and chromosome type of chromosome aberrations (CA), the rate of aneuploid cells was determined as well as the rate of sister chromatid exchanges (SCE).
Our preliminary result shows that there is no significant difference between the exposed and the reference groups in the rate of SCEs. The total rate of CAs was significantly higher ($p<0.01$) in the Hungarian exposed group (2.94%) compared to the control group (1.49%) while in samples from Greece, Poland and Spain the rates of CAs showed slight differences. The total rate of CAs was the highest in both Polish groups compared to samples from other countries. The rate of aneuploid cells did not differ in the exposed and control groups.
Based on the results so far a slight defective effect of pesticides on chromosomes cannot be excluded. Further analysis of our data is needed to reach a final conclusion.

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EVALUATION OF DNA DAMAGE IN SUBJECTS OCCUPATIONALLY EXPOSED TO PESTICIDES USING SINGLE-CELL GEL ELECTROPHORESIS (SCGE) ASSAY

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The comet assay, also called the single-cell gel electrophoresis (SCGE) is a rapid and sensitive method for the detection of DNA damage (strand breaks and alkali-labile sites) in individual cells. The assay is based on the embedding of cells in agarose, their lysis in alkaline buffer and finally subjection to an electric current. In the present study, alkaline SCGE was used to evaluate the extent of primary DNA damage and DNA repair in the peripheral blood lymphocytes of 10 subjects employed in pesticide production in two different periods of exposure: after 6-months spent in the production and after 6 months long period of their absence from the pesticide exposure zone. The subjects are working in three different units of pesticide production (the unit of pesticide synthesis, the unit of emulsion concentrated production, and the unit of powder and liquid pesticide production) and they are simultaneously exposed to atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion. The control group was composed of 10 persons chosen from general population with no history of occupational exposure to neither chemical nor physical agents. After the period of high pesticide exposure lymphocytes of the occupationally exposed subjects manifested increased tail length ($x = 50.13 \pm 9.44$ mm) and tail moment ($x = 60.85 \pm 18.17$) compared with the control group (tail length $x = 13.26 \pm 1.47$ mm; tail moment $x = 10.50 \pm 1.13$). After the workers spent next 6 months out of the pesticide exposure zone both endpoints were still increased (tail length $x = 17.19 \pm 0.44$ mm; tail moment $x = 13.79 \pm 0.39$) in comparison to the control but significantly decreased in comparison to results of the first sample analysis.

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UDS AND PROTEIN SYNTHESIS IN PERIPHERAL BLOOD OF NPP WORKERS

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This investigation is an attempt to assess the genotoxic effect of chronic, fractionated exposure to low-dose radiation by examining unscheduled DNA syntesis (UDS) and protein synthesis (PS) in peripheral blood leukocytes from persons belonging to the operative/repair staff of NPP "Kozloduy".
The workers had an occupational experience of 2 to 20 years and a cumulative dose burden up to 470 mSv. UDS was found to increase progressively with age, cumulative dose and work duration. PS changes were less pronounced and depended only on age. An inverse correlation was observed between UDS and PS when occupational experience exceeded 10 years. For the persons who started working at NPP at a more advanced age, the impact of chronic radiation exposure was more significant.

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THE EFFECT OF IONIZING RADIATION ON THE ANTIOXIDANT CAPACITY OF THE HUMAN BLOOD

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The knowledge on the quantities and effects of the antioxidants in the blood is important both for experimental pharmacology and clinics.
In the present work we have investigated the effect of different doses of ionizing radiation on the antioxidant capacity of the human blood. The results show that the TAS (total antioxidant status) value linearly decreases upto 1 Gy dose. At higher dose (2 Gy) no additional decrease of the TAS value could be detected, where the human blood retains 60-70 per cent of its total antioxidant capacity.
The effect of ionizing radiation on the antioxidant enzymes glutathione peroxidase (GPX) and superoxide dismutase (SOD) have been also investigated. The quantities of GPX and SOD linearly decreases upto 1 Gy dose. Between 1 and 2 Gy doses further mild decrease could be detected.
The data suggest the presence of antioxidant protection barriers in sera with various radiosensitivities.

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CYTOGENETIC LONG TERM FOLLOW UP STUDIES OF BULGARIAN NUCLEAR POWER PLANT WORKERS

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The data on periodical cytogenetic monitoring in the course of 5 years is presented. Chromosome aberrations were studied in peripheral blood lymphocytes of about 400 samplings of 193 workers in the Bulgarian NPP “Kozlodui”. A regular dosimetric control was performed for all the workers. There is no evidence that the annual doses exceed the limit of 50 mSv for any of them. The exposed study group was divided into the following subgroups according to: cumulative dose, work duration, age, smoking, etc. According to the cumulative dose, the following exposed subgroups were evaluated: up to 20 mSv; 21 - 100 mSv; 101 - 200 mSv; 201 - 300 mSv; 301 - 400 mSv; 401 - 500 mSv and > 500 mSv. There was no difference with the control group for accumulated doses up to 20 mSv. The total number of the CA and the dicentrics increased at doses from 20 to 100 mSv, however, insignificantly. A significant increase in the frequency of dicentrics was observed for the subgroups with accumulated doses about > 200 mSv. The highest frequency of dicentrics was found for the exposed subgroup with cumulative dose > 500 mSv. Additional noxae do not seem to exert a significant impact on the frequency of chromosome anomalies.

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TWO HUMAN SUBPOPULATIONS WITH DIFFERENT OXIDANT PHENOTYPES

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Distribution curves for next free radical parameters - sensitivity of erythrocytes to autooxidation, luminol-enhanced chemiluminescence [CL] of blood plasma and salivary fluid - show that healthy human population contains two obvious subpopulations of individuals with partially overlapped diapasons of the parameters. The overlapped maxima might be segregated on X-Y plots of their dependence from some appropriate factors, such as: 1) smoking load (two identical curves for peroxidative hemolysis value, zero point - non-smokers - has appeared to be splitted also); 2) individual psychoemotional load in Levee questionnaire balls; 3) dioxins contents in blood samples of exposed donors - two identical underlying curves in each of the scatterplots for three above listed indices. The revealed bimodality in oxidative phenotype is not coincide with well known human bimodality in chromosomal aberrations (ChAb) level, giving four spots on corresponding scatterplots with relative frequencies resembling ones for ABO blood groups. Nevertheless, direct determination of ABO groups in frozen serum samples have not confirmed this effect. Both “oxidative defenders” and “oxidative forvards” have zones of mutagenic risk on the flanks, however with opposite responses in the levels of blood lymphocytes ChAb in answer to antioxidant vitaminization. The “defenders” seems to have more weak UDS in blood lymphocytes 2 hrs after UV irradiation and increased tendency to pathological serenity. The distance between the subpopulations is possibly a function of earth magnetic field. Irrespective of real mechanisms involved in both discussed phenotypic bimodalities, this knowledge facilitates the analysis of biomonitoring data, especially in small cohorts, and can, in principle, have clinical applications. Hybrid mice F1 CBAxC57Bl were shown previously to express 1:1 opposite parental oxidative phenotypes and so might represent suitable animal model of the new revealed human polymorphism. Author thanks genetists from two Moscow labs for providing of their raw data obtained in shared studies; psychological testing was carried out by F. Ingel and T.Tsutsman.

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SEASONAL DIETARY ANTIOXIDANT LEVELS AND OXIDATIVE DNA DAMAGE IN MIDDLE AGED MEN: A MOLECULAR EPIDEMIOLOGICAL STUDY

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We have used a small-scale epidemiological approach, with about 150 volunteers (50 survivors of myocardial infarction, 50 controls with normal level of lipids, 50 rural controls) taking part in highly focused investigations of their pattern of nutrition in winter and summer. The novel aspect is that we have complemented this nutritional information with measurements of a variety of biomarkers that are believed to represent risk factors for heart disease and cancer. Furthermore, we have carried out an antioxidant supplementation trial, in winter/spring, to boost antioxidant levels to those obtainable in summer. We found that antioxidant intakes and blood concentrations are higher in summer than winter. DNA damage in lymphocytes was measured using the comet assay, in combination with endonucleases specific for oxidised pyrimidines (endonuclease III) or altered purines (formamidopyrimidine glycosylase, FPG). Smokers have substantially higher levels of DNA damage compared with non-smokers. Individual alcohol consumption showed a significant correlation with DNA strand breakage, oxidised bases, and sensitivity to H_2O_2-induced damage in vitro. Plasma levels of a-tocopherol, ß-carotene, vitamin C and total antioxidant capacity (FRAP) increased after supplementation, and there was a decrease in the level of MDA (a marker of lipid oxidation) in plasma. Cytogenetic endpoints were studied in myocardial infarction and rural groups. Antioxidant supplements, as well as increasing corresponding plasma concentrations, led to significant decreases in levels of chromosome aberrations and micronuclei. This is a new interesting finding, as chromosome aberrations are probably the only biomarker to be validated in prospective studies as an index of cancer risk.

Poster session 2

Major topics:

Molecular and cellular mechanisms in mutagenesis and carcinogenesis, Genetic susceptibility, Germ cell mutagenesis, Cytogenetics

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VISUALISATION OF INDUCTION OF THE SOS SYSTEM IN ESCHERICHIA COLI

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The SOS system in E. coli involves more than 30 genes whose expression is induced in response to DNA damage and arrest of DNA synthesis. One of the latest induced genes is sfiA (sulA) encoding a protein that inhibits cell division and causes filamentous growth of the cells. This can be easily observed by fluorescence microscopy. The SOS system is induced in a suspension of E. coli bacteria irradiated with halogen light in an uncovered petri dish. Halogen light emits a wide UV spectrum including UVA, UVB and UVC; a cover glass cuts off the wavelengths below 320 nm. We have examined SOS induction by halogen light irradiation when a suspension of bacteria was uncovered (UVC irradiation) or covered (visible light irradiation) with a glass lid and the effects of hemH1 mutation on SOS induction were investigated. Covering hem^+ bacteria with a petri glass protects E. coli cells from SOS induction, however, it does not fully protect bacteria that bear a hemH1 mutation and slight filamentation is observed. HemH1 mutants are defective in ferrochelatase, an enzyme that by insertion of Fe^{2+} converts protoporphyrin IX to heme. Hem1-defective bacteria accumulate protoporhyrin that produces active oxygen species and are susceptible to and mutated by visible light irradiation. The frequency of mutation is strongly enhanced by transfection of hemH1 bacteria with plasmid pGW2123 bearing umuD'C. We have also tested whether the SOS system is induced after starvation of bacteria. We have found that SOS induction occurs only when bacteria, after starving on plates, are grown in liquid medium containing all of the requirements and glycerol instead of glucose.

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1,N^6-ETHENOADENINE, UNSTABLE DNA LESION, WHICH CONVERTS INTO SECONDARY DERIVATIVES REPAIRED BY E.COLI OXIDATIVE DNA- GLYCOSYLASES

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Several modified DNA bases are chemically unstable and transform into secondary lesions of different structure and coding properties. 1,N^6-ethenoadenine (εA), a highly mutagenic lesion for eucaryotic cells, slowly converts in alkali, but also under physiological conditions into a pyrimidine ring-opened derivative of about 20-fold higher mutagenic potency in E.coli than parental εA. We established the kinetics of εA degradation and identified products using fluorescence spectroscopy, HPLC, TLC, MS and NMR techniques. The glycosidic bond of εdA is about 20-fold less stable than that of dA and in neutral pH, εA either depurinates or converts into 3 products: εdA ->B ->C ->D. Compound B is a mixture of two isomers: pyrimidine ring-opened and closed product of water molecule addition to N(2)-C(3) in εdA. Compound C appears to be a deformylated derivative of ring-opened B compound - 4-amino-5-(imidazol-2-yl)imidazole and compund D depurinated derivative of C. Ethenoadenine is repaired by N-methylpurine-DNA glycosylases. We report here that εA degradation product is not excised from oligodeoxynucleotide duplexes by glycosylases excising εA, but by two E. coli DNA-glycosylases involved in repair of oxidative damage: the Fpg and the Nth proteins. HPLC analysis suggests that both enzymes repair compound B and the Fpg protein another, yet unidentified product. For the Fpg protein, repair is most efficient when excised base is paired with dC (K_m=6.4 nM, k_{cat}=0.42) and dT (K_m=6.94 nM, k_{cat}=0.464), but less favourable when paired with dG (K_m=109 nM, k_{cat}=4.68) and dA (K_m 60 nM, k_{cat}=0.487). The rate of εA degradation in neutral pH, when present in oligodeoxynucleotide is about 2% per week. Taking into account high persistence of εA in DNA and poor excision of its derivatives when paired with A or G, the contribution of εA derivatives to mutagenesis of this lesion should not be neglected.

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THE COMET ASSAY, REPAIR MECHANISMS AND DROSOPHILA MELANOGASTER

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The single cell gel electrophoresis (SGCE) or comet assay was used in D. melanogaster to study in vivo the relationships between two repair mechanisms, the nucleotide excision repair (NER) and a post-replication repair (PRR) mechanism, using respectively mus201 and mus308 mutants, and the induction of DNA strand breaks by three model monofunctional alkylating agents, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS) and N-ethyl-N-nitrosourea (ENU), with different alkylation properties.
Third instar larvae were treated for 12 hours, in chronic treatments, and neuroblast cells, mechanically individualized, were selected to carry out the assay. Whereas two different denaturising conditions, pH 10 and pH 12.6, were used with proficient repair cells, to check for alkali-labile abasic sites (AP), only one of them, pH 12.6, was utilized with repair mutant cells.
Results show: (i) an effect of the pH for MMS, which is evidence of AP site formation; (ii) a clear effect of the NER mechanism in the cases of MMS and EMS, probably due to the lack of repair of N-alkylation damage which could be increasing AP site formation; and (iii) a strong effect of the PRR mechanism for EMS and ENU, indicating that a functional MUS308 protein is required for the repair or process of some of the EMS- and ENU-induced damages, and that these damages, if not repaired, lead to DNA strand breaks, in this assay; furthermore, these results agree with the recent finding of mus308 playing a role in a post-replication bypass of ENU-induced damage, and support the possibility of a recombination-mediated bypass.
In addition to this, this work is a good evidence of the usefulness of the comet assay in Drosophila to carry out mechanistic repair-related studies.

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ROLE OF NUCLEOTIDE EXCISION REPAIR MECHANISM (NER) ON THE REPAIR OF N-ETHYL-N-NITROSOUREA (ENU) INDUCED DNA DAMAGE ON FEMALE GERM CELLS OF DROSOPHILA MELANOGASTER

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Until very recently, the NER mechanism was considered inefficient in the repair of ENU-induced damage, based on experiments of maternal repair analysis, in which postmeiotic male germ cells were treated. These cells have lost their repair capacity, and damages can only be removed, in a short time, by the female repair machinery. We have used as new approach to do this kind of study, the determination of the molecular mutation spectra induced by ENU, in the vermilion system, in female repair active germ cells of D. melanogaster, efficient and defficient (mus201 mutants) in the NER mechanism.
24 hour old NER^+ and NER^- females, treated for 24 hours with 1 mM ENU, were used to carry out at the same time a modified sex linked recessive lethal (SLRL) test. Results show that, in both repair conditions, more than 90 % of mutations occur at AT sites, strongly supporting the influence of the cell stage considered, already found with spermatogonia of Drosophila and mouse.
In addition, our results indicate the influence of NER on the repair of some of the damages induced by ENU. According to SLRL test data, mus201 oocytes present hypomutability with respect to the repair efficient ones, although this hypomutability changes to hypermutability when vermilion mutation frequencies are considered. With respect to the types of recovered mutations and their frequencies, although the same mutations are found, until this moment, in both repair conditions, in NER^- conditions there is an increase in the relative frequency of AT-TA and AT-CG transversions, probably consequence of N-ethylation damage, which is efficiently repaired by NER.

