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Abstracts and participant list

"Environment and human genetic disease - Causes, mechanisms and effects"
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EEMS Fritz Sobels Award Lecture

Traditional approaches and new perspectives in the epidemiology and prevention of mutation-related diseases

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Hippocrates, the Father of Medicine, described the influence of the environment on human health 24 centuries ago. In fact, the man–environment binomial is also known as the “Hippocrates’ dyad”. In those times, the complementary roles of preventive medicine and curative medicine were already delineated, as implicit in the first words of the Hippocratic oath: “I swear by Apollo physician and Asclepius and Hygeia and Panacea and all gods and goddesses...”. Of the Asclepius’ daughters, Panacea was the goddess of therapy, while Hygeia was the goddess of health and preventive medicine. An important turning point occurred by the end of the 19th century with the discovery of bacteria and thereafter of other pathogens. By acting on all three elements (man, environment, and etiological agent), it was possible to decrease the mortality for infectious diseases dramatically. Thus, the 20th century was characterized by a revolution of the epidemiological scenario, with chronic degenerative diseases becoming the leading causes of death in the population (1). Although no generalization can be made, mutations in somatic cells play a key role in the pathogenesis of several diseases. For instance, the use of molecular endpoints enabled us to establish an association between DNA damage and not only cancer (2) but also atherosclerosis (3), degenerative heart diseases (4), and oxidative stress–related glaucoma (5). Moreover, we investigated similar alterations during critical periods of life, such as the perinatal period (6) and ageing (7). The most obvious approach to prevention of cancer and other mutation–related conditions is to minimize exposures at risk. There is growing evidence that a complementary strategy is to render the organism more resistant to the action of noxious agents. This strategy, referred to as chemoprevention, involves the use of cancer preventive drugs and dietary principles. Genomic and postgenomic methodologies have provided new tools to assess both efficacy and safety of chemopreventive agents (8,9), to evaluate the interindividual susceptibility to risk factors, and to predict the response to protective factors. With the precious support of Professor Frits Sobels, we devoted three Mutation Research special issues to this subject (10–12).

EEMS Young Scientist Award Lecture

Recombination repair and a treatment for BRCA2 tumours

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DNA repair and damage response pathways are activated on encountering replication roadblocks in mammalian cells. Here, we report of the chk1 protein activating recombination repair by direct binding to and phosphorylation of RAD51 on the threonine 309 position. We report of homologous recombination pathways and of a repair pathway including the poly(ADP-ribose) polymerase (PARP) involved in replication repair. We propose that, in the absence of PARP-1, spontaneous single strand breaks collapse replication forks and trigger HR for repair. We further show that BRCA2 deficient cells, as a result of their deficiency in HR, are acutely sensitive to PARP inhibitors, presumably because resultant collapsed forks are no longer repaired. Thus, PARP-1 activity is essential in HR deficient BRCA2 mutated cells. We exploit this requirement to specifically kill BRCA2 deficient tumours by PARP inhibition alone. Treatment with PARP inhibitors is likely to be highly tumour specific since only the tumours (which are BRCA2−/−) in the BRCA2+/- patients are completely defective in HR. The use of an inhibitor of a DNA repair enzyme alone, in the absence of an exogenous DNA damaging agent, to selectively kill a tumour represents a new concept in cancer treatment.
Symposium 1
Role of environmental genotoxins in human carcinogenesis
S1
Human cancer from environmental exposures: the epidemiological evidence

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Environmental carcinogens include outdoor and indoor air pollutants, as well as soil and drinking water contaminants. An increased risk of mesothelioma has been shown among individuals experiencing residential exposure to asbestos, while results for lung cancer are less consistent. Results of good-quality studies have investigated lung cancer risk from outdoor air pollution based on measurement of specific agents tend to show an increased risk in the categories at highest exposure, with relative risks in the range 1.5. A causal association has been established between involuntary smoking and lung cancer, with a relative risk in the order of 1.2. Radon is another carcinogen present in indoor air, with relative risk in the order of 1.06 for exposure at 100 Bq/m3. In several Asian populations, an increased risk of lung cancer results among women from indoor pollution from cooking and heating.

There is strong evidence of an increased risk of bladder, skin and lung cancers following consumption of water with high arsenic contamination; results for other drinking water contaminants, including chlorination by-products, are inconclusive. The evidence of an increased risk of cancer from exposure to other environmental agents is inconclusive.

Although the estimate of the global burden of environmental cancer results in less than 1%, these cancers concentrate in subgroups of the population; furthermore, exposure is involuntary and can be, to a large extent, avoided.

S2
Role of environmental genotoxins in human carcinogenesis: evidence from DNA adducts

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DNA adducts are useful markers of carcinogen exposure, providing an integrated measurement of intake, activation and delivery to the target macromolecule in target tissues. Accessible surrogate tissues, such as blood cells, provide the means to investigate occupational or environmental exposure in healthy individuals. Such exposure, e.g. to polycyclic aromatic hydrocarbons, has been demonstrated in several industries and in defined populations, respectively, by the detection of higher levels of adducts. Adducts detected in many tissues of smokers are at higher levels than in non-smokers, although the magnitude of the elevation does not predict the magnitude of the risk. While such associations do not demonstrate causality, they do, importantly, lend plausibility to observed associations between smoking and cancer. However, there is still resistance to the notion that such monitoring can inform, rather than merely confirm, epidemiological investigations. Interestingly, smoking was recently causally linked to cervical cancer after years of being considered a confounding factor; yet smoking-related adducts have been known to be present in cervical epithelium for some time. In some prospective studies, elevated adduct levels have been found in individuals who subsequently developed cancer compared with individuals who did not. The potential for biomarker measurements such as DNA adducts to provide answers to the origin of the many cases of human cancer for which an environmental cause is suspected needs to be exploited more fully in future epidemiological studies.

S3
A cell immortalization assay to investigate mutation signatures in the human p53 gene sequence

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To test hypotheses on the origins of p53 mutations in human tumors, novel strategies are needed for generating mutation spectra experimentally. We developed an assay employing Hupki (Human p53 knock-in) mouse embryonic fibroblasts (HUFs) to examine p53 mutations induced by two human carcinogens, benzo[a]pyrene (BaP), a major tobacco smoke genotoxin, and aristolochic acid (AA), the causative agent of Chinese herbal nephropathy. Immortalized cells from HUF primary cultures exposed to
BaP harbored G to T p53 mutations characteristic of smokers’ lung tumors, strengthening the link between tobacco smoke and cancer mutations. Cell lines derived from AAI-exposed HUF cells harbored primarily A to T mutations, consistent with the presence of persistent AAI-adenine adducts found in DNA of exposed individuals. The HUF assay can be employed to examine mutation signatures in the human p53 gene of other exogenous agents or endogenous risk factors.