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SEXUAL DIMORPHISM OF ^{3}H-THYMIDINE INCORPORATION IN BASOLATERAL AMYGDALA IN ESTROGEN-TREATED RATS

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The amygdaloid complex is one of the brain regions which is under the strong influence of gonadal steroids, especially during neonatal development. The aim of this study was to confirm or exclude the presence of sex difference in ^{3}H-thymidine incorporation after the treatment with a single dose of 1 mg estradiol dipropionate (Galenika, Belgrade) on 3rd day of postnatal life. Simultaneously, treated and control rats were injected i.p. with 30 mCi of (methyl-^{3}H)-thymidine (Amersham, TRK 120. sp.act. 21 C/mmol). The animals were sacrificed by ether anesthesia on 10th day of postnatal life. Brains were isolated, fixed in Bouine solution and processed for standard autoradiography method using paraffin embedding. The analysis of the obtained results points to a higher incorporation of ^{3}H-thymidine in estrogen-treated male rats, compared to treated females. The relevance of increased ^{3}H-thymidine uptake is not clear for the time being. We assume that estrogen may have induced prolonged alteration in chromatin structure. Besides, reactive derivatives created in estrogen metabolism may have induced covalent DNA damage and subsequent activation of DNA repair mechanisms.

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ADAPTIVE RESPONSE AND DSB-REPAIR IN HUMAN LYMPHOCYTES

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An adaptive response induced in human lymphocytes can be revealed by the examination of chromosome damage, survival or mutation frequency. Lymphocytes exposed to very low doses of DNA damaging agents may become less sensitive to subsequent higher doses of the DNA damaging agent. It has been frequently suggested that the adaptive response to ionising radiation involve the induction of DNA repair mechanisms, especially those responsible for DNA strand break rejoining. This hypothesis still remains to be proven.
In our previous experiments we applied the comet assay at alkaline pH and the sandwich ELISA for single-stranded DNA to study the repair of strand breaks in DNA of adapted and non-adapted human lymphocytes. Although, the adaptive response was reflected in a lower micronuclei frequency, no change in the DNA repair rate was observed.
In this work we attempted to find a relation between the adaptation and the level of double-strand breaks (dsbs) in DNA and/or its repair. The initial DNA damage and repair rate was determined by the neutral version of the comet assay. We have assumed that the comet assay at neutral pH estimates mainly dsbs in DNA. To complement these results, we have also applied pulse-field gel electrophoresis (PFGE) - widely accepted as a specific and sensitive method for dsbs study. Lymphocytes were obtained from 5 healthy male donors (non-smokers, mean age 24.8 ± 3.5 years). The presence of adaptive response was confirmed by using the micronucleus test. The frequency of micronuclei in adapted lymphocytes was about 30% lower than that expected for an additive effect of both, adaptive and challenge doses, applied separately.
Lower damage revealed at the chromosomal level in the adapted lymphocytes was unrelated to initial level of dsbs in DNA. The differences between kinetics of DNA repair in the adapted and non-adapted lymphocytes were doubtful.

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COMPARISON OF THE DNA DAMAGES INDUCED IN LYMPHOCYTES BY UV OR X-RAYS AND REPAIR CAPACITIES OF HEALTHY DONORS AND SKIN CANCER PATIENTS

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The aim of this study was to compare variation in the individual susceptibility of various donors to the induction of the DNA damage by genotoxic agents and their cellular capabilities to repair induced damage. The DNA damages induced by UV or X-rays in lymphocytes of healthy donors and persons bearing various categories of skin cancer cells and cellular repair capability of the damages induced were investigated. The single cell gel electrophoresis assay (SCGE) was performed in defrozen lymphocytes from 35 individuals (including nine prior to skin cancer treatment) to evaluate individual DNA damage levels presented in lymphocytes at time of the sample's collection. To compare individual susceptibility to the induction of DNA damage by UV and ionizing radiation, lymphocytes were exposed to dose of 6 J/m^2 of UV or 2 Gy of X-rays and DNA damages were detected again. Additionally, to study variation in the individuals cellular capability to repair damages induced, exposed cells were incubated in presence or absence of phytohemagglutinin. Results showed in untreated lymphocytes of skin cancer patients significantly higher than in the reference group levels of the DNA damages. Significantly different responses to UV and significantly lower capabilities to repair UV induced damage in skin cancer patients were observed. On the average, no significant difference between both groups was observed in the X-rays induced DNA damages expressed by comet tail length and percent of the DNA in the comet tail, however comet tail moment was significantly lower in skin cancer patients. The capabilities to repair X-rays induced damages were lower in the skin cancer patients, both in presence or absence of the agent starting cellular divisions processes.
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INFLUENCE OF ENDOGENOUS NITRIC OXIDE (NO) ON INDUCTION AND REPAIR OF OXIDATIVE DNA DAMAGE

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Nitric oxide (NO) is an endogenous radical formed under physiological conditions. Its reaction with superoxide yields peroxynitrite which is known to be genotoxic. We have analysed the effects of an overproduction of NO in cultured fibroblasts on (i) the steady-state (background) levels of oxidative base modification (8-oxoG) and (ii) the susceptibility of the NO-overproducing cells to DNA damage by xenobiotic oxidants and (iii) the repair kinetics of oxidative DNA modifications.
Steady-state-levels of oxidative DNA damage, measured by means of an alkaline elution assay in combination with Fpg protein to quantify oxidative base lesions, were similar in NO-producing B6 mouse fibroblasts (stably transfected with an inducible NO-synthetase) and in control cells. Moreover, the amount of oxidative DNA damage induced by the photosensitizer Ro 19-8022 + light was similar in the NO-overproducing and control cells. By measuring residual DNA modifications after a repair time of 3 hours, the half-life period of the repair of Fpg-sensitive modifications in both cells lines was determined. No difference was observed between the two cell lines ($t_{1/2}$ 3 hours). Surprisingly, however the induction of DNA single-strand breaks by incubation with hydrogen peroxide was lower in the NO-producing cell line. In accordance with this observation the induction of micronuclei by hydrogen peroxide was significantly lower in the NO-producing cell-line.
These results indicate, that the NO concentrations which are produced by these cell line do not induce significant additional oxidative DNA damage and do not inhibit DNA repair. But endogenous NO prevents the induction of micronuclei by hydrogen peroxide, possibly by radical scavenging.

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GENE EXPRESSION IN NORMAL HUMAN FIBROBLASTS AFTER INDUCTION OF DIFFERENT TYPES OF DNA DAMAGE

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Cisplatin and UVC light give rise to different types of DNA damage. UV-induced DNA-lesions is known to be repaired by Nucleotide Excision Repair (NER). The repair of cisplatin induced DNA damage is not yet fully elucidated, but is thought to depend on NER and recombinational repair. We are currently investigating the transcriptional response in normal human GM38B fibroblasts after treatment with UV-light or cisplatin. By use of Atlas Arrays™, we will identify genes, which are specifically upregulated during cisplatin treatment compared to UV irradiation. We especially focus on genes involved in DNA-repair and cell cycle/apoptosis. The timeperiod between upregulation of p53 and elevation of the p53-inducible gene hdm2 is used to specify the stress response period of the cells and thereby the time window of transcriptional induction. The RNA-polymerase II inhibitor, a-amanitin, will be used to determine whether the actual cellular response is due to specific DNA damage or related to nonspecific blockage of the RNA-polymerase II. By use of the clonogenic survival assay, we have determined the toxic equivalent ID90-values of UVC-irradiation, cisplatin and a-amanitin treatment to 6,7 J/m^2, 1,8 mM and 0,525 mg/ml, respectively. Western blots show that after both UV-irradiation and cisplatin treatment at ID90 dosis, p53 levels peak after 4-6 hours of incubation, while hmd2 levels peak at 6 hours. Current experiments include mRNA extraction from GM38B after UV-irradiation or cisplatin treatment at the specified dose and time, to be used in Atlas Arrays. Ongoing experiments aim to elucidate specific gene inductions of either known or unknown genes, after cisplatin treatment. This provides basic information of both the repair of cisplatin induced DNA damage and of the cellular transcriptional response to cisplatin.

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DETECTION OF UV PHOTOPRODUCT REPAIR OF EUCHROMATIC AND HETEROCHROMATIC GENOME SEQUENCES IN HUMAN CULTURED CELL LINES

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For investigating the repair of transcriptionally inactive regions, we have developed a new quantitative PCR method, which is based on the application of a recently described human low copy number repetitive sequence as a target. The chAB4 repetitive sequence is about 150kb in length, represented with about 50 copies within the haploid human genome with low sequence divergence. A part of this heterochromatic sequence makes possible the examination of repair processes as it is extremely rich in adjacent timidines, the targets of UV irradiation. Since UV-adducts block thermostable polymerase mediated DNA synthesis, adduct dependent reduction in amplification product is observed. To quantitate the amplification we have used a short, 102 bp long internal control product, amplified from the same repeat. It does not contain sites for photoproduct formation. Using this method we studied the repair kinetics of three human melanoma cell lines and have found that two of them show fast repair, similar to control cell line (90% damage removed in 12 hrs) and one cell line shows decreased repair capacity (40% damage removed in 12 hrs). We have studied the transcription-coupled nucleotide excision repair capacity (TC-NER) of these cell lines on the example of the dihidrofolate reductase (DHFR) gene. A dot blot assay has been applied which is based on the 3'Þ 5' exonuclease activity of the T4 DNA polymerase. Since UV-damages block its exonuclease activity the ratio of remaining DNA fragments detected by DHFR gene specific hybridisation probe is proportional to the UV-lesions present in the investigated sequence. Irradiation with UV-C light and culture for different repair periods of the cells before lysis has enabled to study the repair capacity. The bulk repair is rapid and high in each cell line equally but the UV survival is not, Awhich our TC-NER data show good correlation with. The results suggest that melanomas are resistant against UV and several drugs because of their preserved high transcription-coupled repair capacity but the isolated sensitive subclone seems to have lost this ability.

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LOSS OF HETEROZYGOSITY IN THE LARYNX CARCINOMAS

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Head and neck squamous cells carcinoma (HNSCC) represents almost 5% of all malignancies diagnosed in Europe every year. The development of these tumours is highly related to the exposure to environmental mutagens, mainly cigarette smoking and alcohol consumption. However, only a minority of the exposed population develop HNSCC. As with most tumours, only scarce information exists about the molecular mechanisms involved in HNSCC tumorigenesis, despite the fact that both chromosome aberrations and microsatellite instability have been shown in HNSCC cells. Microsatellite instability has been shown to be an early event in HNSCC and probably plays an important role in tumour development, mainly in non-smokers.
Therefore, the aim of present study was to apply automated analysis for searching loss of heterozygosity (LOH) in squamous cells carcinoma of larynx. We chose 11 markers representing regions of potential interest in laryngeal cancer, linked to known oncogenes, tumour suppressor genes, mutator genes and genes involved in metastatic process. The localisation of these loci are as follows: 1q24, 2p16, 3p22, 5q21, 7q31, 8q22, 17q21, 17p13, 18q21. We evaluated specimens from 19 patients. DNA was extracted from both tumour cells and peripheral blood lymphocytes by using standard extraction techniques. The products of PCR were analysed by polyacrylamide gel electrophoresis in an automated DNA sequencer. In our study LOH was observed mainly in the locus D3S1611(3p22), which is linked to the MLH1.

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GENETIC POLYMORPHISMS OF THE P53 GENE AND LUNG CANCER

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The tumor suppressor gene p53 is a cell cycle control gene whose protein product TP53 mediates blockage of cell proliferation. Several germ line polymorphisms representing variable sites in the constitutional genomic DNA have been described for the sequence of the p53 gene. We examined two p53 germline restriction fragment length polymorphisms (RFLP), a BstUI RFLP in exon 4 and a MspI RFLP in intron 6 as potential molecular markers in patients with lung cancer. In our hospital based case-control study 109 lung cancer patients (84 males and 25 females) and 113 controls without malignant diseases have been recruited. The genotype characteristics were determined by PCR-based RFLP methods using DNA extracted from peripheral blood leukocytes. Increased frequencies of the BstUI A2 and MspI A1 alleles were found to be associated with statistically non-significant (P=0.234 and P=0.224) but increased odd ratios for lung cancer (OR 1.28, 95% CI 0.85-1.92; OR 1.38, 95% CI 0.82-2.32). However, there was a significantly higher proportion of MspI A1/A2 heterozygotes (P=0.048, OR 1.83, 95% CI 1.00-3.34) and significantly lower incidence of BstUI A1/A1 homozygotes (P=0.02, OR 0.31, 95% CI 0.11-0.90) in patients but not in controls. The analysis based on haplotype frequencies showed presence of the BstUI-MspI 2-1 haplotype in lung cancer patients (5.4%) in contrast to the absence of this haplotype in healthy controls. The results of this study suggest a certain role of the p53 BstUI and MspI polymorphisms as molecular markers for lung cancer risk.

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IMPAIRED DNA REPAIR CAPACITY IN LUNG CANCER PATIENTS

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The ability for DNA repair is an important host-factor which influences the individual susceptibility to genotoxic carcinogens. Several case-control studies have shown that DNA repair capacity (DRC) is reduced in patients with smoking related lung cancer using cytogenetic endpoints and laborintensive assays. We have standardized an alkaline comet assay to measure more rapidly the cellular DRC in peripheral blood lymphocytes of lung cancer patients and tumor-free control subjects. Additionally, for further characterisation and identification of DNA repair genes which might be responsible for the observed impaired repair, we developed a DNA-array for expression analysis of human DNA repair genes: PCR fragments from I.M.A.G.E. clones of more than 60 known genes, directly or indirectly involved in DNA repair, have been prepared and used for array preparation. Our reproducible and rapid Comet assay enabled us to identify individuals with reduced DRC. So far, in a total of 160 cases and 180 controls we found a significant association between reduced DRC in peripheral blood lymphocytes and elevated risk for non-small cell lung cancer (OR = 2.1, adjusted for smoking, age, gender and smoking status, p<0.0001). Furthermore, expression analyses were performed with mRNA isolated from individuals showing either high or low values of cellular DRC. The reported comet assay results will be presented together with the individual differences in expression of DNA repair genes.

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REPAIR OF DNA LESIONS INDUCED BY OXIDATIVE STRESS: ETHENOADENINE, ETHENOCYTOSINE AND 8-HYDROXYGUANINE IN LUNG CANCER PATIENTS

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Oxidised purines, pyrimidines and DNA ethenoadducts are generated in DNA upon oxidative stress, a process which accompanies certain types of cancers. The lesions induce DNA replication errors and mutations. We measured the activities of repair glycosylases excising eA, eC and 8OHG in tumour and normal tissue from 20 lung cancer patients. The data was analysed in relation to sex, smoking habit and type of lung cancer. High individual variations were observed for each repair activity; ratios between activities towards different lesions seemed to be an individual characteristic. On average, repair of all 3 damaged bases was more efficient in tumour than in normal lung, however, statistically significant differences were observed only for eA. In normal lung, the most efficient repair was that of eC. No statistically significant differences in repair of all 3 damaged bases were observed between sexes and types of cancer. Smoking did not affect repair of eA in normal and cancer lung tissue. In contrast, eC glycosylase activity was significantly higher in normal lung of smokers than in ex-smokers. In normal lung, the 8OHG repair activity was slightly higher in smokers than in ex-smokers (although statistically non-significant, the clear tendency was observed, P=0.069).The level of oxidised bases in DNA was measured by gas chromatography-mass spectrometry with selected ion monitoring - surprisingly, despite of increased 8OHG repair activity in tumour, the 8OHG level is higher in DNA isolated from tumour than from normal lung. This suggests that the extent of oxidative damage to DNA bases in tumour exceeds tissue repair capacity. Our data also suggests that a single lesion and its repair capacity cannot be used as a marker for oxidative damage to DNA and the risk from individual altered bases should be considered separately.

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MUTAGEN-SENSITIVITY OF LYMPHOBLASTOID CELLS WITH A BRCA1-MUTATION

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We have recently demonstrated that lymphocytes from breast cancer patients with a mutation in the breast cancer gene BRCA1 are sensitive to the chromosome-damaging effects of gamma-irradiation and hydrogen peroxide (Rothfuss et al., 2000). We now investigated whether lymphoblastoid cells with a defined heterozygous BRCA1-mutation show the same mutagen-sensitivity. We examined the genotoxic effects of gamma-irradiation and hydrogen peroxide using the micronucleus-test (MNT) and the comet assay. Treatment of a BRCA cell line with gamma-irradiation or hydrogen peroxide induced a clearly enhanced micronucleus (MN)-frequency compared to a normal control cell line. In contrast to the results in the MNT we did not find a significant difference between the two lymphoblastoid cell lines in the comet-assay with gamma-irradiation. Also the DNA-repair (removal of DNA lesions after gamma-irradiation) at different times after irradiation did not show any difference. These results demonstrate that the lymphoblastoid cell line with a BRCA1-mutation shows the same mutagen-sensitivity as lymphocytes from breast cancer patients with a BRCA1-mutation. Therefore, lymphoblastoid cells can be used as an in vitro model to characterize the mutagen-sensitivity of cells with a BRCA1-mutation and to elucidate the mechanism(s) underlying the mutagen-sensitivity.

Rothfuß,A et al., (2000):Cancer Res; 60, 390-394.

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MUTAGEN SENSITIVITY AS A BIOMARKER FOR BREAST CANCER SUSCEPTIBILITY GENES BRCA1 AND BRCA2

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It has been suggested that women with breast cancer are deficient in the repair of radiation-induced DNA damage and it has been shown that hypersensitivity to radiation-induced chromosome damage is a feature of many breast cancer patients. Mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 are responsible for the majority of hereditary breast cancer. BRCA1 seems to participate in the repair of DNA double-strand breaks (DSB) and/or oxidative DNA damage while BRCA2 has been shown to affect DSB-repair. We have investigated whether mutagen sensitivity is related to mutations in BRCA1 and BRCA2. Using the micronucleus test (MNT) and the comet assay, we studied the induction and repair of DNA damage in lymphocytes of 36 women from 18 families with familial breast cancer. Total sequencing of BRCA1 and BRCA2 revealed 12 different BRCA1 mutations and three different BRCA2 mutations in these families. Our results indicate that about 90% of women with a BRCA mutation showed elevated radiation sensitivity in the MNT compared to concurrent controls (i. e. healthy women without family history of breast cancer). Radiosensitivity was generally not observed in relatives from breast cancer families without the familial mutation. Hypersensitivity toward hydrogen peroxide was also observed for lymphocytes carrying a mutation in BRCA1 or BRCA2, indicating that the mutagen sensitivity is not solely due to a defect in the repair of DNA-DSB. In contrast to the results with the MNT we did not find significant differences between women with and without a BRCA mutation with respect to the induction and repair of DNA damage in the comet assay. This finding suggests a normal rate of DNA damage removal and points to a disturbed fidelity of DNA repair as direct or indirect consequence of a BRCA mutation. Our results support the usefulness of induced micronucleus frequencies as a biomarker for cancer predisposition and its application as a screening test in breast cancer families.

Ref.: Rothfuß et al. Cancer Res. 60,390-394 (2000).

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ACRYLAMIDE: METABOLISM AND CHOICE OF MODEL FOR CANCER RISK ASSESSMENT

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Acrylamide, a known neurotoxic substance, gives rise to increased cancer incidence in animal tests. The metabolite, glycidamide, is reactive toward DNA, in contrast to acrylamide itself (Segerbäck et al, 1995), and is assumed to be the genotoxic agent. As for the carcinogenic action of acrylamide experimental data show that, per administered dose, mice are 10-20 times more sensitive than rats (Törnqvist et al, 1998), although the U.S. EPA procedure would predict that rats are about two times more sensitive than mice (U.S. EPA, 1990). According to a new linear multiplicative model for the prediction of cancer risks from genotoxic agents, the incremental cancer risk is proportional to the target dose of genotoxic compound and to the background risk (Granath et al 1999).
Hemoglobin adducts in mice and rats were determined by the N-alkyl Edman method, after i.p. injection of acrylamide (0.3-1.5 mmol/kg). From the adduct levels the relative in vivo doses of acrylamide and glycidamide were inferred. In agreement with the relative sensitivities of mice and rats to acrylamide, the dose of glycidamide, per administered dose, were found to be more than 10 times higher in mice than in rats. This results contribute thus to the validation of the multiplicative model and confirms the role of glycidamide as the genotoxic factor in acrylamide exposure.

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ACRYLAMIDE - A COOKING CARCINOGEN?

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The observations that smokers compared to non-smokers (Bergmark 1997) and unexposed humans and laboratory animals compared to wild animals (Tareke 1998) have higher hemoglobin (Hb) adduct levels from acrylamide (AA) led to the hypothesis that AA could be formed during cooking of food. This hypothesis was tested by feeding rats on fried or unfried animal standard diet. Blood was collected from experimental rats and the adduct, N-(2-carbamoylethyl)valine (CEV) to the N-termini of Hb was measured by gas chromatography-tandem mass spectrometry according to the N-alkyl Edman method. The level of the studied Hb adduct was considerably higher (8-10 times) in rats fed fried diet than in rats fed unfried diet (Tareke et al. 2000).
The product ion spectrum of the studied adduct was identical with that of a verified standard and was interpreted. At the same time AA was pinpointed as the causative reactive factor, through the demonstration that AA is formed during heating of the feed. The level of AA in the fried feed is compatible with the measured levels of the CEV adduct to Hb.
An evaluation of cancer tests of AA and available data for its metabolism to glycidamide leads to the estimation that the background dose of AA in humans is associated with a considerable cancer risk. The magnitude of the risk increment due to background exposure to AA is under investigation. Studies of AA in human diet as well as the mechanism of its formation has been initiated.

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EFFECTS OF GRISEOFULVIN IN RAT LIVER MEDIUM-TERM CARCINOGENIC ASSAY: INDUCTION OF gGT AND GST-P ENZYME-ALTERED FOCI

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Griseofulvin (GF), widely used as a pharmaceutical drug in veterinary and human medicines, was investigated using a rat liver medium-term carcinogenic assay. Fisher F344 male rats were given a single intraperitoneal injection of diethylnitrosamine (DEN, 200 mg/kg bw.) and received GF at 2000 mg/kg bw by gavage two weeks later and for 12 weeks (IGF group). 3 control groups were included in this study: with carboxymethylcellulose (CTL group) or with only DEN (DEN group) or GF without DEN (GF group) as indicated in this model. All rats were subjected to two-thirds partial hepatectomy at week 3 and were sacrificed at week 14. Liver sections were then examined for the expression of two markers for preneoplastic lesions in rat hepatocarcinogenesis: gamma-glutamyl transpeptidase (gGT) and glutathione S-transferase placental form (GST-P). The number of positive gGT and GST-P foci was significantly increased in the IGF group when compared to the DEN group. However, in animals which only received GF without DEN administration (GF group), an increase of gGT positive foci was observed when compared to CTL or DEN groups but no increase in the number of GST-P positive foci was observed when compared to the CTL group. These results may suggest that GF exerts a liver tumour promotion potential.

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BLEOMYCIN-ASSAY AS A PREDICTIVE BIOMARKER IN ENVIRONMENT - RELATED DISEASES

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Hungary is one of the first countries in world-wide cancer mortality statistics. To demonstrate the existence of elevated mutagen sensitivity in environment-related diseases such as head and neck cancer and alcohol-related liver diseases occurring due to strong alcohol drinking and smoking, beside spontaneous chromosomal aberrations (CAs) bleomycin-induced break/cell (b/c) values i.e. the “bleomycin assay” reported first by T.C.Hsu were studied in different groups:
1./Head and neck cancer patients (HNCPs-111 patients) 2./Heavy alcoholics with liver diseases (ALCs - 44 patients) 3./Non smoking healthy controls (106 patients) 4./Smoking, but not drinking controls (124 patients).
When the spontaneous rate of CAs was studied, the highest frequency of aberrant cells (3.34%) was found in the HNCPs followed by the values of smokers (2.88 %) and ALCs (2.73%). Non-smokers showed the lowest aberration frequency (2.35%). Smoking and alcohol consumption in HNCPs and ALCs resulted cancer on one hand, and liver disease on the other. Such kind of life-style is really a risk-factor not only in the mutagenesis but also in the carcinogenesis, showing correlation with significantly elevated frequencies of CAs.
According to the bleomycin-sensitivity assay, the mean of b/c values revealed significant differences between HNCPs (1.16 b/c) and both controls (1.02 b/c). The age, and the smoking status did not influence the mutagen sensitivity, indicating reliabilty of the assay as a predictive biomarker of cancer risk, as it was expected. However, ALCs (1.34 b/c) did not differ significantly from the HNCPs. At the moment it is not known whether ALCs will later develop any type of cancer. Significantly lower sensitivity to bleomycin among smoking controls (1.02 b/c) may be related to a genetically more controlled sensitivity to tobacco exposure, than in patients where alcohol potentiated the action of tobacco. Concerning the proportion of hypersensitive persons, 64% of HNCPs and ALCs were hypersensitives to bleomycin, while 45% of the controls showed also hypersensitivity. This is a two-fold value of the international average in controls. Hungarian control groups seem to be more sensitive to environmental stress-situations, and thus, the cancer proneness is more emphasized in this population than anywhere else. The less reliability of the biomarker for the risk assessment of cancer susceptibility for the Hungarian population can not be excluded either.

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CHROMOSOMAL ABERRATIONS AND MICRONUCLEI STUDIES OF THYROID CANCER PATIENTS

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High frequencies of chromosomal aberrations in peripheral lymphocytes have been found in some cancers. For non-familial thyroid cancers the information available is sparse and equivocal.
The aim of this study was to assess the possible chromosomal instability in peripheral blood lymphocytes from 22 patients suffering from non-familial thyroid cancer (17 papillary carcinoma and 5 follicular carcinoma) compared to a control group of healthy subjects. In this study, two cytogenetic assays, the chromosomal aberrations assay and cytokinesis blocked micronucleus assay were used. The frequency of chromosomal aberrations excluding gaps (%) was 1.68 ± 1.39 (mean value (SD) for the patients'group vs. 2.20 ± 1.87 for the control group. The frequency of binucleated lymphocytes with micronuclei (‰) was 5.41 ± 3.51 (mean value (SD) for the patients group vs. 5.37 ± 3.21 for the control group. The results obtained revealed no significant differences between both groups. This study reinforces the idea that constitutional chromosomal instability in peripheral blood lymphocytes is not visible in non-familial thyroid carcinomas.

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INDIVIDUAL CYP1B1 GENE EXPRESSION AND SUSCEPTIBILITY TO DNA-ADDUCT FORMATION

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Adducts to DNA are commonly used in occupational biomonitoring studies as internal biomarkers of exposure to PAH. Variations in the expression of biotransformation genes, due to genetic polymorphisms and seasonal variations, may contribute to differences in the formation of aromatic DNA adducts after PAH exposure and to the observed problems with reproducibility. In an ongoing 18 month study, blood samples are drawn every second month from 16 subjects genotyped for CYP1A1, -1B1, -2A6, -2E1, mEH, NQO1, GSTM1, -T1, -P1 and AhR. The mononuclear cells are incubated for 3h with 250 µM 7,12-dimethylbenz(a)anthracene (DMBA) dissolved in DMSO. DNA is analysed with a ^{32}P-HPLC based method for DMBA-DNA adducts. Total RNA is prepared from untreated cells and mRNA levels of CYP1A1, CYP1B1 and GSTP1 are determined by competitive RT-PCR. A clear seasonal variation in DMBA-DNA adducts seems to exist, with the highest adduct levels observed in late summer and fall. The CYP1B1 expression shows a similar seasonal pattern with highest mRNA levels in the mononuclear cells sampled in August and October. The CYP1A1*1/*2 genotype and the GSTM1 null genotype may also influence the susceptibility to DMBA-adduct formation ex vivo. We conclude that the seasonal pattern earlier described for AHH inducibility in cultured human lymphocytes, seems to coincide with increased CYP1B1 expression and susceptibility to form PAH-DNA adducts.

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INFLUENCE OF DEFECTED GENOTYPES OF DETOXIFYING ENZYMES ON DNA ADDUCT FORMATION INDUCED BY ENVIRONMENTAL POLLUTANTS

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The study was aimed for an estimation of susceptibility to environmental mutagens. The study group was consisted of 72 children living in Upper Silesia that is a highy urbanized, industrialized and polluted region of Poland. The group was divided further according to concentration of lead ions in peripheral blood. Using PCR-based method the following polymorphic genotypes of detoxifying enzymes were determined: glutathione S-transferase (GST M1, M3, P1 and T1), N-acetyltransferase (NAT2) and epoxide hydroxylase (EPHX). Aromatic DNA adducts level was determined in blood lymphocytes by ^{32}P-postlabelling.
Distribution of gene defect in the studied group did not differ from the data for caucasian population. Mean DNA adduct level for the whole group equaled to 9.2/10^8 nucleotides. In the groups with the defected genotype GST M1, GST M3, GST T1 and EPHX, determining poor detoxification, mean DNA adduct level was higher than in respective groups with a corresponding wild-type gene. The defects in GST M1 and GST T1 tended to coincide that was followed by a significant increase of DNA adduct level.
The established data provide a further evidence for genetically determined susceptibility to mutagenes/ carcinogens present in a polluted human environment.

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EFFECT OF GENETIC POLYMORPHISMS ON MUTAGEN SENSITIVITY

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Mutagen sensitivity, thought to reflect individual DNA repair capacity, has been associated with increased risk of several environmentally induced cancers. Mutagen sensitive individuals show a high frequency of chromosome breaks after in vitro treatment of lympocytes with bleomycin. It is conveivable that in combination with carcinogenic exposure, this susceptibility may greatly influence individual cancer risk. However, possible individual differences in bleomycin metabolism have not been taken into account when bleomycin sensitivity has been measured, and this might have caused some bias in the outcomes of previous studies on mutagen sensitivity and individual cancer proneness. Detoxification of bleomycin is known to be catalyzed by N-acetyltransferases (NATs) in bacteria and by glutathione S-transferases (GSTs) and bleomycin hydrolase (BLMH) in humans. On the DNA level, bleomycin induces oxidative base damage and strand breaks, which are repaired by base excision repair (BER) and homologous recombination repair (HR), respectively. Many of these metabolic enzymes and DNA repair pathways are genetically polymorphic in humans. To examine the possible effect of metabolism and DNA repair on bleomycin sensitivity, 80 healthy Hungarian volunteers and 34 head and neck cancer patients with known bleomycin sensitivity were analyzed for their BLMH, GSTM1, GSTM3, GSTP1, GSTT1, NAT2, XRCC1 (involved in BER) and XRCC3 (involved in HR) genotypes. No genotype-related differences in bleomycin induced break frequencies were detected in the patients. Controls heterozygous for variant allele of XRCC1 codon 280 displayed a higher frequency of bleomycin induced breaks than controls homozygous for normal allele ($p<0.05$ in non-smokers). A significant interaction between XRCC1 and GSTP1 genotypes was also found.

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GENETIC POLYMORPHISMS OF BIOTRANSFORMATION ENZYMES IN POPULATION OF CZECH REPUBLIC AND THEIR USE IN EPIDEMIOLOGICAL AND MUTAGENESIS STUDIES

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The genetically variable biotransformation enzymes: cytochromes P450 (CYP), epoxide hydrolase (EPHX), and glutathione S-transferases (GST) metabolize drugs, carcinogens, and natural products. In addition, most probably up to 90% of human cancers result from exposure to environmental carcinogens suggesting that individual effectiveness in detoxification of chemicals may influence susceptibility to malignant disease. The aim of our study was to determine frequencies of the above listed polymorphisms of biotransformation enzymes in the population of the Czech Republic and to compare these frequencies with data on other ethnic groups.
Polymerase chain reaction-restriction fragment length polymorphism based genotyping assays were used to determine the frequency of polymorphisms in CYP1A1 (3'-flank), CYP2E1 (5'-flank and intron 6), EPHX (exon 3 and exon 4), GSTM1 (deletion), GSTP1 (exon 5), and GSTT1 (deletion) in a group of 416 Czech individuals. We have found the following frequencies of mutated alleles : CYP1A1-m2, 0.097; CYP2E1-C, 0.077; CYP2E1-c2, 0.023; EPHX(exon 3)-His, 0.383; EPHX(exon 4)-Arg, 0.198; GSTM1-null, 0.509; GSTP1-Val, 0.301; GSTT1-null, 0.167. These values are similar to those presented in other studies in Caucasians. Based on the results of EPHX genotyping, the activity of its protein product was deduced and individuals were divided into subgroups with: low, medium, and high EPHX activity. 42.5% of population would fall into low, 44.4% medium, and 13.1% high EPHX activity group. Presented set of results may prove to have a great potential in future epidemiological studies conducted on the Czech and supposedly other related populations of Slavic origin (Bulgarian, Polish, Russian, Slovak, etc.). First applications comprised of 1/ the case-control study of patients with lymphomas and 2/ research on the influence of genotypes on susceptibility to styrene-induced mutagenesis in exposed workers. The work at this project was supported by grants IGA 1850-5 and GACR 313/99/1460.