S4
Metabolic genes as markers of biochemical susceptibility: A mechanistic approach

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Metabolic genotypes have been implicated as cancer risk factors. However single locus variants evaluated in case control studies, tend to show very weak penetrance, often with borderline significance. More fruitful and biological meaningful approaches to the study of variation in genetic susceptibility include the use of specific target populations, the use of biochemical or molecular endpoints instead of disease associations, and the analysis of complex genotypes composed of multiple loci or haplotypes. In each case, mechanistic considerations can allow for hypothesis testing related to the known function of genetic variants in metabolic pathways. Examples of this approach include studies of infant leukaemia in patients without a chromosomal rearrangement, sensitivity analysis for metabolites of benzene and benzopyrene DNA adducts in exposed workers, and the use of cluster analysis to identify candidate multiple locus genotypes. In these and many other examples, the role of single and multiple locus genotypes of polymorphic metabolic genes can be seen to be significant and informative regarding both mechanisms of genotoxicity and the origins of differential genetic susceptibility to the effects of environmental agents.
Symposium 2
Biomarkers and molecular epidemiology: Present state and future trends
S5
The use of biomarkers to predict cancer risk

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The ultimate biomarker is supposed to reflect both exposure and the risk of developing the adverse health effect, e.g. risk of cancer. Several biomarkers for genotoxic exposure have been developed and their role as predictors for cancer risk has been explored in epidemiological studies. Bulky carcinogen-DNA adducts has for more than a decade been used to assess the exposure for genotoxic compounds present in ambient air and specific occupational settings. Bulky adducts are not a clear indicator for exposure as no association between the level of specific carcinogenic PAH and PM2.5 has been established. This has been explained by the fact that adduct level is a function of exposure and individual susceptibility, i.e. metabolic capability and repair. In several cohort studies a high DNA adduct level has been associated with an increased risk of cancer, e.g. lung cancer. One of the biological consequences of adducts are induction of mutations and chromosomal damage. Whereas these biomarkers cannot be linked to specific exposure, mutation in P53 gene in blood cell DNA and chromosomal aberration have been linked to increased cancer risk.

S6
Applicability of transcriptomics in biomonitoring studies

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There is increasing interest in the development and application of biomarkers for the purpose of risk assessment among human populations exposed to adverse agents as present in the food and the environment. A major area for application is environmental carcinogenesis. In this respect, various cytogenetic biomarkers of genotoxic risk have been repeatedly applied in classical molecular epidemiology studies in which their usefulness has been demonstrated. However, these markers in general monitor a single genotoxic event while not being very specific. Therefore, there is a need for new biomarkers which should provide more generic and more mechanistic information on adverse health effects in humans. It is widely felt that these may be developed by the emerging subdiscipline of toxicogenomics. By transcriptomic analysis, the genome-wide response to genotoxic exposure may be harvested from target as well as from surrogate tissue thus yielding insight in molecular effects on multiple, mechanistically relevant genetic pathways. An approach to generate gene expression profiles from model environmental carcinogens in human blood cells as biomarkers for genotoxic events will be described, and first results of transcriptomic analysis of lymphocytes from carcinogen-exposed human populations will be presented.

S7
Genomic imprinting: A marker of environmental epigenetic effects.

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Genomic imprinting is an essential mechanism in mammals that gives rise to allelic expression of genes depending on the parental origin of the allele. Some eighty imprinted genes have been identified in mice and humans to date. Many of these play important roles in extra-embryonic and embryonic development. In humans, deregulation of genomic imprinting is involved in growth- and neuro-developmental syndromes. Aberrant imprinting is frequently observed in cancer as well. The control of imprinted genes is highly complex and involves DNA methylation and covalent histone modifications (1). Different kinds of environmental stress, such as embryo culture and assisted reproduction technologies, may perturb the somatic maintenance of these epigenetic marks. Also diet may disrupt the maintenance of epigenetic marks, and can thus heritably affect gene expression. Studies in the mouse suggest that genomic imprinting is particularly susceptible to perturbation in the trophoblast. Possibly, this could be explained by the finding that, in contrast to the embryo and the adult animal, imprinting in the placenta does not involve
DNA methylation (2).


S8
Molecular epidemiology: new rules for new tools?

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Molecular epidemiology uses molecular and genetic markers in the study of the environmental and genetic determinants of cancer and other diseases. The potential advantages associated with these biomarkers are manifold and include: a) increased sensitivity and specificity to carcinogenic exposures; b) more precise evaluation of the interplay between genetic and environmental determinants of cancer; c) earlier detection of (pre)cancerous changes; d) characterization of disease subtypes-etiologies patterns; e) evaluation of primary prevention measures. These, in turn, may translate into better tools for etiological research, individual risk assessment, and, ultimately, primary and secondary prevention. The traditional classification in markers of exposure/dose, markers of damage/effect, and markers of susceptibility is not straightforward, and often difficult to apply in practice. An area that has not received sufficient attention concerns the validation of these markers, which can be considered as a specific type of intermediate, or surrogate, endpoints (SE). Validation of a SE is the demonstration that it possesses the properties required for its use. The principles underlying the validation of a SE, and the associated problems, underwent remarkable developments and discussion in therapeutic research. However, the challenges posed by the application of these principles to epidemiological research, where the basic tool for this validation (i.e., the randomized study), is seldom possible, have not been thoroughly explored. The main differences between clinical and epidemiological SE, together with the methodological implications, are discussed, and some suggestions for further research are provided.
Symposium 3
Epigenetics and genetic instability
S9  
Epigenetic alterations causing genetic lesions in human cancer

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Tumor cells frequently present genetic alterations affecting genes involved in cellular proliferation and death. Methylation is the main epigenetic modification in mammals and abnormal methylation of the CpG islands located in the promoter region of the genes leads to transcriptional silencing. Examples include the p16INK4a, p15INK4B, p14ARF, Von Hippel-Lindau (VHL), the estrogen and progesterone receptors, E-cadherin, death associated protein (DAP) kinase and the first tumour suppressor gene described, retinoblastoma (Rb). Moreover, epigenetic lesions may also drive genetic lesions in cancer. For instance, the promoter hypermethylation of the DNA mismatch repair gene hMLH1, the DNA alkyl-repair gene O(6)-methylguanine-DNA methyltransferase (MGMT), the detoxifier glutathione S-transferase P1 (GSTP1) and the familial breast cancer gene BRCA1 increase the frequency of genetic lesions such as microsatellite instability, G to A transitions, steroid-related adducts and double-strand breaks in DNA. The environmental impact on the epigenetic status makes the story even more fascinating because proposes alternative ways to explain genetic alterations in human cancer.

S10  
Chromatin Remodelling and Nucleotide Excision Repair in the yeast Saccharomyces cerevisiae

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We employ the MFA2 mating type locus as a model to examine relationships between NER and chromatin remodelling. MFA2 is repressed in α haploid cells and transcribed in a mating type. With α cells chromatin immunoprecipitation shows Lys9 and/or Lys14 of histone H3, but not the relevant sites of histone H4 in two nucleosomes at the repressed MFA2 promoter are hyperacetylated following UV. This histone hyperacetylation diminishes as repair proceeds. Accompanying this, chromatin in the promoter becomes more accessible to restriction following UV and returns to the pre-UV state gradually. UV-related histone hyperacetylation and chromatin remodelling in this promoter are dependent on Gcn5p and partially on Swi2p respectively. Deletion of GCN5, but not of SWI2 impairs only the local repair of DNA damage. These post-UV chromatin remodelling events do not require damage recognition by Rad4p or Rad14p. However, in rad4/rad14 NER defective mutants the remodelled chromatin does not return to its pre-UV status. Intriguingly, although Gcn5p also has a role in transcription of MFA2 in a cells (the gcn5δ has only 20% of wild-type MFA2 mRNA), when Gcn5p functions during repair of the repressed MFA2 there is no transcription. These experiments suggest a whole spectrum of gene products exists that can influence DNA repair and whose activity will be important for efficient NER.

S11  
(Phospho)-proteomics of cellular stress responses

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Acute and chronic cellular stress activates a variety of signal transduction pathways that mediate the cellular response to injury. Such a response directs the cell to either repair and survival or cell death (apoptosis). We have been using proteomics analysis to study in more detail the altered expression and in particular the phosphorylation status of proteins in the cellular stress response to toxicants. Protein tyrosine kinases are an important class of kinases that are crucial in cellular responses to the environment as well as in the development of cancer. These kinases mediate the phosphorylation of tyrosine residues in a variety of proteins thereby (in)directly affecting either gene expression or other cell biological events. The use of highly specific antibodies that recognize phosphorylated tyrosine residues in combination with proteomics technology allows the detailed identification of post-translational modified proteins in the cellular stress response. We have used this approach to better understand the molecular mechanism of toxicant-induced perturbations of cellular interaction (cell-cell and cell-ECM) in relation to cell death of
renal epithelial cells. So far our results indicate that phospho-proteomics can play a prominent role in unravelling relevant signalling pathways and/or events that regulate the cellular response to harmful insults.

**S12**

**Control of DNA Damage Tolerance by Ubiquitin and SUMO**

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Tolerance to replication-blocking DNA lesions is achieved by the ubiquitination of PCNA, the eukaryotic processivity clamp for replicative DNA polymerases. While monoubiquitination in response to treatment with genotoxic agents induces mutagenic lesion bypass by means of damage-tolerant polymerases, multiubiquitination facilitates an error-free damage avoidance mechanism that uses the genetic information encoded by the undamaged sister chromatid. During S phase in the absence of exogenous DNA damage PCNA is modified by the ubiquitin-like protein SUMO. As both modifiers target the same site on PCNA, an antagonistic relationship had previously been postulated; however, the physiological consequences of sumoylation are still poorly understood.

We have now investigated the relevance of SUMO for the function of PCNA in the absence and presence of DNA damaging agents. We present genetic and biochemical evidence that the apparent negative effect of SUMO on lesion bypass is mediated by the helicase Srs2p, which affects genome stability by suppressing unscheduled homologous recombination. We show that Srs2p physically interacts with sumoylated PCNA, which contributes to the recruitment of the helicase to replication forks. Our findings suggest a mechanism by which SUMO and ubiquitin cooperatively control the choice of pathway for the processing of DNA lesions during replication.
Symposium 4
Systems biology and genetic toxicity
S13
Understanding stress response networks in yeast: models of toxicity for arsenic or iron

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Arsenic is a nonmutagenic carcinogen affecting millions of people. The cellular impact of this metalloid in Saccharomyces cerevisiae was determined by profiling global gene expression and sensitivity phenotypes. Expression data was mapped to the yeast network of 20,985 protein-protein/protein-DNA interactions. This analysis indicated that arsenic likely channels sulfur into glutathione for detoxification, leads to indirect oxidative stress by depleting glutathione pools, and alters protein turnover via arsenation of sulfhydryl groups on proteins. Phenotypic profiling mapped to the metabolic network composing of all biochemical reactions in yeast, and revealed two significant networks: 1) shikimate; and 2) serine, threonine, and glutamate biosynthesis. These pathways were upstream of differentially expressed ones, indicating that transcriptional and phenotypic profiling implicate distinct, but related pathways. With a similar approach, we have studied the cellular impact of mitochondrial iron overload by using yeast as a model for Friedreich’s Ataxia. This disease is characterized by a decreased expression of the mitochondrial iron binding protein, frataxin. It has been shown that progressive loss of frataxin in yeast leads to a rapid increase in mitochondrial iron, oxidation of mitochondrial proteins, and mitochondrial and nuclear DNA damage. Mapping of global gene expression data onto the yeast interactome revealed several transcriptional activators (ATF1, HAP4, and CAD1), mitochondrial ribosomal proteins, TCA proteins and oxidative phosphorylation proteins, as important centers of activity.

S14
Gene-interactions in DNA damage response pathways identified by genome-wide RNAi

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Recent progress in reverse genetic approaches, principally RNA interference (RNAi), now allows the analysis of gene function on a genomic scale in an animal system. We set out to identify the complete repertoire of genes that protects an animal’s genome against environmentally and/or endogenously induced DNA damage by performing genome-wide RNAi screens using the multi-cellular model organism C. elegans for phenotypes associated with DNA damage repair. To increase throughput we first improved RNAi technology such that worms were cultured in liquid in a 96-well format, with each well containing a different RNAi clone. Subsequently, by systematically inactivating almost all genes encoded by the C. elegans genome, we identified many genes that protect the genome against DNA damages that occur upon DNA transposition, ionizing radiation and/or treatment with the alkylating agent MMS; some of these knockdowns also have defects in the apoptotic and/or cell cycle response.
In addition, we have developed several transgenic reporters (in the worm) that allow the visualization of repair of specific DNA damages (such as double-stranded breaks) and monitor the stability of specific instable DNA sequences (micro-satellites and G-tracts).
Location, location, location and toxicity modulation


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Alkylating agents represent endogenous and environmental toxicants that can cause damage through the direct transfer of alkyl groups to nucleophilic centers found in cellular macromolecules. We have performed high throughput screens using a library of ~4,700 *Saccharomyces cerevisiae* gene deletion strains to identify proteins that modulate the toxicity of the alkylating agents. These high throughput screens, which we have termed genomic phenotyping, allow for a systematic analysis of gene products that modulate toxicity and have been used to show that over 1,400 proteins respond to insult from the alkylating agent methyl methansulfonate (MMS). To help interpret the data, we have designed and implemented computational sub-cellular localization and protein interactome mapping techniques to recognize statistically significant trends and identify MMS-toxicity modulating protein pathways and protein complexes (i.e. networks). In addition, these computational approaches have been used to assemble a global picture detailing toxicant exposure. Specifically, we have identified cellular locations containing a statistically significant over representation of toxicity modulating proteins and for MMS they include the nucleus, microtubule, vacuolar membrane and endosome. We also identify toxicologically-important protein complexes and pathways operating in these locations and demonstrate that pathways associated with DNA repair, cell cycle checkpoints, transcription, macromolecular trafficking and vacuole function can now be counted among the many responses that prevent carcinogen-induced toxicity.