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POLYMORPHISMS OF DRUG METABOLIZING ENZYMES AS GENETIC FACTORS AFFECTING INDIVIDUAL SUSCEPTIBILITY TO ORAL CANCER

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Excessive alcohol consumption, in combination with smoking habits, has been found to be associated with increased risk of developing oral cancer. Besides those exposures, a possible role can be played by genetic factors, affecting the overall capacity of metabolising alcohol and many carcinogenic compounds contained in cigarette smoke. In fact, several enzymes involved in the biotransformation of these substances show a considerable extent of polymorphism. Therefore a large fraction of the population is defective for some enzymatic activities and this might account for a differential susceptibility to alcohol- and smoking-induced adverse health effects.
Aim of this study was to investigate on the role of genetic polymorphism in drug metabolising enzymes as potential biomarkers of susceptibility to oral cancer. The genotype at CYP2A6, CYP2D6, CYP2E1, ADH2 and ADH3 loci has been determined by PCR-based methods in oral cancer patients and healthy matched controls. Several biological and life style factors have also been taken into account as possible confounders. Genotype frequencies at CYP2A6, CYP2E1, ADH2 and ADH3 loci are comparable in the two samples, thus there is no evidence of an influence of these polymorphisms on the risk of developing oral cancer. In contrast, the results support a possible role of CYP2D6 polymorphism, which should be regarded as potential risk factor (odd ratio: 4.5, $p<0.03$ for heterozygotes) for alcohol- and smoking-related cancer.

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COMPARISON OF SHORT-TERM GENOTOXICITY OF 4-AMINOBIPHENYL IN BLADDER CELLS OF FAST AND SLOW ACETYLATOR CONGENIC MICE

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A study aimed at evaluating the role of N-acetyltransferase polymorphism on the genotoxicity of aromatic amines is being carried out in a mouse model of congenic strains with different alleles of the Nat-2 gene. Previously, a borderline significant difference had been observed between the two strains for the induction of bone marrow micronuclei by 4-aminobiphenyl (4-ABP), with fast acetylator mice showing a higher effect than slow acetylators. In view of the epidemiologic suggestion of a modest but consistent increase in bladder cancer risk for slow acetylators, the study was extended to measure the induction of micronuclei in bladder cells. In both strains a significant increase of micronucleated cells was observed with respect to controls ($p<0.005$); slow acetylator mice showed a mean frequency of 7.8% compared to 6.3% of fast acetylators, although the difference was not statistically significant. A comet assay was conducted in liver, bladder, bone marrow cells and spleen lymphocytes. Liver and bladder cells were apparently unaffected, while small but significant effects were measured in spleen (both strains) and bone marrow (fast acetylators) cells. Finally, a comet assay was carried out in peripheral blood cells sampled from the tail vein at multiple times after treatment, to identify the maximum expression time of 4-ABP-induced comet damage. So far, data have been obtained for slow acetylators: the kinetics of individual responses were heterogeneous, suggesting interindividual variations in the absorption and distribution of orally administered 4-ABP and pointing out the difficulty, under these experimental conditions, to identify the optimum sampling time; nevertheless, paired comparisons between individual baseline and maximum tail moments showed a significant difference.
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DETERMINATION OF CYP1A1 GENOTYPES AND FREQUENCY OF SCEs IN COKE OVEN WORKERS

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Complex interactions between environmental and genetic factors are involved in the induction of cytogenetic effects. In the present study the association between genetic polymorphism of cytochrom P 4501A1 gene and frequency of SCEs was investigated. A total of 82 male workers from coke-oven department of steel factory and reference group of 25 university workers were included to genotype and cytogenetic study. The frequency of the CYP 1A1 genotypes in exposed group was as follows: AA 56.5%, AG 42.1%,GG 1.4% and in the control: AA 47.3%,AG 36.8%,GG 15.9%.The frequency of SCEs was analysed in the particular group of exposure. The mean number of SCEs was increased in the group of workers exposed 10-15 years compared to control /SCE per cell: 6.70-6.21 and 3.9 resp./. This difference is significant, but there are no such significant differences between groups of exposure. Our data do not show the association between genotype and cytogenetic effect. The genotype did not significantly influence the SCE level / $p> 0.05$/.

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NAD(P)H:QUINONE OXIDOREDUCTASE (NQO1) POLYMORPHISM AND LUNG CANCER

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The enzyme NQO1 or DT-diaphorase catalyses the two-electron reduction of quinoid compounds to hydroquinones, which in most cases decreases the reactivity of the compound, but sometimes causes bioactivation. A base substitution polymorphism in the NQO1 gene has been identified. This C-to-T transition at base pair 609 of exon 6 causes a change in amino acid 187 from wild-type proline to serine, which is associated with decreased NQO1 activity. Individuals homozygous for the variant form seem to entirely lack NQO1 activity. Ethnic differences in the prevalence of the variant allele have been reported. Since NQO1 is involved in the metabolism of many compounds present in tobacco smoke, and is highly expressed in human lung, it is of interest to study if the NQO1 polymorphism is a risk factor for lung cancer. This question has previously been addressed in a few studies on ethnically mixed populations with divergent results. In the present study the NQO1 polymorphism was analysed by PCR/RFLP in 422 lung cancer patients of Swedish origin. The genotype- and allele-frequencies among these patients were compared with those found in a group of patients with chronic obstructive lung disease (n=71) and with those of a control group consisting of healthy individuals (n=672). No statistically significant differences in genotype or allele frequencies were found between these groups. The frequency of the variant S allele among controls was 0.16, which is similar to what has previously been reported in other Caucasian populations. In the subgroup of lung cancer patients with small cell carcinoma the PS or SS genotype was found to be significantly more common in older (> 65 yrs) patients compared to younger (=< 65 yrs) patients and also compared to older controls. In conclusion, the NQO1 P/S polymorphism does not seem to be any major risk factor for lung cancer, with the possible exception of small cell carcinoma.

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MICROSOMAL EPOXIDE HYDROLASE POLYMORPHISM AND SUSCEPTIBILITY TO LUNG CANCER

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Microsomal epoxide hydrolase (mEH) catalyses the hydrolysis of a wide variety of reactive epoxide intermediates including epoxides formed from polycyclic aromatic hydrocarbons in tobacco smoke. Although the action of mEH often is regarded as protective it sometimes leads to metabolic activation. Two single-base pair polymorphisms in the mEH gene that result in amino acid substitutions have been identified. The Tyr113His exchange in exon 3 has been associated with decreased mEH activity, whilst the His139Arg substitution in exon 4 has been reported to increase the mEH activity. In the present study the relationship between mEH polymorphisms and susceptibility to lung cancer was investigated. The study population consisted of 235 Swedish lung cancer patients and 513 healthy Swedish control subjects. Statistical analyses of the exon 3 and exon 4 polymorphisms were performed separate but also combined into indices of predicted mEH activity. No statistically significant differences were observed in the distribution of genotypes, alleles or predicted mEH-phenotypes between lung cancer patients and healthy individuals. Stratification by histological subtypes did not reveal an increased lung cancer risk with any specific mEH genotype or phenotype. The mEH polymorphisms did not appear to affect the smoking behaviour. Among individuals with intact GSTM1, high predicted mEH activity was less frequent in lung cancer patients than in controls. In conclusion, the mEH polymorphisms did not appear to be a major modifier of susceptibility to lung cancer.

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GENETIC POLYMORPHISM IN XENOBIOTIC METABOLISM ENZYMES AND INDIVIDUAL SUSCEPTIBILITY TO LUNG AND BLADDER CANCERS

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Several genes involved in the metabolism of carcinogens have been found to be polymorphic in the human population, and specific alleles are associated with increased risk of cancer at various sites. A combined analysis of polymorphic enzymes - microsomal epoxid hydrolase (mEH), glutatione S-transferase m (GST M1) and u (GST T1) and their implication as cancer risk factors was performed in a case-control study of lung and bladder cancers. The genotype frequencies were determined by PCR-based methods in 180 lung cancer patients, 90 urinary bladder cancer patients and in a community-based sample of 355 healthy, unrelated individuals. Among patients with lung cancer, the genotype determining very slow mEH activity was associated with increased risk of lung cancer (OR=2.71, CI=1.20-6.43). As related to bladder cancer risk, our study demonstrate overall GSTT1 effect on bladder cancer risk. Individuals with the GSTT1 null genotype are at approximately 1.9-fold higher risk (OR=1.86, CI=1.1-3.13) of developing bladder cancer. On examination of the genotype combination, a significant association between a combined GSTM1 null genotype and genotype determining very slow mEH activity and increased lung cancer risk was found (OR=5.23, CI=1.38-29.24).

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CODON 72 ALLELIC POLYMORPHISM OF p53 TUMOR SUPPRESSOR GENE IN COLORECTAL CANCER PATIENTS, AND ITS INTERACTION WITH POLYMORPHISM OF METABOLYZING ENZYMES

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Somatic mutations of the p53 tumor suppressor gene can be found in several tumors, and certain hereditary cancer syndromes are caused by inherited mutations of the p53 gene. In such cases the function and activity of the p53 protein is strongly disturbed or totally lacking. In contrast to the point mutations, allelic polymorphisms of the p53 gene lead to minor conformational and functional variation of the protein, thus causing different susceptibility to certain cancers. Role of codon 72 (Arg/Pro) polymorphism of the p53 gene was mainly studied in lung and cervical cancer. In Caucasian populations the Pro allele is the rare variant, and it is probably associated with more frequent occurrence of lung cancer.
In the present case control study we determined the occurrence of p53 codon 72 alleles in a healthy Hungarian population and among colorectal cancer patients. Allelic polymorphisms of metabolizing enzymes (glutathione-S-transferases M1 and T1, cytochrome P450 1A1 and 2E1) were also determined. p53 and CYP 1A1 were genotyped with an allele specific polymerase chain reaction, CYP 2E1 with RFLP, and the GST homozygous deletions with a combined PCR in the presence of an internal control.
The Pro allele was found more frequently among colorectal cancer patients than controls, which suggests an association between presence of Pro allele and the susceptibility to colorectal cancer. In addition, an interaction between p53 codon 72 polymorphism and GSTM1 Ins/Del polymorphism was detected, since presence of both high-risk allele (homozygous 0 genotype for the GSTM1, Pro allele of the p53 gene) has lead to a multiplicative risk increase.

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CYTOGENETIC ALTERATIONS AND DNA DAMAGE IN SOMATIC CELLS OF PARKINSON'S DISEASE PATIENTS

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The involvement of increased levels of oxidative stress in neuronal degeneration is one of the leading hypotheses in the pathogenesis of neurodegenerative disorders, such as Parkinson's (PD). The formation of free radical species by oxidative stress can induce genotoxicity and a reduction or absence of protective mechanisms against oxidative damage in some individuals may result in their increased susceptibility to genotoxic damage. Thus, genotoxic changes might represent one of the crucial events in the pathogenesis of neurodegenerative diseases. We have investigated the presence of cytogenetic alterations and primary DNA damage in somatic cells of a group of 15 subjects affected by Parkinson's disease (not under theraphy) using the human lymphocytes micronucleus (MN) test and the comet assay (single cell gel electrophoresis, SCGE), respectively. The MN assay is able to detect the presence of structural and/or numerical chromosomal aberrations in interphase lymphocytes. We used a modified version of the comet assay that allows to detect besides DNA strand breaks (SSB), the presence of oxidised bases, i.e. oxidised pyrimidines (endonuclease III-sensitive sites) and altered purines (sites sensitive to formamidopyrimidine glycosylase, including 8-Hydroxy-2'-deoxyguanosine, a typical indicator of oxidative damage). Compared to healthy controls (paired for sex, age and smoking habit) PD patients show higher frequencies of MN and a four-fold increase in the levels of SSB. Significant differences were also obtained in the distribution of oxidised purine bases between the two groups. As unrepaired primary DNA lesions generate chromosome fragments which in turn, can be included into micronuclei, we hypothesise that the presence of elevated levels of primary DNA lesions can explain at least partially, the increased MN frequency observed in PD patients. In order to confirm these findings, fluorescence analysis of MN with a pancentromeric DNA probe is in progress to detect if MNs are mainly due to chromosome breakage or to chromosome loss.

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MOLECULAR ANALYSIS OF THE REVERSION OF THE WHITE-IVORY ALLELE OF DROSOPHILA MELANOGASTER

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The induction of reversion of the white-ivory allele in Drosophila melanogaster has been used as a good indicator of mutagenic activity. Nevertheless, the molecular mechanism of this reversion is still not well understood.
The white-ivory phenotype is due to the duplication of approximately 2.96 kb in the region of the white locus positioned between +2795 and -173 (according to the Levis coordinates). The reversion to the wild phenotype seems to be due to the excision of one of the copies of the duplication.
In order to find out the cause of this reversion and, in case of loss of one of the copies, the precision with which such excision occurs, we have isolated and analysed revertant lines obtained after the treatment of a white-ivory mutant strain with alkylating agents.
The Southern blot analysis, and the results from the amplification by PCR of the three specific regions, that include the beginning and the end of the duplicated region as well as the internal junction region of the duplicated sequence, shows that the revertants analysed have lost one of the characteristic white-ivory duplicated copies. No alterations in other regions of the white locus were detected when analysed by Southern blot.
The sequencing of the fragments of amplified DNA, manifests that the revertants analysed present the precise excision of the duplication. In addition, our results show slight differences with the published sequence of the white-ivory mutation and with the wild type sequence of the white locus.

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THE MAJOR DNA ADDUCT OF BUTADIENE IS N7-(1-(HYDROXYMETHYL)-2,3-DIHYDROXYPROPYL)GUANINE, A POTENTIALLY HIGHLY MUTAGENIC DNA ADDUCT ORIGINATING FROM 1,2-EPOXY-3,4-BUTANEDIOL

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Mice are more susceptible to the rodent carcinogen 1,3-butadiene (BD) than rats. Genotoxic epoxides of BD, 1,2-epoxy-3-butene (BMO), 1,2-epoxy-3,4-butanediol (EBD) and 1,2:3,4-diepoxybutane (DEB), have been suggested as the ultimate carcinogens. Reaction of dG or DNA with BMO gives equal amounts of N7-(2-hydroxy-3-butenyl)guanine (G1) and N7-(1-(hydroxymethyl)-2-propenyl)guanine (G2). The major adduct (>80%) formed when DEB is allowed to react with dG is N7-(2,3,4-trihydroxybutyl)guanine (G3), while N7-(1-(hydroxymethyl)-2,3-dihydroxypropyl)guanine (G4) is formed as a minor adduct (< 15%). For EBD the opposite was found: reaction with dG leads to G4 as the major adduct with G3 as minor adduct. 2D-Total correlation ^{1}H-NMR spectroscopy of G4, indicated that the N7-alkyl group is in a virtually fixed conformational state and may interact, by virtue of its hindered rotation, with the O^{6} of guanine, which would imply a higher degree of mutagenicity of G4 than of any other N7-guanine adduct observed in this study.
In rats and mice exposed to [4-^{14}C]-BMO (1-50 mg/kg, i.p.) DNA adduct profiles in rat and mouse were similar in liver and lung, with G1 and G2 as main, and G4 as minor adduct. In rats and mice exposed to 200 ppm [2,3-^{14}C]-BD by nose-only inhalation for 6 h, G1 and G2 were minor adducts in liver (1.9 and 8.0 per 10^{8} bases for rats and mice, respectively) and lung (1.6 and 6.6 per 10^{8} bases). G3 was present in mouse, but not rat, liver and lung at levels of 20 and 12 adducts/10^{8} bases. The major adduct was G4 which accounted for 13 and 90 (liver) and 11 and 139 (lung) adducts/10^{8} bases in rats and mice, respectively. These results indicate that the major DNA adduct following exposure to BD is from EBD, and not from BMO or BDE. In addition, NMR data suggest that this adduct is highly mutagenic. These findings have important implications for the understanding of the mechanism of carcinogenicity of BD.