Predicting radiation toxicity by gene expression profiling

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Severe late toxicity is the dose-limiting factor in radiotherapy. Two years after treatment approximately 5% of prostate cancer patients suffer from grade III complications (over-responders), while a similar fraction of patients do not develop any adverse effects (non-responders). There is increasing evidence that genetic predisposition may play an important role in the variation of the radiation response. Our aim is to detect genetic risk factors for late radiation toxicity to individualize therapy. We hypothesize that genetic differences underlying the difference in development of radiation toxicity will be reflected in the cellular response of normal tissue cells to irradiation. We performed retrospective gene expression profiling using commercial microarrays. In total the expression profiles of ex vivo irradiated and mock irradiated lymphocytes of 20 prostate cancer patients of either group were investigated. Using a random validation strategy, we confidently classified the responder status of the majority of the patients. In order to get a general insight in the pathways of stress responses elicited by exposure to DNA damage agents we used mouse ES cells as a working model. We will show that gene set enrichment analyses reveals the connectivity of identified differentially expressed genes in a time and dose dependent way.
Workshop1
Acute DNA damage responses
W1
Nucleotide excision repair: from DNA lesion to biological effects

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Nucleotide excision repair (NER) is a multiprotein repair system capable to remove a variety of DNA lesions from the genome. Two NER sub-pathways have been identified dealing with repair of DNA lesions in bulk chromatin (global genome repair, GGR) or with repair of lesions in the transcribed strand of active genes (transcription-coupled repair, TCR).

Over the past years our laboratory has investigated the mechanisms underlying DNA lesion recognition by NER in expressed and nonexpressed DNA sequences by a variety of approaches. The rate of global genome repair is dictated by the helix distorting properties of DNA lesions, whereas transcription-coupled repair is thought to be triggered by stalling of RNA polymerase II at the site of DNA lesions and subsequent recruitment of the NER repair machinery. In this presentation I will focus on the assembly of the GGR and TCR NER complex at sites of DNA damage in mammalian cells and on the contribution of TCR and GGR to repair, mutagenesis and cancer in transgenic mice with defined deficiencies in the NER sub-pathways employing different genotoxic agents. The key role of TCR in alleviating the adverse effects of DNA damage is illustrated by a 10-fold higher resistance of TCR proficient mice to acute toxic effects of DNA damage compared to TCR deficient mice. Expression of GGR (and to lesser extent TCR) is critical for countering cancer in mice. The results will be discussed in relation to induced DNA damage, inhibition of transcription, restoration of DNA damage-inhibited RNA synthesis and apoptotic response.

W2
Base excision repair: mechanisms and biological relevance

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One specific feature of base excision repair (BER) is its apparent redundancy. DNA glycosylases present broad substrate specificity and different DNA polymerases and DNA ligases can perform the resynthesis and ligase step, respectively. It has been suggested that this redundancy might underscore functional differentiation. We provide evidence that DNA polymerase β operates mainly during a pre-replicative BER whereas replicative DNA polymerases are likely to be involved in the repair of lesions that are formed or persist at replication forks. To increase the complexity of this repair pathway, proteins outside BER may increase the efficiency of the process. We show examples of nucleotide excision repair components that participate to oxidative damage repair by stimulating the activity of specific DNA glycosylases.

Growing evidence indicates that the BER repair intermediates, more than the modified bases, are the lesions responsible for the majority of the adverse effects of DNA damaging agents. A provocative hypothesis has now emerged in which BER enzymes, more than controlling the integrity of the genome, may create unintended consequences. Adaptive increases in the major AP site endonuclease, APE1, and 3-methyladenine DNA glycosylase have been shown to generate microsatellite instability in chronic inflammation (Hofseth et al., 2003). Similarly, we detect up-regulation of BER genes in association with microsatellite instability in an inflammation related cancer (i.e. stomach cancer). Future research should address the mechanistic basis of this phenomenon that might be of great relevance in human pathology.

W3
The role of homologous recombination in the repair of DNA damage in mammalian cells

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In the last few years it has been found that homology-directed repair (HDR) of DNA damage forms an important component of survival in somatic mammalian cells. For example, the disruption of key HDR genes such as RAD51 in mice leads to early embryonic lethality. More surprisingly, a similar phenotype is found for loss of genes that facilitate RAD51 activity, such as the RAD51-like genes XRCC2, RAD51L1, and RAD51L3, and the human breast cancer susceptibility genes BRCA1 and BRCA2. Recent structural studies suggest that BRCA2 regulates HR activity through binding to RAD51 self-assembly sites, but the biochemical functions of most of the HDR proteins are not fully understood. HDR is particularly important
in recovery from damage during DNA replication; however, research in my laboratory has also shown the
importance of the HDR proteins in the proper segregation of chromosomes in mitosis (Griffin et al. 2000
Nature Cell Biol., 2:757-761). Loss of XRCC2, for example, leads to chromosomal instability and
aneuploidy, and we have linked this phenotype to abnormalities of centrosome function (Deans et al. 2003
Cancer Res., 63: 8181-8187). Centrosome disruption distinguishes HDR-defective cells from those lacking
other pathways of DNA break repair, such as non-homologous end joining. Current knowledge of the
relative importance of these DNA damage-response pathways and their connections to genetic instability
and cancer will be highlighted.

W4
DNA Repair in Drug Resistance and Apoptosis: the O6-methylguanine response

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DNA damaging agents, including environmental carcinogens and many anticancer drugs, induce apoptosis
that may counteract mutagenesis. To identify specific DNA lesions triggering apoptosis, we investigated
cells exhibiting various DNA repair defects. Data show that critical DNA lesions such as O6-methylguanine
(O6MeG) and misrepaired N-methylation lesions trigger apoptosis in rodent cells by activating the
mitochondrial damage pathway. We propose that DNA double-strand breaks (DSBs) are distal apoptosis-
inducing lesions arising from primary lesions during DNA replication. For O6-methylating agents, an
additional requirement is mismatch repair giving rise to DSBs that trigger Bcl-2 decline, caspase-9/-3
activation and DNA degradation. This can occur independent of p53. Since p53 knockout fibroblasts are
more sensitive to methylating agents (and UV light) than p53 wt cells, p53 appears to play a protective
rather than a pro-apoptotic role in this cell system, presumably by stimulation of DNA repair. This is in
contrast to lymphoblastoid cells (e.g. TK6 and WTK1 which are wt and p53mt respectively), human
lymphocytes and brain tumor cells in which p53 participates in apoptotic signaling. In lymphocytes MNNG
provokes O6MeG-triggered apoptosis at low dose range only in stimulated cells whereas at high dose
levels apoptosis is also induced in resting cells, presumably due to non-repaired N-alkylations. This
contrasts ionising radiation which induces more apoptosis in resting lymphocytes. O6MeG-triggered
apoptosis in lymphocytes and tumor cells was preceded by p53 and Fas upregulation, indicating the
involvement of death receptors. Overall, the data support a model of O6MeG-triggered apoptosis in which
MGMT, MMR, receptor and mitochondrial apoptotic proteins interplay. The role of ATM, XRCC2, DNA-PK
and the MAP kinase pathway in the O6MeG response will also be discussed.