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BIOMARKERS OF EXPOSURE TO BUTADIENE AS BASIS FOR CANCER RISK ASSESSMENT

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1,3-Butadiene (BD) is carcinogenic in mice and rats, with mice being considerably more sensitive than rats. Urine metabolites are 1,2-dihydroxybutyl mercapturic acid (DHBMA) and a mixture of monohydroxy-3-butenyl mercapturic acids (MHBMA). The reactive metabolite 1,2-epoxy-3-butene forms 1- and 2-hydroxy-3-butenyl valine adducts in haemoglobin (MHBVal). The aims of the study were: (1) to compare the suitability of MHBMA, DHBMA and MHBVal as biomarkers for low levels of exposure to BD, and (2) to explore relative pathways of metabolism of BD in humans for comparison with mice and rats, which is important in relation to cancer risk assessment in man. MHBMA, DHBMA and MHBVal were determined in 2 studies in workers engaged in the manufacture and handling of BD. Airborne BD was assessed by personal air monitoring. MHBMA was more sensitive than DHBMA for monitoring recent exposures to BD and could measure 8-h time weighted average exposures as low as 0.13 ppm. The sensitivity of DHBMA was restricted by relatively high natural background levels in urine, of which the origin is currently unknown. MHBVal adducts in haemoglobin was a sensitive method for monitoring cumulative exposures to BD at or above 0.35 ppm. Statistically significant relationships were found between MHBMA and DHBMA, between either of these variables and 8-h airborne BD levels and between MHBVal adducts and average airborne BD levels over 60 days. The data on biomarkers demonstrated a much higher rate of hydrolytic metabolism of 1,2-epoxy-3-butene in humans compared to mice and rats, which was reflected in a much higher DHBMA / (DHBMA + MHBMA) ratio and in much lower levels of MHBVal in humans. Assuming a genotoxic mechanism, the data of this study coupled with other published data on DNA and haemoglobin binding in mice and rats, suggest that the cancer risk for man from exposure to BD is expected to be less than for the rat and much less than for the mouse.
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FORMATION OF DNA ADDUCTS, DNA STRAND BREAKS AND MICRONUCLEI IN MICE EXPOSED TO STYRENE BY INHALATION

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7-substituted guanines are the main adducts from alkylation of DNA by styrene 7,8-oxide (SO), the major in vivo metabolite of styrene. The formation of the b-isomer of 7-SO-guanine in male NMRI mice was studied in an inhalation study. The mice were exposed to styrene in concentrations of 750 mg/m^3 or 1500 mg/m^3 for 21, 7, 3 and 1 days (6 h/day, 7 days/week). The 7-SO-guanines were analysed from the lung and liver tissues by the ^{32}P-postlabelling method with anion-exchange cartridge enrichment. The adduct levels were markedly higher in the lungs as compared to livers. In the lungs the levels of 7-SO-guanines, after 21 days of exposure, were 2.3 adducts / 10^7 normal nucleotides (1500 mg/m^3) and 0.6/10^7 (750 mg/m^3). The adducts were identified at all time points, the levels increasing with longer exposures. When the single-strand breaks (SSB) were analysed with Comet assay as endonuclease III -sensitive sites, a dose dependent response was observed in bone marrow of exposed mice, the levels of SSB's slightly increasing with time. The inhalation of 1500 mg/m^3 of styrene induced significant increase of micronuclei (MN) after 7 days of exposure (10.4 ± 2.5/1000 cells, i.e. twice higher MN frequency than in controls). After 21 days of inhalation no significant difference between the control group and both exposed groups was observed. Whether this decrease after 21 days of styrene inhalation was due to the inhibition of cell proliferation caused by styrene exposure or due to the effective repair process, remains to be studied.

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EFFECTS OF DIETARY SELENIUM ON ENDOGENOUS DNA ADDUCTS IN THE RAT

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Studies of DNA adducts from environmental carcinogens have revealed that there are also endogenously generated adducts produced. The origins of these background levels have to some extent been identified and major sources are probably reactive oxygen species, lipid peroxidation products and other reactive species formed as a consequence of normal (and abnormal) cell metabolism. Formation of many of these adducts can be prohibited by addition of antioxidants and other radical scavengers. The essential trace element selenium is one important component of these defence systems, e.g. glutathione peroxidase and other selenium containing enzymes. In order to test the effect of selenium on endogenously generated DNA adducts male Wistar rats were given normal, half and double amounts, respectively, of selenium in their diet. After 18 weeks on these three different diets the animals were sacrificed and DNA adducts in the liver and the lung were analysed using the ^{32}P-postlabelling assay with nuclease P1 enrichment. HPLC analysis of the labelled digests indicated 5 adducts in the lung and 7 adducts in the liver, which were consistently present. The pattern and levels of adducts in the liver did not alter with selenium content of the diet. However, in the lung 3 adduct peaks were detected which decreased and 2 adduct peaks which increased with increasing selenium. Two of the adducts decreasing were also formed during Fenton type of reaction with DNA in vitro, indicating that they represent adducts formed by reactive oxygen species. The reason why two adduct peaks increased with increasing selenium in the diet is unknown, but since the source and identity of those are unknown it is possible that they represent some kind of shift in balance of enzymatic and/or chemical processes in the cell. The present study shows that even minor changes in the dietary concentrations of selenium could have a profound effect on levels of endogenously generated adducts which might be harmful to carry in the genome. Future studies will hopefully reveal if selenium content of the diet will have these effects also in humans.
Abbreviation: HPLC, high performance liquid chromatography

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PARP OR MDM2-P19^{ARF} SYSTEMS ARE NOT INVOLVED IN BENZO(A)PYRENE (BP) INDUCED P53 RESPONSE IN MOUSE SKIN

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Metabolism and DNA-binding of BP are the prerequisites for BP-induced carcinogenesis. Chemically induced cancer is probably dependent on the loss of the function of p53 tumour suppressor protein. Since the response of p53 protein to DNA damage has been mainly studied in vitro, we have used the mouse skin model. p53, poly(ADP-ribose) polymerase (PARP), MDM2 and p19^{ARF} proteins after BP treatment were studied by Western blotting. B57BL/6 male mice were treated with 500 µg of BP and/or 5 mg of 3-aminobenzamide (3AB) in acetone by painting on the shaved back skin during the non-growing phase of the fur growth. BP clearly induced p53 protein in agreement with our earlier studies (Bjelogrlic et al. 1994, Tapiainen et al. 1996, Serpi et al. 1999). The inhibitor of PARP, 3AB, did not affect the BP-induced increase in p53 protein at any of the studied time points (6 or 2 hours before BP-treatment, or 2, 6, 9, 12, 15, 24, 48, or 72 hours after BP-treatment) implicating that PARP is not essential for BP-induced p53 induction. BP-treatment induced also an increase of p21 and MDM2 proteins. Since p53 protein is a transcription factor of these proteins, the BP-induced p53 protein in mouse skin is in functional conformation. p19^{ARF} protein was not affected, and thus it is unlikely that the inhibition of p53 degradation by MDM2-p19^{ARF} system is the reason for the increase of p53 by BP in mouse skin.

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MECHANISMS OF OXIDATIVE STRESS-INDUCED MUTAGENICITY AND ADAPTIVE RESPONSE

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We have shown that hyperbaric oxygen (HBO) treatment of mammalian cells is a well suited model for studying the genetic consequences of oxidative stress. (Rothfuß et al., 1999; 2000). Despite a strong clastogenicity, HBO did not markedly increase mutant frequencies in the HPRT test with V79 cells. As gross genetic alterations are generally not detected with the HPRT test, we now investigated the effect of HBO treatment in the mouse lymphoma assay (MLA) with L5178Y tk+/- cells. Our results demonstrate a clear mutagenic effect of HBO. This effect was solely due to the induction of small colony mutants with loss of heterozygosity (LOH) in the TK region, indicating that induction of chromosomal mutations account for the observed mutagenic effect. As the same treatment also clearly induced oxidative base damage, including 8-oxoguanine, we conclude that the induction of point mutations as a consequence of induced 8-oxoguanine seems to be of minor importance for oxidative mutagenesis. Exposure of human lymphocytes to HBO led to the induction of an adaptive response, similar to that observed in our previous in vivo experiments (Rothfuß et al., 1998). Northern Blot analysis demonstrated an induction of the stress gene heme oxygenase 1 (HO1), possibly indicating a protective mechansim via the increase of ferritin levels. In contrast, a comparable protection was not found in V79 cells, indicating a cell-specific difference in expressing an adaptive response.

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THREE POSSIBLE HUMAN BIOMARKERS FOR COLON CANCER AND THE CHARACTERISATION OF 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4,5-B]PYRIDINE (PHIP)

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Colon cancer is a multistage process. Normal mucosa develops into polyps, which may develop into tumours. DNA adducts are biomarkers, biologically linked to chemical exposure, tumour formation and clinically observed cancer. They have been detected in the mucosa of colon cancer patients. A possible correlation between dietary heterocyclic amines and human cancers has been found, as well as DNA adduct formation of heterocyclic amines in human tissues. Levels of DNA adducts in colon mucosa from non-cancer controls, polyp-, and cancer patients were analysed. There was no significant difference between any of the groups. Two single DNA adducts detected with the ^{32}P-HPLC method revealed that control mucosa had higher adduct levels compared to polyp and tumour mucosa. One DNA adduct was only found in non-tumour and tumour mucosa. The three DNA adducts together could be biomarkers for colon cancer. Standards of B[a]P and the heterocyclic amines 4,8-diMeIQx, PhIP and AaC were compared with the colon DNA adducts. PhIP showed to be present in all stages in the colon cancer process, namely controls, polyp- and cancer tissues.

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CYTOGENETIC CHANGES IN CHROMOSOME 6 DO NOT CORRELATE WITH THE LEVEL OF MANGANESE SUPEROXIDE DISMUTASE IN MALIGNANT MESOTHELIOMA

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Malignant mesothelioma is an aggressive tumor which originates from mesothelial cells of the serous cavities. In most cases it is associated with occupational exposure to asbestos fibers. Manganese superoxide dismutase (MnSOD) plays an important role in protecting cells and tissues from oxidant injury and hyperoxia. When compared to healthy pleura or nonmalignant mesothelial cells, the level of MnSOD is high in mesothelioma tissue and in cultured mesothelioma cell lines. The MnSOD gene is located in 6q25. In order to study the possible role of numbers of chromosome 6 in high MnSOD expression, four human mesothelioma cell lines (M14K, M24K, M25K and M38K) were hybridised with a chromosome 6 specific paint by fluorescence in situ hybridisation (FISH). We also performed a comparative genomic hybridisation study (CGH) to detect DNA copy number changes in these cell lines. MnSOD levels were measured by Western blotting. A high MnSOD expression, as compared to non-tumorigenic mesothelial cell line (MeT-5A), was detected in all four mesothelioma cell lines. The expression was highest in M24K and M38K cell lines. No extra copies of chromosome 6 or translocations of this chromosome were found in the mesothelioma cell lines using FISH. CGH detected, on average, more losses than gains of genetic material. However, no losses or gains were found in chromosome 6 in any of the cell lines. Losses in chromosome 14 were found in all four mesothelioma cell lines. No cytogenetic changes could be detected in the MnSOD gene region of chromosome 6. We conclude that the level of MnSOD does not depend on the number of copies of chromosome 6, and the regulation of MnSOD is not dependent on amplification in 6q25 either.

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DIFFERENT REGULATION OF LABILE IRON POOL IN L5178Y CELL LINES DETERMINES THEIR SUSCEPTIBILITY TO HYDROGEN PEROXIDE

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Minimising the concentration of “free iron” present in so-called labile iron pool (LIP), a low-molecular-weight cytosolic pool of weakly chelated iron that rapidly transits through the cell is one of the main protective strategies against oxidative stress. The regulation of physiological LIP has to be tightly controlled and it seems that constitutive level of LIP is midway between the cellular need for iron and the hazard of excessive generation of OH.
We characterised and compared the regulation of iron metabolism in intact L5178Y (LY) cell lines to elucidate the contribution of iron to their differential sensitivity to H_2O_2. We showed that the level of iron in LIP is more than 3-fold higher in H_2O_2-sensitive, LY-R cells than in H_2O_2-resistant, LY-S cells. In parallel, we found that the expression of ferritin (FR), main intracellular iron sequestering compound, at both mRNA and protein level is markedly greater in LY-S than in LY-R cells, which confirms a putative role of FR in detoxyfying iron and its role in antioxidant defence. Treatment of LY cells with the iron chelator desferroxamine (DFO) confirmed further the role of high LIP in the establishment of pro-oxidant status in LY-R cells. Pretreatment of LY cells with DFO followed by H_2O_2 treatment gives a considerable sparing effect, which is substantially greater for the LY-R than for the LY-S line. This is reflected in the initial DNA damage as well as in the cell survival. Simultaneously, we observed that DFO reduces LIP level by 70% and only by 20% in LY-R and LY-S cells, respectively. These different changes in LIP levels upon treatment with DFO were associated with the induction of IRE-binding activity of IRP1 in LY-R cells without any detectable effect in LY-S cells suggesting the inhibition of FR synthesis in the former line at the level of IRE-IRP1 post-transcriptional regulatory mechanism.

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BUFFER-MEDIATED MODULATION OF DNA CARBOXYMETHYLATION AND METHYLATION INDUCED BY DIAZOACETATE AND ITS SIGNIFICANCE

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Potassium diazoacetate (KDA), a nitrosated glycine derivative has been shown to form O6carboxymethyldeoxyguanosine (O^6CMdG) and O6methyldeoxyguanosine (O^6MedG) adducts with DNA [1]. The aim of this study was to investigate to what extent the incubation buffer would affect the level and profile of O^6-alkylguanine adducts. Adduct levels of O^6CMdG and O^6MedG were determined by HPLC fluorescence after immunopurification. 8mM KDA treatment of DNA dissolved in Tris-EDTA (TE, pH 7.5) seemed to reduce the extent of O^6CMdG formation and increase methylation when compared to the reaction in phosphate buffered saline (PBS, pH 7.4). The ratio of O^6CMdG to O^6MedG was approximately 14:1 in PBS, whereas in TE buffer the ratio was only 2:1 (data uncorrected for recovery of immunoaffinity columns). Adduct levels appeared to be also affected by the concentration of the buffer system. Both carboxymethylation and methylation were highest in 0.5xTE and lowest in 2xTE. In 0.5xTE, about three times more O^6CMdG was formed compared to 2xTE. Methylation showed a similar pattern, although to a lesser degree. Relative proportions of O^6CMdG to O^6MedG ranged from 2.8:1 (0.5xTE) to 1.9:1 (2xTE). One explanation could be that TE buffer was reacting with the alkylating agents generated from KDA. KDA-induced p53 mutational spectra were obtained by treating a p53 containing plasmid (pLS76) dissolved in either TE or PBS [2]. Despite the expected differences in O^6CMdG and O^6MedG levels the mutational spectra obtained using the two buffers were statistically indistinguishable.
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DIESEL PARTICLES INDUCE A CYTOKINE RESPONSE IN HUMAN TYPE II ALVEOLAR LUNG EPITHELIAL CELLS AND MURINE LUNG TISSUE

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Exposure to diesel exhaust particles (DEP) seems to be related to the increased prevalence of pulmonary diseases, and animal models with repeated exposure have shown DEP to be carcinogenic. The mechanisms are unknown, but may involve inflammation and the generation of reactive radicals. Inflammation is regulated, in part, by cytokines, which attract and activate inflammatory cells, such as macrophages and neutrophile granulocytes, in the airways. Cells recruited to the airways are capable of producing reactive oxygen and nitrogen species, which are directly genotoxic and can cause genetic damage in lung cells. We investigated the effects of DEP (10-500 µg/ml) on cell viability and cytokine mRNA expression (IL-1 a, IL-6, IL-8, TNF-a) in human A549 type II alveolar lung epithelial cells. All cytokines were measured after 24 hrs of exposure, and IL-6 was furthermore measured after 2 and 5 hrs.
No cytotoxicity was observed of any dose tested. DEP induced a dose- and time-dependent cytokine response in A549, and the cytokines increased between 4- and 18-fold at highest DEP dose compared to cells incubated without DEP. The Comet assay will be used for studying DNA damage.
Parallel to our in vitro findings, we found that DEP inhalation in mice with a single dose of 10 mg/m^3 for 2 hrs increased IL-6 mRNA production in lung tissue 4-fold compared to control mice.
The present results indicate that DEP can induce a cytokine response in the alveolar epithelium, which may contribute to the adverse respiratory effects of these particles.