W5
The disappearance of the phosphorylated form of H2AX (γH2AX) monitors DNA double strand breaks repair (DSBs) only at low levels of DNA damage

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After induction of DSBs, histone H2AX phosphorylation is rapid and extensive producing foci that can be
visualized microscopically. It is now admitted that there is a strong, even direct, correlation between the
number of DSBs and the number of γH2AX foci. On the opposite, it remains open whether H2AX
disappearance is an event that reflects the kinetics of DNA DSB repair since apparently contradictory
results were obtained from different studies. To answer this question, γH2AX disappearance was studied
after exposure to increasing doses of IR and calicheamicin γ1 (CLg1) in DNA DSBs deficient and proficient
cell lines. Our results indicate that the disappearance of γH2AX reflects the DNA DSBs repair activity only
for low doses of CLg1 (40 pM) and IR (2 Gy) independently of the experimental approach used
(immunofluorescence, flow cytometry or Western Blot). At higher cytotoxic doses, the disappearance of
γH2AX is no longer correlated to DNA repair and our results suggest a threshold effect in DNA damage
signalling. Finally, in DNA repair proficient cells, the early decrease in foci intensity observed after
exposure to 2 Gy without significant changes in the global γH2AX phosphorylation levels suggest that loss
of foci is mediated not only by γH2AX dephosphorylation but also by redistribution of γH2AX in chromatin.
Workshop 2
Genetic biomarkers of biological effects and cancer risk
W6
DNA adducts as biomarkers of cancer risk

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Much work has been conducted examining whether the presence of carcinogen DNA-adducts in human tissues is a marker of cancer risk. Promising results have been found for aflatoxin-DNA adducts as a predictor of hepatocellular carcinoma, with the presence of aflatoxin-N7-guanine in urine being associated with increased risk. However, research on “bulky” DNA adducts indicative of exposures to aromatic compounds has been less promising. Most studies have investigated smoking related cancers, although three have investigated breast cancer, and have measured adduct levels in tissues among the cases at cancer diagnosis. Only two studies, both of smoking related cancers, have had prospective designs with adducts measured in biological samples collected years prior to diagnosis. Associations between adduct levels and smoking related cancers have been seen among current smokers but not among non- or ex-smokers. It is not clear whether this represents biological phenomena and provides mechanistic insight or reflects measurement issues and attenuation of observed associations in non-smokers. In breast cancer two studies that measured adducts in breast tissue have found case-control differences, but no associations were seen in two analyses that measured adducts in white blood cells. In general, it is not clear to what extent case-control differences in non-prospective studies reflect tumor effects on adduct levels. Issues that need to be addressed include: possibilities of reverse causality, substantial batch variability and incorrect interpretation of statistical analyses of antecedent exposures and biomarker data.

W7
Micronuclei as biomarkers of cancer risk

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Agents that induce breaks in DNA or disrupt the mitotic machinery lead to the formation ofacentric chromosome fragments and whole chromosomes that fail to engage with the spindle and are not included in the main nuclei. The lagging chromosomes form micronuclei and gene dosage in the main daughter nuclei is altered. Micronuclei are also induced by deficiencies in micronutrients such as folate and vitamin B12 which leads to chromosome breaks as well as hypomethylation of cytosine which causes centromere dysfunction and chromosome loss. Because micronuclei provide a measure of DNA mis-repair they have also been used successfully as a method of identifying individuals with inherited defects in DNA repair such as truncation mutations in BRCA1 and BRCA2 breast cancer genes. The cytokinesis-block micronucleus assay is an efficient “cytome” method for scoring a comprehensive array of chromosomal instability (micronuclei, nuclear buds, nucleoplasmic bridges, reduced apoptosis) that is commonly observed in cancers. Recent studies have shown that the micronucleus assay “cytome” is not only sensitive to factors that are associated with increased cancer risk such as ageing, deficiency in micronutrients, smoking, alcohol, radiation and other environmental carcinogens but can also successfully detect gene-nutrient and gene-toxin interactions that affect chromosomal instability. In addition the results of recent prospective studies (HUMN project and EU Cancer Risk Biomarkers programme) have shown that the micronucleus index in lymphocytes predicts cancer risk prospectively which is the ultimate test of a predictive biomarker.
Chromosomal aberrations and SCEs as biomarkers of cancer risk

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Previous studies have suggested that the frequency of chromosomal aberrations (CAs), but not of sister chromatid exchanges (SCEs), predicts cancer risk. We have further examined this relationship in European cohorts comprising altogether almost 22,000 subjects. The results confirm the CA-cancer association and indicate that it is not explained by undetected cancer. Chromosome-type CAs may have a more pronounced predictive value than chromatid-type CAs, showing a particular association with gastrointestinal cancers. Various genetic polymorphisms of xenobiotic metabolism, DNA repair and folate metabolism affect the level of CAs and might collectively contribute to the cancer predictivity. Uncontrollable variation in SCE level seems to invalidate SCEs as a biomarker of cancer risk.

(CancerRiskBiomarkers, QLK4-CT-2000-00628)

Chromosomal aberrations and cancer risk: a multicentric study from Central Europe

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Chromosomal Aberrations (CA) in Peripheral Blood Lymphocytes (PBL) of healthy individuals might represent a marker of susceptibility to cancer, based on the concept that genetic damage in PBL reflects similar damage in cells undergoing carcinogenesis. We conducted a study based on cohorts from Croatia, Hungary, Lithuania, Poland and Slovakia to study the association between CA levels and (specific) future cancer risk. 8,430 subjects were included and followed from the date of the cytogenetic test until the date of death, cancer diagnosis, emigration, 85th birthday or end of follow up. Total CA was defined as the number of cells with aberrations, excluding gaps, per 100 cells and categorized in tertiles by laboratory. 224 subjects were diagnosed with cancer or died from a malignant tumour. The relative risk was 1.55 (95% confidence interval 1.07, 2.26) in the medium tertile and 1.71 (95% confidence interval 1.17, 2.50) in the upper tertile, as compared with the lower tertile. No significant effect modification by either sex (p=0.26), smoking (p=0.66), age at test (p=0.86), or time since test (p=0.55) was observed. The analysis of specific types of cancer showed a relationship between CA frequency and cancer of the stomach and the colorectum, while for lung cancer the results were only suggestive of an association. No increased risk of head and neck cancer, lymphatic and haematopoietic neoplasms, breast or skin cancer was detected. The present study showed that a high frequency of CA in PBL is associated with an increased risk of cancer.
W10
Molecular markers of tumor progression and prognosis established in head and neck cancer

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An efficiency of head and neck cancer treatment is low. Occurrence of multiple primary tumors (MPT), local metastasis and tumor relapse are the main causes of treatment failure. An identification of genetic markers of all the listed symptoms is a task for molecular epidemiology and experimental oncology studies.

The efforts are focused on identification of a specific genotype predisposing MPT formation, differentiation between MPT and metastasis, discovering of entering into micrometastasis, identification of genetic background of tumor tendency to relapse. Altogether, a battery of tests is under studies to find the goals.

The main own results are as follows:
1. An amplification of 11q13 and other chromosome rearrangement involving this region determined together with 3q gain is a reliable marker of poor prognosis.
2. Cytogenetic findings indicate for the regions of oncogenes amplification and tumor suppressor genes deletion to be studied further for loss of heterozygosity. The latter technique appears to be well informative concerning disease prognosis.
3. Comparative genomic hybridization appears to be a proper method to determine cancer cells clonality and to differentiate between metastasis and MPT.
4. Genotyping of selected genes coding carcinogen metabolizing enzymes and DNA repair enzymes did not show any distinct genotype predisposing to MPT.
Workshop 3
Air pollution and cancer
W11
A prospective study on air pollution and lung cancer in Europe: THE GENAIR PROJECT

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GenAir is a case-control study nested within the EPIC cohort, aiming at studying the relationship between air pollution or ETS and cancers of the bladder, lung, oral cavity, pharynx, larynx or leukemia, all newly diagnosed after recruitment. Also deaths from respiratory diseases (COPD and emphysema) were identified and included since they are suspected of being associated with air pollution or ETS exposure. Only never smokers or ex-smokers since at least 10 years have been included. We have matched three controls per case for exposure assessment and the analysis of questionnaire data, and two controls per case for laboratory analyses. Matching criteria were gender, age (plus or minus 5 years), smoking status (never/former smoker), country of recruitment, and time elapsed since recruitment (months). Laboratory analyses include cotinine, bulky DNA adducts, hemoglobin adducts, a number of metabolic polymorphisms, DNA repair polymorphisms, and mutations in plasma DNA. Exposure to air pollution has been assessed by experts at the Utrecht University. Air pollution - We found a non-statistically significant association between lung cancer and residence nearby heavy traffic roads (odds ratio=1.46, 95% confidence interval, CI, 0.89-2.40). Exposure data for single pollutants were available for 197 cases and 556 matched controls. For NO₂ we found an odds ratio of 1.14 (95% CI 0.78-1.67) for each increment of 10 μg/m³, and an odds ratio of 1.45 (1.07-1.95) for concentration changes of 30 μg/m³ (preliminary analyses). No clear association was found with other pollutants. DNA adducts - Leukocyte DNA adducts were analysed blindly using the nuclease P1 modification of the 32P DNA postlabelling technique. The intensity of adduct patterns was generally stronger in the chromatograms of healthy non-smokers who developed a lung cancer in the following years in comparison with the other samples. The observed adduct profile has been previously described among subjects environmentally exposed to air pollution. Adducts were associated with the subsequent risk of lung cancer, with an odds ratio of 1.86 (95% CI 0.88-3.93) when comparing detectable versus non-detectable adducts. The association with lung cancer was stronger in never smokers (OR=4.04; 1.06-15.42) and among the younger age groups. After exclusion of the cancers occurring in the first 36 months of follow-up, the OR was 4.16 (1.24-13.88). A positive association was found between DNA adduct levels and the concentration of O₃.

W12
Biomarkers of dose, effect and susceptibility to PAHs in a population exposed to environmental pollution: The EXPAH project.

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Biomarkers of exposure, effect and susceptibility were compared in populations exposed to different levels of polycyclic aromatic hydrocarbons (PAHs), associated with air pollution in Czech Republic, Slovak Republic and Bulgaria. Personal exposure monitoring and stationary monitoring of ambient air were carried out. Particulate matter extracts were analysed for PAHs. The relationship between exogenous DNA damage caused by exposure to PAHs and oxidative DNA damage was evaluated. ³²P-Postlabelling was the most revealing biomarker of exposure, and demonstrated an increase in DNA damage associated with PAH exposure. FISH was more sensitive than conventional cytogenetic methods as a biomarker of effect. The frequency of translocations by FISH related to levels of a B(a)P-like DNA adduct. Oxidative DNA damage induced by the exposure was less predictive as a biomarker, and 8-hydroxy-2'-deoxyguanosine was negatively correlated with total and B(a)P DNA adducts. Genetic susceptibility factors were identified for all biomarkers.

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W13
Mechanisms of genotoxicity of ambient air particles

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Ambient particulate matter (PM) comprises a heterogeneous mixture of substances including ultrafine carbonaceous particles, metals and organic matter, whose composition varies according to the predominant source of particles, season and prevailing weather. Recent epidemiological studies have demonstrated an association between PM and lung cancer, but the responsible mechanisms remain largely unknown, and crucial causal constituents are still to be identified.

In vitro and in vivo studies with coarse and fine PM sampled at various locations and seasons as well as model PM composed of ultrafine carbon black which has been coated with polycyclic aromatic hydrocarbons and/or metals have revealed a likely role for multiple constituents and mechanisms in particle genotoxicity. PM-associated transition metals, as well as particle-cell interactions lead to reactive oxygen species formation and subsequent induction of oxidative DNA adducts and strand breakage. Furthermore, PM samples can carry mutagens into the lungs, which may lead to formation of bulky DNA adducts. Apart from these genotoxic effects, PM has also been shown to activate signalling pathways (NFκB, AP-1) which are associated with inflammation, cell cycle regulation and proliferation. Importantly, ultrafine particles (<100nm) represent a major component of PM, which despite a negligible contribution to the over all particle mass, represent an extremely large surface area. This surface area and associated surface reactivity has been held responsible for adverse effects of ultrafine particles, possibly including lung tumourigenesis.

W14
In vitro effects of PAH exposure in liver cellular models – a combination of genotoxic and nongenotoxic events

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We studied toxic modes of action associated with genotoxicity and tumor promotion in immature progenitor liver WB-F344 cells exposed to 80 PAHs, methyl-PAHs or complex environmental mixtures and compared the results with effects of PAHs in established hepatoma cell models. PAH genotoxicity and their potency to activate aryl hydrocarbon receptor (AhR) led to distinct patterns of apoptotic and proliferative responses in both liver progenitor and hepatoma cells. Several PAHs, including dibenzo[a,l]pyrene, benzo[a]pyrene, benzo[g]chrysene and 7,12-dimethylbenz[a]anthracene were strong genotoxins in WB-F344 cells; formation of DNA adducts corresponded with high increase in S-phase, phosphorylation of p53 and apoptosis induction. A majority of PAHs and methyl-PAHs induced various nongenotoxic modes of action, including induction of CYP1A1/1A2/1B1 and AKR1C9 mRNA expression, the release of the WB-F344 cells from contact inhibition, and/or inhibition of gap junctional intercellular communication. Effects of extracts of complex environmental mixtures, such as airborne PM10 particles and river sediments, included both AhR activation and inhibition of GJIC, however, these effects were not additive when compared with chemical data on concentrations and in vitro toxic potencies of individual PAHs. [Supported by the EU project ModelKey, grant No. SSPI-CT-2003-511237-2.]

W15
DNA-adduct and tumor formation by the urban air pollutant 3-nitrobenzanthrone in rat.