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HSP70 OVEREXPRESSION PROTECTS CELLS AGAINST HEAT SHOCK, BUT DOESN'T PROTECT MITOTIC SPINDLE AGAINST DAMAGE INDUCED BY TOXIC AGENTS

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HSP70 is one of heat shock proteins that are known to act as molecular chaperones. Their level is significantly increased when cells are subjected to stress conditions including heat shock, exposure to free radicals, infection by pathogens and tissue injuries. In our previous studies we showed that exposure of Chinese hamster V79 cells to air-borne pollutants disturbs mitotic apparatus. It was manifested by multipolar or uncomplete spindles what leads to aneuploidy and, in consequence, makes these cells potentially tumorogenic.
The aim of our study was to check if constitutively expressed HSP70 gene could protect cells against spindle-deteriorating agents. V79 cells were transfected by electroporation with pR70.1/DNA3 construct containing rat HSP70 gene under the control of a constitutive promotor CMV and a gene conferring neomycine resistance. Stably transfected clones were outgrown in the presence of neomycin as a selectable marker. The clone with immunohistochemically detected highest overexpression of HSP70 was subsequently used for assessment of cytoprotective role of HSP. To find out whether overexpression of HSP70 protects against heat shock cells were exposed to 42^{o}C and their persistence was measured by MTT test. As expected, HSP70 producing cells were more resistant to high temperature than control cells. Next, mitotic spindle succeptibility for air-borne pollutants was measured both in transfected cells overexpressing HSP70 gene and in reference cells transfected with empty vector only. We found no statistically significant differences in mitotic spindle damage (assessed by counting cells with invalid spindles) between control and HSP70 overexpressing cell lines. Our data show that cytoprotective function of HSP70 protein may not be effective against mitotic spindle malformations induced by chemical mutagens.

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ANALYSIS OF MUTATIONAL EFFECTS AT THE HPRT LOCUS IN HUMAN LYMPHOCYTES IRRADIATED IN VITRO WITH GAMMA RAYS

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The mutational effects of ionising radiation at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus were studied in peripheral blood lymphocytes (PBL) irradiated in vitro with g rays. To assess the persistence of radiation induced mutants, the cells were cultured up to one month after irradiation and the HPRT mutants were selected every week on the basis of 6-thioguanine resistance (Albertini et al., 1982). After an expression time of 7 days there was a dose related increase of 7.6 x 10^{-6} mutants/Gy. Mutant frequency (MF) and cloning efficiency (CE) decreased with time in cultured cells, and there was no evidence of a delayed induction of mutations. The mutational spectrum of the HPRT gene was determined by PCR analyses in a total of 99 mutant clones derived from irradiated PBL (Gibbs et al., 1990). The independent origin of mutant clones carrying the same mutation was assessed by analysing the TCR ggene rearrangements (de Boer et al., 1993). The results showed a dose-related increase of deletions, in particular of the whole gene. Two preferentially deleted regions were identified; one involving the HPRT exon 3, and another one the 3'-terminal and the 3'-flanking regions of the gene. One complex mutation involving a non-contiguous deletion of exons 2-5 and 7-8 was observed among the mutants isolated after 3Gy irradiation.

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DECREASED MITOTIC ACTIVITY OF HUMAN LYMPHOCYTES TREATED WITH EPINEPHRINE IN VITRO

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Epidemiological and endocrinological studies have pointed to a strong influence of hormones in the development of malignant diseases. Hormones can exert mitiogenic effects and thereby act as tumor promoters. Besides, steroid hormones act as complete carcinogens capable of inducing mutations due to their metabolic conversion to reactive derivatives. The objectives of this investigation were to evaluate mitogenic effects of epinephrine in cultured human peripheral blood lymphocytes. Six experimental concentrations were used (range from $5x10^{-10}$ M to $1.5x10^{-4}$ M). Both positive (10^{-6} M MNNG) and negative controls (placebo) were used. The cultivation vials were set up according to standard procedure for sister-chromatid exchange test. Mitotic index (MI) was determined on at least 1000 cells per each culture, whereas proliferation index (PI) was calculated on at least 200 cells per culture, so as to reflect the ratio of cells in first, second and third mitotic cycles. Control values were MI=5.91%, and PI=1.84. Epinephrine caused significant antimitogenic effects as well as cell-cycle delay at higher concentrations applied ($5x10^{-6}$ M, $5x10^{-5}$ M and $1.5x10^{-4}$ M). It can be concluded that epinephrine interferes progression through the cell cycle and decreases mitotic index at concentrations higher than $5x10^{-6}$ M. Molecular mechanisms for such effects remain unclear, for the time being.

IN VITRO INDUCTION OF CELL-CYCLE DELAY IN PERMETHRIN-TREATED HUMAN LYMPHOCYTES

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Permethrin is a broad spectrum insecticide widely used in pest control and against various ectoparasites. It has been shown that permethrin causes cytogenetic changes in cultured human lymphocytes. The aim of the present study was to evaluate possible changes in cell-cycle kinetics of human lymphocytes treated with permethrin. Four experimental concentrations of permetrhin were used: 1.2, 3, 6 and 12 mg/ml. Both positive (10^{-6} M MNNG) and negative contols (acetone, the solvent) were used. The cultivation vials were set up according to standard procedure for sister-chromatid exchange test. Since the cultures were not synchronized it was possible to observe cells in different mitotic cycles. Cell-cycle kinetics was analysed by proliferation index, a parameter that expresses the ratio of cells in the first, second and third mitotic cycles. The analysis of proliferation index revealed significant cell-cycle delay at all concentrations of permethrin except the lowest one.

DNA ADDUCTS, p53 AND $p21^{WAF1}$ EXPRESSION, AND CELL CYCL ALTERATIONS AFTER EXPOSURE OF EMBRYONAL HUMAN LUNG FIBROBLASTS TO PAHs

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Polycyclic aromatic hydrocarbons (PAHs) are activated into diol-epoxides before interacting with DNA. The mechanism contributing to their carcinogenic action is still unclear, although it may be related to their ability to damage DNA without eliciting G1arrest ("stealth properties").
Dibenzo(a,l)pyrene (DB(a,l)P) and benzo(a)pyrene (B(a)P) were investigated in an in vitro system using multiple end-points. Growing as well as near-confluent human diploid embryonal fibroblasts (HEL cells, USOL, Prague) were PAH-treated for time- and dose-dependent responses: DNA adducts were analysed by ^{32}P-postlabeling, p53 and $p21^{WAF1}$ by Western blotting with ECL immunodetection, and the cell cycle by flow cytometry (DNA content).
The exposure to 0.1 µM DB(a,l)P and 1.0µM B(a)P for 24 h induced maximal DNA adduct levels, although the binding activity of DB(a,l)P was ~8-10 times higher as compared with B[a]P in both growing cells and at near confluence. The levels of p53 and $p21^{WAF1}$ increased in a time- and dose-dependent manner and correlated with DNA adducts (p53, $r = 0.832$, $p < 0.001$; $p21^{WAF1}$, $r = 0.808$, $p < 0.001$). Treatment of growing HEL cells with 0.1 µM DB(a,l(P resulted in major cell cycle alterations starting from 12 to 16 h of treatment. After 24 h of exposure, compared to controls, there was a high level of DNA adducts (935 ± 193 /10^8 nucleotides) accompanied by an increase in S phase cells ($24.5 \pm 1.5\%$), both G0/G1 and G2/mitosis being decreased. Similar trends were observed at near confluence and after exposure to 1.0 µM B[a]P, but differences were more subtle (0 to 10% range).
These data suggest that exposure of HEL cells to DB(a,l)P, and less so to B[a]P, induces DNA adduct formation, p53 and $p21^{WAF1}$ expression without eliciting transient G1 arrest, but rather an S phase delay/arrest. It is unclear if the increases in p53 and $p21^{WAF1}$ are responsible for the S phase delay to allow DNA repair before further progression of the cell cycle.

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NIJMEGEN BREAKAGE SYNDROME AND ATAXIA TELANGIECTASIA CELLS DIFFERED IN THEIR G2-PHASE CHECKPOINT

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It has been shown that Nbs1 and ATM, the gene products deficient in Nijmegen Breakage Syndrome (NBS) and Ataxia Telangiectasia (AT), play a role in the maintainance of genome integrity. It has been shown, that many hallmarks for cellular radiosensitivity are sahared by the two syndromes. Nevertheless, cell cycle regulation has been poorly investigted. In former investigations, we could show that the p53 response appears markedly different between NBS and AT cells (Antoccia et al I999) and our recent experiments were aimed to analyse the G2-phase checkpoint in more detail. Experiments were performed on LCLs from an AT patient as well as on LCLs from three NBS patients with different mutations: ZRA with the slavic founder mutation, 668/97 a north african patient with private mutation (Maraschio et al. J. Med.Genetics,2000) and 96B453, an atypicalNBS-patient without a detectable mutation in Nbs1 gene. A similar rate of chromatid damage was induced by X-rays in NBS as well as in AT cells. However, short time after irradiation NBS cells ZRA and 96B453 showed a stronger inhibition of entry into mitosis and later on a lower extent of G2-phase accumulation, in comparison to AT cells. In contrast, NBS 668/97 behaved in a intermediate manner between AT and normal Cyclin-B1 antibody imunofluorescence staining was performed to analyse the extent G2-phase accumulation. The observed amounts in cytoplasmic accumulation of this protein, supported the observed differences between the different NBS cell lines. Furthermore, the level of cyclin-B1 and phosphorylation status of p34CDC2 (CDC2-P-Tyr) were evaluated by western blotting in fractionated cytoplasmic and nuclear extracts of irradiated cells. In contrast to normal and NBS cells, AT cell sustained a lower accumulation of cytoplasmic cyclin-B1 and a lower extent of nuclear CDC2-P-Tyr. Further experiments are in progress to evaluate the function of phosphatase CDC25 which act upstream of p34CDC2.

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MEIOTIC RECOMBINATION IN DROSOPHILA MELANOGASTER UNDER THE INFLUENCE OF THREE MUTAGENIC COMPOUNDS

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In order to study the influence of different mutagenic compounds in meiotic recombination in germ cells of D. melanogaster, we have used three alkylating agents with different mechanisms of action: N-ethyl-N-nitrosourea (ENU), diethyl sulfate (DES) and hexamethyl phosphoramide (HMPA). 24 hours old females, heterozygous for y w ct m f X chromosome markers, were treated with the mutagens and afterwards they were mated to hemizygous y w ct m f males. The progeny was scored to calculate recombination frequencies between contiguous loci. The progeny was fractionated following a brood pattern to score in each experiment separately the progeny from oocytes and oogonias. However, since there are no biological reasons to expect differences between the control frequencies obtained in oocytes and in oogonias, we decided to join both progenies as a unique control.
In oocites we did not find differences between control and induced recombination frequencies. In oogonias the obtained data reveal that in the y-w interval in the case of ENU there is an increase in the recombination frequency. With the other chemicals, there are not significance differences maybe due to the low number of scored individuals.
Parallely, we perform a similar study using a fly strain where the y-w interval is placed in a different region from its usual position in X chromosome, using the In(1)sc$^{[8]}$, sc$^{[8]}$ inversion. The recombination frequencies obtained in this case were lower than those obtained in the former experiments, and we never found differences between control and treatments neither in oocytes nor oogonias. The lack of frequency increase, in the case of ENU, can be explained if chromatin condensation is higher in this new position, with regard to normal location, preventing the interaction between DNA and the chemical, and therefore decreasing the process of recombination.

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SOME ANTIOXIDANTS INHIBIT EMS-MUTAGENESIS IN DROSOPHILA GERM CELLS

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The effects of two antioxidants (AO) 2,6-dimethyl-3,5-diethoxycarbonil-4(Na carboxylate)-1,4-dihydropyridine (DHP) and glutation (GP) were compared under different conditions: (1) the treatment of adult males or (2) their larvae; (3) the treatment of females. Then adult males were exposed to ethyl methanesulfonate (EMS). Germ cell mutability was estimated by sex linked recessive lethals (SLRLs) that are due to intralocus alterations. Besides, the embryonic (EL) and postembryonic (PEL) lethals caused by chromosome breaks were scored

When adult males being fed by AOs, these compounds didn't influence EMS mutagenic and clastogenic effects in both test-systems. Larval pre-treatment with AO reduced the chromosome break level as well as the frequency of SLRLs. For example, DHP (90 mM) decreased the EMS induced embryonic lethality from 20.4 to 16.8% under 7-8 day-storage of spermatozoa and this AO reduced the EMS-induced SLRL frequency by more than 50% in sperm cells without storage. The protective effect of GP (10 mM) was found in both test systems: this AO reduced EMS mutagenicity by ~20% in SLRL test (z=2.29* by Cochran) and by~30% in chromosome break test after storage (z=2.67** by Cochran). Significant reduction of the chromosome breakage and SLRL rates was usually observed when spermatozoa exposed to EMS were stored in normal females treated with AOs. The lack of maternal repair systems inhibited the sensitivity of females mei-9 and mei-41 to AO protective action.

Thus, the data obtained suggest DHP and GP action is mediated by defense systems. The antioxidants tested seems to affect repair pathways involved in chemical mutagenesis in Drosophila melanogaster.

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SOME FEATURES OF DNA REPAIR INVOLVED IN CHEMICAL MUTAGENESIS IN DROSOPHILA GERM CELLS

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The effects of the monofunctional alkylating agent ethyl methanesulfonate (EMS) were estimated by sex-linked recessive lethal mutations (SLRLs) and embryonic, late embryonic and postembryonic offspring lethality (EL, LEL, PEL) after feeding adult males. SLRL-test allows detection of intralocus alterations, e.g. point mutations, whereas EL, LEL, PEL, as a rule, are caused by chromosome breaks (CBs). The relationship between mutation rates and O^6-alkylguanine production is known. In contrast, EMS-clastogenicity is supposed to associate with 7-ethylguanine and AP-site formation. The latter was confirmed by increasing CB frequency when treated males were mated with excision repair-deficient females mei-9^{L1}. A minor but verified reduction of their frequency, when using females mei-41^{D5}, was more unexpected fact which suggested involvement of a recombinational repair pathway in EMS-clastogenesis.

Some approaches were used to study the adaptive response to EMS in Drosophila germ cells: the treatment of adult males or females with adaptive mutagen doses (0.05-0.1 mM), whereas adult males were exposed to the challenger dose (usually, 10 mM). When males being fed by the mutagen in both doses, the SLRLs were scored at different spermatogenesis stages (spermatozoa and spermatogonia, alternative in repair capacity). When females being exposed to an adaptive dose and males being done to the challenger one, only sperm cell mutability was analyzed. Unfortunately, all our attempts to reveal the adaptive response were failed. These data support the assumption made by Guzder et al. (1991), that neither species of Drosophila alkyltransferase appear to be inducible.

Guzder S.N., Kelley M.R., Deutsch W.A. (1991) Mutat. Res.,Vol.255, No.2, 143-153.

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DROSOPHILA MUTAGEN-SENSITIVITY DEPENDING ON SPERMATOGENESIS STAGES AND MALE GENOTYPE

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Repair capacity of germ cells was studied by detecting mutagen-sensitivity of Drosophila germ cells at the different spermatogenesis stages depending on the male genotype (wild type, y or y ct v, y mei-9). Adult males were exposed to ethyl methanesulfonate (EMS). At the end of the feeding period they were mated to the virgin females Basc. For 10 days 5 broods were made and two of them (the first and fifth ones) were analyzed for sex-linked recessive lethal (SLRL) mutation rates.
The mutagen-sensitivity of spermatozoa was shown to be 5 times as much as spermatogonia in repair-proficient males of wild type. The differences in sensitivity to EMS between these spermatogenesis stages in yellow males were less pronounced. With deficiency of excision repair, males y mei-9^a responded to EMS action with similar SLRL frequencies at both pre- and postmeiotic stages. The data obtained indicate the lack of any repair pathway which seems to be associated to yellow locus.
1,4-dihydropyridine derivative glutapiron (GP) was used to modulate repair processes. GP reduced the frequency of EMS-induced mutations at spermatogenesis premeiotic stages in repair-proficient males, but not in sperm cells. In males y mei-9 this compound affected EMS-induced SLRL frequency neither in spermatozoa nor in spermatogonia. Thus, the antimutagenic actions of GP appear to be mediated by DNA repair.

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EFFECT OF PATERNAL SUBCHRONIC EXPOSURE TO X-RAYS ON DOMINANT LETHALITY AND F1 ABNORMALITIES

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Genetic damage produced in a sperm or egg could affect the next generation, for example by miscarriages, birth defects or childhood diseases. The usual form of human exposure is chronic exposure to low doses of mutagen.
Male Pzh:Sfis mice were exposed subchronically (8 weeks, 5 day/week) to low doses of X-rays. Daily doses were 0.05 Gy, 0.10 Gy and 0.20 Gy, during 8 weeks 2 Gy, 4 Gy and 8 Gy, respectively. Males were mated to unexposed females. They were killed on day 17 of pregnancy and examined for incidence of postimplantation deaths, dominant lethal mutations and gross congenital malformations in live foetuses.
The percent of pregnant females was decreased in the group exposed daily to 0.20 Gy. There were no effect on male infertility in control as well in experimantal groups. The cumulative dose of 8 Gy resulted in decrease of total and live implantations and induced over 50 % dominant lethal mutations. Also decreased of number of live foetuses was observed after exposure to 4 Gy. In all experimental groups increased the number of early deaths. There were no effect in congenital malformations and no effect in late deaths.
The results showed that subchronic irradiation of males to low doses of X-rays did not induce infertility of males nor abnormalities of their live foetuses, but caused significant increase of early deaths. In human it can risk of spontaneous abortion.

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DIFFERENTIAL CHROMOSOME BEHAVIOR IN MAMMALIAN OOCYTES EXPOSED TO THE TRANQUILIZER DIAZEPAM IN VITRO

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Aneugenic chemicals may differentially affect specific chromosomes (Xi et al., 1997). 25µg/ml of the sedative diazepam (DZ) delayed cell cycle progression, interfered with chromosome congression, and affected mitochondrial function. The drug induced aneuploidy and presegregation in mammalian oocytes spontaneously maturing in vitro (Yin et al, 1998). In this study, immunofluorescence staining for a-tublin was combined with FISH using chromosome specific probes to study the influence of DZ on the behavior of individual chromosomes in mouse oocytes. It was demonstrated that the chromosome X, unlike chromosomes 16 and 8, was more often displaced from the spindle equator in meiosis I arrested oocytes than expected by chance ($p<0.05$); DZ also caused predivision of chromosomes X, 16, and 8 (in 1.9, 2.3, and 3.0% of oocytes, respectively). Predivision has been suggested to be one cause of trisomy formation with advanced maternal age in the human. Therefore, DZ activity induces an aged phenotype and, as maternal age, may differentially predispose individual chromosomes in oocytes to errors in segregation during maturation.

Xi, L. et al. (1997) Induction of chromosome-specific aneuploidy and micronuclei in human lymphocytes by metabolites of 1,3-butadiene. Carcinogenesis, 18,1687-93.
Yin, H. et al. (1998) Diazepam induces meiotic delay, aneuploidy and predivission of homologues ad chromatids in mammalian oocytes. Mutagenesis, 13(6), 576-80.

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FREQUENCY OF ANEUPLOID SPERMATOZOA STUDIED BY MULTICOLOR FISH IN SERIAL SEMEN SAMPLES

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Three-probe fluorescence in situ hybridization was used to assess interindividual variation in the frequencies of aneuploidy for chromosome X, Y, and 8 in human sperm and peripheral lymphocytes. Fifteen healthy 20-year-old men were sampled on seven occasions over a two-year period. All the donors lived in the same community and were sampled at the same times. At the end of the study, slides were coded and randomized, and 10,000 spermatozoa per sample were scored.
Significant interindividual differences were observed for sex chromosome disomies and diploidies ($p<0.001$). Intraindividual differences were not found during the two year interval. The donors with consistently elevated sex chromosome disomes also exhibited significantly higher frequencies of sex chromosome aneuploidy in peripheral lymphocytes.
These individuals did not exhibit elevated sperm aneuploidy for other autosomes studied (8, 16, 18, 21). However, the frequency of 21 aneuploidy in spermatozoa was significantly higher than that of the other autosomes.
These data support the hypothesis that the sex chromosomes are more prone to aneuploidy in sperm as well as lymphocytes .
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CYTOGENETIC ANALYSIS AND COMET ASSAY IN MULTIPLE ORGANS OF MICE AFTER 4 WEEKS TREATMENT WITH MELPHALAN

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Within the collaborative framework of a ongoing project aimed at evaluating alternative methods for carcinogenicity testing, several specific endpoints have been measured in order to identify relevant short-term biomarkers wich may prove useful for mechanisistic interpretation.
In a preliminary toxicokinetic study prior to carcinogenicity testing, groups of three mice received 0.5, 1, 2 or 4 mg/kg body weight of melphalan by oral gavage once a week for 4 consecutive weeks. Several tissues were sampled for further analysis. The induction of micronuclei and of chromosomes translocations were assessed cytogenetically, whilst the induction of DNA single-strand breaks was assayed using an alkaline comet assay.
Data analysed so far for micronuclei in bone marrow cells sampled 24 h after the last treatment, show a marked increase at all doses, without an obvious dose relationship at higher dose levels. The alkaline comet assay performed on bone marrow, splenocytes, bronchoalveolar macrophages, liver and bladder cells harvested 2 hours after the last treatment. At an intermediated dose level there was a marked increase in DNA damage in liver cells, and a smaller, but statistically significant effect in bone marrow.
The positive control for comet assay performed on the same organs from mice treated with a single intraperitoneal injection of methylmethanesulfonate, showed a marked effect on all organs.
Analysis for translocation involving chromosomes 2 and 8 in the bone marrow cells is currently in progress.

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RADIATION INDUCED CHROMOSOME ABERRATIONS IN MOUSE SPLENOCYTES AS MEASURED BY C-BANDING PROCEDURE: EFFECT OF BRDU IN CELL CULTURE

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Radiation induced chromosome aberrations (CA) correlate with cancer incidence in exposed individuals and the occurrence of genetic diseases in their progeny. Lab mouse strains are established models for radiogenic risk assessment in humans, therefore it is necessary to pool new data on induction and longevity of CA in different mouse tissues in vivo and in vitro. One of the recently developed assays is the metaphase CA assay in mouse splenocytes. This is a presentation of our cytogenetic investigations on dose-effect relation in mice C57Bl. The experimental animals were gamma-irradiated with doses of 1, 2 or 3 Gy in vivo. BrdU was administered at a final concentration of 5 mM to part of the cell cultures at 19h post-stimulation. Chromosome preparations were done according to a standard protocol at 36 or 44 hours post-stimulation. They were stained by a C-banding procedure and analysed for CA (dicentrics, fragments, metacentric Robertsonian-like configurations, etc.). Our results show that the frequency of unstable CA in C-banded chromosome preparations increased with the dose with the exception of the metacentrics. By the C-banding procedure few stable aberrations (reciprocal translocations) could be visualized. BrdU enhanced the yield of CA in splenocytes from non-irradiated controls and radiation induced CA in exposed animals.

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EFFECT OF WR-2721 ON THE FREQUENCIES OF g-RAY INDUCED DICENTRICS AND TRANSLOCATIONS IN MOUSE SPLENOCYTES ANALYSED BY FISH

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Ionizing radiation induces two main classes of chromosomal aberrations in GO cells - dicentrics and translocations, implicated in both biological dosimetry and cancer risk assessment. Inhibitors of DNA repair have been shown to influence differentially the frequencies of radiation induced dicentrics and translocations. It has been proposed that different types of repair kinetics could be involved in the formation of these two types of exchange aberrations. The radioprotector WR-2721 [S-2-(3-aminopropylamino)ethyl phosphorothioic acid] is well known with its action as a free-radical scavenger in the repair of DNA damage. The purpose of the present study is to evaluate the effect of WR-2721 on the frequencies of radiation induced dicentrics and translocations. Mice were whole-body irradiated with three doses (1, 2 and 3 Gy) of ^{137}Cs X-rays. WR-2721 was administered 15 min prior to irradiation. Fluorescence in situ hybridization (FISH) with DNA libraries specific for chromosomes 1, 11 and 13 was employed to analyse the frequencies of translocations. Dicentric yields were analysed using C-banding. The results show that WR-2721 decreases the frequencies of both the dicentrics and translocations at all doses studied by a factor of 1.5 on average. There is no significant difference in diminution of dicentrics in comparison to translocations.

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COMBINATION OF MN TEST WITH FISH USING (TTAGGG)n TELOMERIC PROBE AS CENTROMERE DETECTION SYSTEM IN CHINESE HAMSTER V79 CELL LINE FOR ANEUPLOIDY VERSUS CLASTOGENICITY DISCRIMINATION

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In Chinese hamster V79 cells, mammalian (TTAGGG)n telomeric sequence is also present at centromeric regions. In the aim to evaluate the suitability of the (TTAGGG)n probe as centromere detection system in V79 cells for aneugens versus clastogens discrimination, FISH was combined with the MN test. A comparative study was performed using FISH on human lymphocytes with the human pan-centromeric probe. Known and suspected genotoxic compounds were tested in both V79 cells and human lymphocytes. The MN test in combination with FISH on human lymphocytes was performed as described previously in Elhajouji et al (1995). The MN test with V79 cells was performed according to standard protocols (Miller et al., 1997). The (TTAGGG)n probe was hybridized in situ with V79 cell preparations. On most centromeres (except the large four chromosomes) the signals were very strong and appeared as wide bands, while no hybridization spots were observed at the telomeres under the hybridization conditions used. Similar signal distribution in chromosomes of V79 cells was previously published by Simi et al (1998). A strong concordance was found between results obtained with the two MN systems (V79 cells and human lymphocytes). Indeed compounds that were detected as aneugens in human lymphocytes using the pan-centromeric probe were also classified as aneugens in V79 cells using the mammalian telomeric probe. Classic clastogens such as ethyl methanesulfonate and cyclophosphamide were also detected as clastogens in V79 cells. Despite the absence of signals from the four large chromosomes, the combination of FISH with (TTAGGG)n probe with the MN assay using V79 cell line was found to be a useful tool for aneugens versus clastogens discrimination.
1.) Elhajouji et al. (1995) Env. Mol. Mutagenesis, 26:292-304. 2.) Miller et al. (1997) Mutat. Res., 392:45-59. 3.) Simi et al. (1998) Mutat. Res., 397:229-233

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EVALUATION OF CHROMOSOME INSTABILITY INDUCED BY IONISING RADIATION IN HUMAN LYMPHOCYTES

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To understand the delayed effects of ionising radiation, human lymphocytes were irradiated in vitro with low energy protons or grays, and then grown either as massive or clonal cultures. Chromosome preparations from the g-irradiated cell population (0-4 Gy) were obtained after 9-28 days of culture. 15 days after irradiation with 2 or 3 Gy, the chromosome aberration frequency was still significantly higher than in the matched control culture. Aberrations were mostly unstable and chromatid-type, therefore formed de novo in the course of cell proliferation. From a different subject, clonal cultures obtained from lymphocytes irradiated with 2 Gy of g rays were scored for chromosome aberrations (300 metaphases per clone); heterogeneous values ranging from 1.33 to 11.30% were observed in 8 irradiated clones, while in non irradiated clones the frequency of aberrant metaphases appeared as low as expected in standard control cultures (1.00-3.67%). These data support the view that ionising radiation can induce chromosome instability in the progeny of irradiated cells (Kadhim et al., 1992). By chromosome painting, chromosome 9 was found to be more frequently involved than expected in chromosome breaks, at both early and late time intervals after irradiation. This observation suggests that chomosome 9 is intrinsically more prone to chromosome breakage events than other human chromosomes. To evaluate possible dose- and LET-depending effects, chromosome analysis is in course from a number of clones obtained after proton irradiation (0-2 Gy, 31 keV/µm) or (rays (0-2 Gy).

Kadhim M.A. et al. (1992) Nature 355: 738-740.

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CYTOGENETIC AND OXIDATIVE DAMAGE STUDIES IN HUMAN LYMPHOCYTES AFTER IN VITRO TREATMENT BY PLATINUM, RHODIUM AND PALLADIUM METAL SALTS

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A cytogenetic study carried out by our group showed both a clastogenic and an aneuploidogenic activity for some analysed Platinum (Pt), Rhodium (Rh) and Palladium (Pd) metal salts. In order to confirm the results of the previous study we tested the genotoxic potential of other Pt, Rh and Pd salts in different oxidation states: $PtCl_4$; $PtCl_2$; $(NH_4)_3RhCl_6$; $(NH_4)_2PdCl_4$; $(NH_4)_2PdCl_6$.We assessed mutagenic properties of the new metal salts by using the human lymphocyte micronucleus assay (HLMA) coupled with the fluorescence in situ hybridisation (FISH) using a digoxigenin-labeled pancentromeric DNA probe. Recent studies showed that toxicity of some metals is also due to oxidative damage induction by production of O_2 reactive species. To test the possible role of oxidative damage in genotoxicity of $PtCl_4$, $PtCl_2$, $(NH^4)_2PdCl_4$, $(NH_4)_2PdCl_6$, $RhCl_3$ and $(NH_4)_3RhCl_6$, we used an alkaline modified version of the single cell gel electrophoresis (SCGE). This version provides for the use of two enzymes involved in the excision repair, they are endonuclease III (endoIII) and formamidopyrimidine-glycosylase (FPG), which recognise and cut respectively oxidised pyrimidines and purines. Enzymes convert these lesions in DNA single strand breaks (SSBs), producing fragments, which migrate towards anode during electrophoresis, making up the tail of comet. Our results show a significant induction of MN for Pt and Rh compounds as compared to the control (Fisher's exact test). Pd (II) salt shows a weak significant MN induction at only one dose (500µM).Data concerning FISH do not show any significant difference in the frequency of MN C+ and MN C- for three tested compounds (two Pt salts and a Rh one). These results indicate that MN induction is due to both a clastogenic and an aneuploidogenic mechanism. Data concerning SCGE show a significant increase in the level of DNA oxidative damage only for the two Rh(III) compounds. All the other tested metal salts are not able to induce oxidative damage.

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DETERMINATION OF HIGHEST CONCENTRATION FOR ANALYSIS IN THE HUMAN LYMPHOCYTE IN VITRO CYTOGENETICS ASSAY

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Primary human lymphocytes are a commonly used cell type for regulatory in vitro cytogenetics (IVC) assays. Regulatory guidelines recommend that toxicity in these cultures is determined by measurement of the depression in mitotic index (MI) produced by a test substance relative to the concurrent solvent control. The highest concentration used for metaphase analysis should produce a depression in MI of at least 50%. Examination of a large number of data sets within this laboratory showed that depression in MI alone was the criterion for dose selection in only 6% of the cases. For the majority of test substances cytotoxicity, as manifested by changes in chromosome morphology (e.g. fuzzy or indistinct chromatids) was the main criterion for choice of highest concentration. In some cases this coincided with an appropriate reduction in MI. In most cases, 50% MI depression was not achieved but analysis of higher concentrations was not possible due to the observed cytotoxic effects on the chromosome morphology. We recommend that the highest concentration analysed in these assays be chosen based on an assessment of both chromosome morphology and MI depression.

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ELIMINATION KINETICS OF CHROMOSOMAL ABERRATIONS (CAs) FOLLOWING CHEMICAL- AND RADIATION EXPOSURES

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Conventional chromosome staining in cytogenetic biomonitoring is still a useful method, especially in cases when the follow-up of people exposed to genotoxicants was started a long time ago, and the kinetics of CAs must be studied in progress. However, the half-life of peripheral blood lymphocytes (PBLs), and thus the time-course of the elimination of CAs has not been fully discovered. This question is important when we are looking for the relationship between high frequency of CAs and risk for cancer. The half-life of PBLs was studied mostly when ionizing radiation was the inductor of aberrations, and approximately 1500 days were considered as the time of disappearence of 50% of dicentric and ring aberrations. It is still not known

1.How long CAs, induced by chemical mixtures causing so many kinds of DNA damage as radiation does, persist

2.Whether the localization and volumes of irradiation have any role in CA production

3. Whether the dose of irradiation has any significance in the persistence of CAs.

As models, testicular cancer patients treated either with vinblastin+cisplatin+bleomycin, or irradiated in pelvic area in 2 different volumes (7500 cm^3 and 5000 cm^3) with 41Gy or 26 Gy, and thyroid cancer patients irradiated with 50 Gy in the neck- and upper mediastinum area (1500 cm^3), were analyzed and compared for CA frequencies immediately, and up to 6 years after the termination of treatments. The chemotherapy, containing a radiomimetic component bleomycin, induced 5-times less, but more long-lasting CAs than the radiation therapy caused in any of the above cases. Radiation doses and the volumes did not, but the localization of the irradiated area (neck+upper mediastinum with the remaining function of the thymus in the adulthood!) affected both the yield and the elimination rate of CAs. The half life of PBLs seems to be shorter than one year following radiation therapy, while, though rather equivocal, it is about 4 years after chemotherapy. The elimination rate of CAs is probably mostly influenced by the degree of irreparable DNA damage and cell death as it is seen in irradiated patients. This phenomenon occurs not only in PBLs, but also in precursor cells, where the mechanisms of aberration formation, reparation and apoptosis differ significantly from those induced by chemical mixtures.