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3-Nitrobenzanthrone (3-NBA) has been identified as a direct-acting mutagen and genotoxic agent. In this study we evaluate both the in vivo genotoxicity and the carcinogenicity of 3-NBA in a situation corresponding to inhalation; i.e. a combined short-term and lifetime study with intratracheal instillation in female F344 rats. DNA-adduct formation, a marker for the primary effect after single instillation, showed
four major DNA-adducts with a rapid increase and peak after 2 days, followed by a decline. No DNA-adducts above background level were observed after 16 days. The highest DNA-adduct formation was observed in lung, followed by kidney, whereas liver contained 1/10 of the levels of DNA-adducts found in lung. The tumor study was performed with repeated instillations (0.5 mg 3-NBA/ml) to a total dose of 1.5 and 2.5 mg, respectively. Squamous cell carcinomas were found after 7-9 months and after 10-12 months in the high and low dose group, respectively. In conclusion, 3-NBA forms DNA-adducts in the lung immediately after intratracheal administration. Due to an efficient DNA-repair, almost all DNA-adducts were eliminated after 16 days. Tumor formation in two dose groups was observed in a dose-dependent manner with squamous cell carcinomas as the predominant tumor type.
Workshop 4
Nutrigenomics and dietary modulation of carcinogenesis
W16
Vegetable-induced gene expression changes in anticarcinogenic pathways in human and mouse colon.

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Abundant epidemiological evidence exists that colon cancer is influenced by environmental factors, with diet as a major determinant. Particularly, vegetable consumption is associated with reduced colon cancer risk. Vegetables contain a wide variety of substances with anti-carcinogenic properties, and some of them may influence gene activities. In order to investigate this, a human dietary intervention study was conducted to identify genes differentially expressed by vegetables in vivo in human colon epithelium by microarray technologies. By comparison of pre- and post-intervention values and also the experimental dose groups, genes deregulated by vegetables were identified. Many modulated genes are known to be related to (colon) carcinogenesis. Almost all the effects can be mechanistically linked to cellular processes that explain either prevention of colorectal cancer risk by high vegetable intake or increased colorectal cancer risk by low vegetable intake. In addition, mouse studies were performed, in which for many genes a significant dose-dependent, though not linear, effect was detected. Furthermore, several of these genes were also regulated by one or more of the specific vegetables. Also here, the altered gene expression can indeed explain reduced cancer risk. Few genes, however, are similarly affected in human and mouse.

W17
Effects of dietary factors on genomic stability in humans: results of controlled intervention trials

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The SCGE technique has been used in human intervention trials to identify DNA protective dietary constituents. Comparisons of DNA-damage in lymphocytes before and after interventions provide information on DNA lesions which can be detected with SCGES (single-/double strand breaks), restriction enzymes enable to monitor endogenous formation of oxidized purines and pyrimidines, additionally also alterations of the ROS sensitivity and repair can be investigated. About 45 studies with vitamins and foods have been published, in half of them protective effects were found. We used SCGE-assays to investigate the effects of Brussels sprouts, coffee and of a common spice (Rhus coriaria). In the latter case, it was possible to identify gallic acid as the active principle whose antioxidant activity in humans was found 50-fold stronger as that of vitamin C. In all our interventions, protection of endogenous formation of oxidized bases and a decrease of the ROS sensitivity was detected which could be attributed to scavenging effects and induction of ROS protective enzymes (SOD). Recently we showed that SCGE assays also enable also detection of antigenotoxic effects towards dietary carcinogens such as PAHs, HAs and other food carcinogens (acrylamide, heavy metals). In the case of Brussels sprouts we found strong protection towards the most abundant HA, PhiP; which could be attributed to inhibition of SULT required to activate this amine; in the case of coffee, protection towards BPDE could be explained by induction of GST pi which detoxifies the diol. Results of animals studies showed that these effects are paralleled by cancer protection.

W18
Chemopreventive potential of tea

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The anticarcinogenic activity of tea, against a number of chemical carcinogens, has been amply demonstrated in animal models, but epidemiological studies have failed to provide unequivocal evidence.
for its effectiveness in humans.

One of the principal mechanisms of the anticarcinogenic activity of tea is inhibition of the initiation stage of carcinogenesis, by modulating the activity of xenobiotic-metabolising enzyme systems, such as the cytochromes P450 and the conjugation systems, so as to favour the deactivation of chemical carcinogens at the expense of activation. Exposure of rats to tea as their sole drinking fluid influenced the excretion of mutagens in the urine, following treatment with the dietary carcinogen 2-amino-3-methylimidazo-[4,5-f]quinoline (IQ). However, no such effect was observed when decaffeinated black tea was used, emphasising the role of caffeine.

Similarly, a cross-over study was conducted in human volunteers to establish whether black tea intake, as part of an otherwise normal diet, modulates the metabolism of heterocyclic amines consumed in the form of well-cooked beef burgers, as exemplified by the excretion of mutagens in urine. Mutagens were extracted from urine using blue rayon, and mutagenic activity was determined in the Ames test, employing the S. typhimurium O-acetylase over-expressing YG1024 bacterial strain. Volunteers consumed three well-cooked beef burgers, whereas a concurrently cooked fourth burger was analysed for mutagenic activity, so as to relate urinary mutagenicity to the intake of heterocyclic amines.

W19
Relevance of apple consumption for protection against DNA damage induced by hydrogen peroxide in human lymphocytes

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We performed a single-dose placebo-controlled cross over study designed to investigate the ability of apple fruit to protect against peroxide-induced damage to DNA in humans. Six healthy, non-smoking male volunteers (age: 29.7 ± 4.5; Body mass index 23.5 ± 2.0 kg/m²) were placed for 2 days on an antioxidant-poor (AP) diet. After 48 h of AP diet the volunteers were required to consume 800 ml of homogenized unpeeled apple or placebo (500 ml water) and blood samples collected 0, 3, 6 h post-consumption while still on AP diet. From blood of each subject collected at the various time-points we measured: total antioxidant activity in the plasma; reactive oxygen species (ROS) generation in human lymphocytes treated in vitro with hydrogen peroxide (H₂O₂); micronuclei (MN) formation in lymphocytes incubated in plasma and challenged with H₂O₂. Preliminary results indicated that there was a strong inhibition of H₂O₂-induced micrornucleated cells by the plasma samples collected 3h (~50%) and 6h (~40%) after apples consumption as compared to plasma samples collected after placebo consumption. MN frequency induced by H₂O₂ was significantly influenced by plasmatic total antioxidant activity (r=0.961, P=0.039) and by the increase of intracellular ROS formation (r=0.954, P=0.045). These findings can be useful to understand the relationship between the antioxidant capacity and DNA damage in humans.

W20
Effects of grape extracts from Greek varieties of V. vinifera and plant polyphenols on mutagenicity caused by reactive oxygen species in Salmonella typhimurium TA102 cells.