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CYTOGENETIC ASPECTS OF ASTHMA

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Nowadays, bronchial asthma (BA) is considered to be an infectious - allergic disease, in the development of which a great role is played by hereditary factors. The aim of this work is to determine the frequency of chromosomal aberrations and acrocentric chromosomal associations (ACA) of peripheral blood lymphocytes with a combined examination of clinical, immunological and biochemical characteristics among 95 BA patients. The control group is comprised of 40 healthy persons of the same age range. In the period of asthmatic attacks before the beginning of medical treatment, the average frequency of lymphocytes with chromosomal aberrations has constituted 8.91% with fluctuations in certain patients ranging from 3.62 to 1.80% (1.63 - 1.92% in healthy persons). Among the chromosomal aberrations, we have observed tghe prevalence of the acentric fragments (solitary and paired ones) 54.07 and 38.67% respectively. The aberrations of the exchangeable type have comprised 6.09 - 8.32%.The interrelation between the degree of mutagenic activity and the character of certain allergens have been revealed, which testifies to the endogenic mechanisms of injuries arising in the proces of allergy and inflammation development. The objective markers of BA seriousness have served the determination of ACA which coincided with the development of immunological reactions. We have also traced fluctuations of lymphocyte frequency with a varying number of ACA; lymphocytes with both ACA (20.5 ±1.31) occur more frequently, while solitary ones - with 6 acrocentrics. As a result of the treatment therapy we have traced an ACA decrease ranging from 4.56 ±0.12 to 3.91 ±0.11.The data received correlate with the main clinical indicators (dyspnea attacks frequency and duration decrease), improvements of living conditions.

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CYTOGENETIC INVESTIGATIONS OF BALKAN HONEYBEE ECOTYPES

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Comparative biometric and ultrastructural studies of the chromosomes of three Balkan honeybee ecotypes were undertaken. We investigated following ecotupes: Balkan, Timok and Syenichko - Peshterski ecotype. The conducted biometric analyses point to the existence of differences in relative chromosome length and centromere index (arm ratio) between investigated ecotypes' chromosomes. These results motivated us for further - ultrastructural investigations, because we wanted to detect some of mechanisms which might be the cause of the biometric chromosomal differences. Ultrastructural chromosome analyses of the three studied indigenous honeybee ecotypes showed that biometric differences of chromosomes are caused by ultrastructural variations i.e. by differences in the distribution of euchromatin and heterochromatin.

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APOPTOSIS IN NERVOUS SYSTEM AND IN PLACENTA

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With help of the special histological methodes and electronic microscope we have studied qualitative and quantitative changes in the simple reflex arch's component parts (motor and sensor neurons, nerve fibers of the ventral and dorsal roots and median nerve) on the level C_5 - T_1 of the 143 dog's spinal cord during postnatal period of the ontogenesis. We have distinguished that apoptosis is the main cause of the motor and sensor neurons and nerve fibers death during early period of the postnatal development (from day of the birth to 2 years). During this period the number of the motor neurons in the spinal cord decreased from 7795±197,0 to 6839±207,3, the number of the sensor neurons in the spinal ganglions - from 27734±317,7 to 24874±235,1, the number of the nerve fibers in ventral roots decreased from 26810±423,2 to 21370±299,7, in dorsal roots - from 65795±916,9 to 56360±770,4, in median nerve - from 30760±454,0 to 24835±329,7
In the adult dogs (from 2 to 8 years) apoptosis of the nerve cells and fibers has gone out slowly. In the old (10 years) and senil (15 years) animals apoptosis provoked 63-72% and 25-32% cell's death among common cell's death. Apoptosis had has place in cellular componentes (0,6-2,2%) of the placenta during physiological pregnancy and delivery. Apoptosis had has higher degree (5-24%) in cellular componentes of the placenta in the women with pyelonephritis and gestosis
Apoptosis of the nerve cells and placenta cells had has specific structural manifestation. Nuclear chromatine of that cells had has appearance of the dense granules, which were condensed on the inner surface of the nuclear membrane. Cytoplasmic organelles: endoplasmic granular net, ribosomes, mitochondrii, Golgy's apparatus were dense and disorganized. Than such cells wrinkled and fragmented, formed aggregates of the apoptotic bodies, which were consisted from some cytoplasmic organelles and chromatin granules, framed with membrane. Such bodies were took up by the phagocytes and were eliminated quickly over the tissue. So, apoptotic cells never provoked inflammation.

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ISOLATION OF PRECURSORS OF 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE(MX) FROM DOMESTIC SEWAGE

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MX has been received much attention because of the strong genotoxic potency and the presence in chlorine-disinfected tap water. The origin of MX is mainly humic substances containing humic acid and fulvic acid. MX was also isolated from swimming pool water and river water. In this study, the isolation and identification of other precursors of MX were tried by using domestic sewage including several kinds of tea and bean. Each sample was treated with sodium hypochlorite and the reaction solution was applied to HPLC. The fraction having the same retention time to that of MX was methylated with CH_3OH-H_2SO_4 and then submitted to GC-MS-SIM analysis. MX was detected from green tea, black tea, pu-ehr tea, Rooibos tea, soybean and adzuki bean. Several polyphenols containing catechins and flavonoids might be potent precursors of MX. Therefore, the treatment of domestic sewage is essential task to prevent the formation of MX.

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COB(I)ALAMIN USED FOR TRANSALKYLATION OF METHYL GROUPS IN DNA FORMED IN VITRO AND IN VIVO BY N-METHYL-N-NITROSOUREA

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The alkylating properties of phosphotriesters was confirmed through transfer of the alkyl group to both standard nucleophiles and the supernuclophilic cob(I)alamin (Haglund et al., 1997; Haglund et al., 2000). The latter was shown to transfer methyl and ethyl groups from model phosphotriesters within minutes at room temperature. This method is now being developed for transalkylation of adducts to DNA phosphates. DNA alkylated in vitro and in vivo by N-methyl-N-nitrosourea was enzymatically (DNAse, venom phosphodiesterase and alkaline phosphatase) digested for the formation of nucleosides and phosphotriesters (Swenson and Lawley, 1978). The DNA digest was transalkylated with cob(I)alamin showing both disappearance of the phosphotriesters and formation of the expected level of methyl-cobalamin. Alkyl-cobalamins in the transalkylation solution can be directly analysed by liquid chromatography/mass spectrometry. This work shows the usefulness of cob(I)alamin for transalkylation of DNA-phosphate adducts for determination of degree of alkylation in DNA.

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ABOUT THE POSSIBILITY OF COMPLEX GENETICO-PSYHOLOGICAL HUMAN STUDY RESULTS OVERALL ESTIMATION

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Complexity of the interpretation of multifactorial studies results is connected with the necessity to generalize different dimensioned and different ranged test data. The correct comparison could be done only in frame of one test, so usually it can get only qualitative evaluation of complex study results such as, for example, - the situation in Town X is totally better than the one in Town Y. In this investigation for more correct multifactorial data comparison we used the approach consists in additional ordering of all of the individual data and in special ranked data summation. In frame of this approach nomograms for interpolation of individual data into dimensionless indexes were plotted for each test before ordering. Each nomogram was plotted in Dekart' coordinates when axis included possible diapason of test data variability and ordinate was ranged in interval from 0,0 to 1,0. The sense of this interpolation was in primary data transformation into indexes having qualitative estimation. So, ordinate magnitudes 0,8-1,0 means "normal", 0,0 - 0,2 means "very bad". For example, chromosome aberration level below 2% was determined as "normal" and level >10% as "very bad". All of the ordering calculation were made using primary data of human genetico-psychological studies of 8 groups of workers of 3 Russian towns - totally 128 persons. In these investigations were tested 2 - 7 parameters for each person - chromosome aberration and UDS DNA level in blood lymphocytes, urine mutagenesity in Ames' test, hair heavy metal contents, social adaptivity and alarming levels. As a result of calculations we quantitatively compared human genetic hazard in 3 regions of Russia. We suppose that this kind of ordering could be applied for all kind of complex investigation from clinical and epidemiological to hygiene, biochemical, physiological, psychological and so on. And there is one limitation - for the analysis can be used only data which has correct qualitative interpretation.

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TESTICULAR CELLS FROM RATS REPAIR OXIDATIVE DNA LESIONS

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Cellular DNA is damaged by xenobiotics but also by the endogenous formation of reactive oxygen species leading to oxidative DNA damage of which 8-OH-guanine (8-oxo-G) is particularly important. Testicular cells may be protected against some xenobiotics by the blood-testis barrier. Hence, the requirement for nucleotide excision repair (NER) may be limited - in accordance with our recent observation that NER is deficient in testicular cells from both rats (Brunborg et al., Mutat.Res. 342, 157-70, 1995; Jansen et al., unpubl.) and humans (Olsen et al., unpubl.). On the other hand, oxidative damage is evidently induced and must be eliminated. We have hence studied the relevant DNA glycosylase enzymes involved in base excision repair of oxidative DNA lesions in vitro and in cells ex vivo.
Testicular cells were isolated from Wistar rats by enzymatic tissue digestion. From such cells protein extracts were prepared and used in an in vitro incision assay with a defined oligonucleotide as a DNA substrate. The results show that 8-oxo-G lesions are excised by the testicular cell extracts, at equal or higher efficiency compared to extracts from somatic rat hepatocytes.
Functional repair in whole cells was also studied. For this purpose, testicular cells were treated with a photoactive chemical (Ro 19-9786) plus cold visible light. Thereafter cells were incubated in medium without the drug for 0-3 hours and ultimately analysed for DNA lesions with alkaline elution. The alkaline elution process included treatment of some samples with a formamido pyrimidine glycosylase (fpg) extract prepared from an fpg overproducing E. coli strain. The data show that very few direct DNA breaks (or alkali labile sites) are induced by the Ro 19-9786 + light treatment alone, but frequent breaks were detected when the fpg enzyme treatment was included. This strongly indicates a specific formation of oxidative (8-oxo-G) DNA lesions that are recognised and incised by the fpg enzyme. During the 3-hour repair period, about 50% of the initial fpg-sensitive lesions were removed. In conclusion, oxidative DNA lesions are repaired efficiently in rat testicular cells.

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INVESTIGATIONS INTO THE ADDUCT FORMATION POTENTIAL OF SELECTED PHARMACEUTICALS

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^{32}P-postlabelling is a well established method in the monitoring of environmental mutagens. We are interested in how far this method can be used for the early identification of carcinogenic pharmaceuticals with a genotoxic mechanism of action. For that purpose we established a list of pharmaceuticals used with a molecular weight in the range of 100 to 600 g/mol to assure detection in the postlabelling assay. This list was narrowed down for compounds which were on the German market for at least 29 years, thus being able to obtain data on potential carcinogenicity in the epidemiologic literature. From this list of compounds (n=734), we selected those which showed structural alerts for genotoxicity or carcinogenicity in an in silico prediction system (DEREK, Lhasa Ltd., U.K.) or for which already indications of carcinogenic effects existed in the literature (van den Eden and Friedman, 1995). Finally by using the mentioned selection criteria a list of 56 pharmaceuticals was created, which we expected to have a genotoxic or carcinogenic effect.
Up to now we were able to test 14 compounds from this list. Eleven compounds (including such well known drugs as paracetamol, chloral hydrate, clindamycin and isoniazid) were negative in the postlabelling assay despite the fact that for some of these positive mutagenicity data are existing. Three compounds were positive including tamoxifen, hydro-cortisone and potassium canrenoate. On the basis of these preliminary data, we will assess the predictivity of the ^{32}P-postlabelling method for drug screening of mutagenic and carcinogenic effects.

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APPLICATION OF THE UMU-ASSAY FOR MEASURING THE GENOTOXIC POTENCY OF COMPLEX MIXTURES

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Hazard characterization of complex mixtures of environmental pollutants is often based on a classical compound by compound analysis of the individual constituents. In many cases, such an approach is insufficient, because the identity, concentration and toxicity of all individual chemicals are unknown, let alone the mixture toxicity. Two examples are presented in which the umu-assay (Oda et al, 1985) is used to determine the genotoxic potency of a complex mixture of pollutants.
In the first example (De Maagd et al, submitted), the suitability of the umu-assay for the purpose of Whole-Effluent Assessment (WEA) has been compared to two other primary DNA damage tests, i.e. the SOS-chromo-test and the Mutatox test. Comparison was based on the criteria quantitative sensitivity, reproducibility, fitness for quantitative use and practicality and cost-efficiency. For WEA purposes, the umu-test seemed best fit for genotoxicity assessment due to its higher quantitative sensitivity compared to the SOS-chromo-test. The Mutatox test was regarded unfit due to its low reproducibility and hard to interpret dose-response curves.
In the second example (Hamers et al, 2000), the genotoxic potency of airborne particulate matter (APM) was tested, which was collected by high-volume sampling at highway and background sites under different wind directions. The umu-assay enabled discrimination between APM collected down-wind and up-wind from the highway and between APM collected at different sites. The results further confirmed earlier observations that APM from northern (marine) wind directions is relatively clean in The Netherlands, whereas APM from eastern wind directions has varying composition and related genotoxic potency on different sampling days. This variation may be caused by incidental passages of polluted air from the industrial Ruhr-area in Germany.

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PRELIMINARY STUDY ON CHANGES IN p53 AND p21 GENE EXPRESSION IN HUMAN LYMPHOCYTE CULTURES EXPOSED TO STYRENE-7,8-OXIDE

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Styrene is used in the manufacture of many commercially important products like rubber, plastics and several resins. The highest human exposures to styrene take place by inhalation during the production of fibreglass reinforced plastics. The genotoxic effects detected in workers from such factories are attributed to its first metabolite styrene-7,8-oxide (SO), produced by cytochrome P450 monooxygenases.
In this study human lymphocyte cultures from 4 donors were exposed to 50 mM and 200 mM SO, 1% DMSO being the control. In order to determine the expression of some genes involved in apoptosis and cell cycle regulation, aliquots of the lymphocyte cultures were taken at six different time points. Total mRNA was extracted in each one of them and RT-PCR was carried out to analyse the expression of the genes p53 and p21.
In addition, the cytokinesis block proliferation index (CBPI) has been calculated using the cytokinesis-block micronucleus assay, as an indication of cell proliferation kinetics. Moreover, morphological assessment of apoptotic cells in each 72 h culture has been performed.
In our results, a high interindividual variation in the expression of the studied genes was observed. Expression curves obtained for the two genes, together with the data of CBPI and apoptotic cells scored, suggest that exposure to high levels of SO may induce a delay in the cell cycle, probably directed to allow repair systems to act over the genotoxic damage produced, more than drive cells towards programmed cell death.

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PRACTICAL INTERPRETATION OF THE REVISED OECD GUIDELINES FOR GENOTOXICITY TESTING. THE RECOMMENDATIONS OF THE INDUSTRIAL GENOTOXICITY GROUP

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Revised guidelines for the core genotoxicity assays were published by the OECD in 1998. As with all regulatory guidelines there are areas where legitimate alternatives are allowed. However, there are also points which are open to a number of interpretations. The IGG convened a panel of experienced genetic toxicologists to review the guidelines to make recommendations on best scientific practice to aid in consistent interpretation.
General points covered by the review included: use of historical control data; definition of maximum dose level; evidence of absorption in in vivo assays; use of a single sex in in vivo assays; repeat assays in in vitro assays; testing of insoluble materials.
Assay specific recommendations included the following topics. Bacterial mutation assay: strategy for anti-bacterial compounds; choice of strains. In vitro cytogenetics assay: polyploidy; test design; cell cycle measurement. Mammalian cell gene mutation assay: colony size data for L5178Y; design of repeat test. Erythrocyte micronucleus test: choice of dose route. In vivo UDS: study design
The details of the panel's recommendations will be given in this presentation to enable them to be discussed within the genetic toxicology community.

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