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The detection and identification of agents, known as antimutagens, which inhibit mutagenesis, are considered important since DNA damage is believed to be a crucial step in a variety of diseases and degenerative processes such as ageing. For example, oxidative DNA damage caused by reactive oxygen species (ROS) such as hydroxyl radical (OH•), superoxide radical (O₂•-) and hydrogen peroxide (H₂O₂) has been used as a biomarker for cancer risk in humans. Especially, those antimutagen agents present in human dietary products are of great importance because they could act as preventive substances. Thus in the present study, the antimutagenic efficacy of methanolic and aqueous grape extracts, from two Greek varieties of Vitis vinifera (Assyrtiko Santorini and Mandilaria Santorini), polyphenol enriched fractions of these extracts and plant polyphenols present in them, was tested against oxidative mutagenicity caused by bleomycin (blm) and H₂O₂ using the Ames test in Salmonella typhimurium TA102 cells. Mutagenicity caused by blm was inhibited by methanolic and aqueous grape extracts. On the other hand, some of the polyphenol enriched fractions enhanced blm-induced mutagenicity, while others had no effect. No protection was also observed for the plant polyphenols resveratrol, quercetin, catechin, epicatechin and
ellagic acid. The induction of mutagenicity by \( \text{H}_2\text{O}_2 \) was prevented by methanolic grape extracts, while aqueous grape extracts, polyphenol-rich fractions and resveratrol caused an increase in mutation frequency. Epicatechin, protocatechuic acid and gallic acid did not affect mutagenicity. These results indicate that the protection against ROS mediated mutagenicity may be one of the mechanisms by which grape extracts exert their chemopreventive and anticarcinogenic activity observed by in vitro and in vivo studies. Furthermore, the pro-oxidant action of some polyphenols and polyphenol-rich fractions could account for the cytotoxic and apoptosis-inducing properties of plant polyphenols against cancer cells under experimental conditions specified.
Workshop 5
Environmental genotoxins and children's health
W21
Trends in childhood disease

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There is clear evidence of increasing rates of asthma in various countries during the last decades, although rates in some countries may now have stabilized or even decline as recent UK data indicate. Data also suggest that rates of other atopic disorders such as upper respiratory and food allergies as well as atopic dermatitis may be increasing. Although an increase in the frequency of neurodevelopmental disorders such as autism and attention deficit disorder has frequently been discussed, the limited data in this field does not justify such a conclusion.

While geographic heterogeneity regarding reproductive outcomes is apparent, global trends have not been identified. Interpretation of the available information is hampered by inconstant diagnostic criteria over place and time and the lack of population-based surveillance data, which makes it impossible to ascertain trends in actual disease frequency.

Data indicate that developed countries have a gradually increasing incidence in leukemia with a corresponding drop in the incidence of lymphoma. Increases in brain tumor frequency may be related to the development and wide application of new diagnostic capabilities, rather than a true change in the incidence of malignant disease. With a better prognosis for childhood cancer survival, secondary cancers following chemotherapy appear to be increasing.

A wide range of environmental factors (nutrition, lifestyle, socio-economic status, behavior choices, industrial compounds) is thought to have an impact on children’s health. Industrial chemicals seem to play a minor role.

W22
Children’s susceptibility

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Children exhibit a higher daily intake of air and food per kg body weight, and as such they may have a proportionally higher intake of airborne and food borne toxic compounds than adults. The air intake of a resting infant of an age less than one year is twice that for an adult.

In general, children have higher levels of physical activity and spend more time outside and for that reason they inhale more outdoor air. Behaviour of children is age-specific and the differences in behaviour between adults and infants are obvious; e.g., in ability to remove themselves from a noxious environment, hand-to-mouth behaviour and playing on the floor.

Children are considered more susceptible than adults since they exhibit physiological differences related to their size, growth, development and immaturity of organs, resulting in alterations of target organ and central nervous system susceptibility. Eighty percent of alveoli are formed during postnatal development. Hence, changes in the lungs continue, and exposure to harmful air pollutants during childhood has an important impact on lung development. Children exhibit immaturity of some metabolic pathways and their ability to detoxify and excrete some chemicals differs from that of adults. Children show interindividual susceptibility varying with age. Genetic damage early in life and susceptibility of children exposed to genotoxins may influence risk of carcinogenesis as indicated by data from pilotprojects in the CHILDRENGENONETWORK (QLK4-CT-2002-02198).

W23
Evidence of effects of ambient environment on children’s health

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The molecular epidemiology methods were used to analyze the impact of air pollution in pregnancy outcome studies in the Czech Republic. Organic compounds adsorbed to air particles (PM10) induced DNA adducts and embryotoxicity in in vitro studies. The carcinogenic polycyclic aromatic hydrocarbons
(carc-PAHs) were mostly responsible for the genotoxic activity, contributing to 45-50% of all DNA adducts induced by these complex mixtures. The placental bulky DNA adducts have been studied in relation to metabolic genotypes CYP1A1, EPHX, GSTM1 and NAT2. DNA adducts were determined by 32P-postlabeling assay. DNA adducts in placentas were affected by air pollution, smoking, genotypes, vitamin C levels. Higher DNA adducts were observed in nonsmoking mothers delivering children with IUGR (intrauterine growth retardation). In the Pregnancy Outcome Project, an increased risk of IUGR was established for mothers who were exposed to carc-PAHs > 15 ng/m3 during the first month of gestation. Using multiple regression analysis, GSTP1, EPHX and CYP1A1 MspI genotypes decreased newborn birth weight in polluted district. These are new results, indicating the significant impact of carcinogenic PAHs to the early stages of fetus development. They should be used for the risk assessment, as the impact of carc-PAHs to the fetal development was not evaluated, yet.

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W24
Experimental models to study human transplacental exposure to genotoxic agents

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Human placenta differs more than any other organ between species. Thus, for evaluation of the exposure of human fetus to genotoxic agents, human tissues and cells are the best. Since placenta is anatomically complex and highly polarized in its functions, experimental models that retain the tissue structure are most valuable. Such models include human placental perfusion and tissue explant cultures. Primary trophoblast cells and most cell lines of trophoblast origin do not grow into confluent monolayers in culture. This inhibits studies on polarized transport of nutrients and other compounds.

Human placental perfusion, theoretically, can model the transplacental transfer including both maternal-placental and placental-fetal interphases. Xenobiotic metabolism and tissue retention and binding can be studied in placental perfusion. Mechanistic studies on placental transporters, amended by, for instance, proteomics, are also feasible using placental perfusion. Furthermore, by perfusing placentas from mothers smoking, using illegal drugs or with a disease, the impact of these factors on the fetal exposure can be studied. In the validation of the placental perfusion model, relevant considerations include both the evaluation of the placentas at the start of the perfusion and various technical details of this complex model. An important consideration is the known interindividual variation among humans and the related question of how many placentas are needed to reliably reflect the range of variation.

Compared to in vitro-models, placental perfusion is more physiological. Compared to in vivo studies in humans, safety considerations for the mother and the baby are generally not an issue. Also toxic compounds, like environmental carcinogens, can be studied.

W25
Environmental genotoxins/carcinogens and children’s health: Current gaps in scientific knowledge

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Most of the common, serious children’s health problems, such as neurological disorders, asthma, diabetes, and cancer, show variation in incidence among human populations and/or over time, and therefore would seem to have preventable causes. Cancer is among the most intensively studied endpoints in this regard and will be the representative topic for this presentation. It is important to discover the extent to which childhood cancer risk is linked to environmental genotoxic agents: this is the first critical knowledge gap to be filled. Viable alternative explanations include nutritional effects, and exposure to infectious agents. Both human molecular and mechanistic animal model studies indicate that genotoxic events may contribute to childhood cancer risk, but epidemiology has thus far not definitively pinpointed causative environmental agents. There are potentially gap-filling investigations that might facilitate more precise epidemiological study of possible associations. Such investigations include discovery of molecular changes in childhood cancers and perinatally-caused animal tumors; description of the ontogeny of DNA repair enzymes in humans and rodents; study of association between SNPs in relevant enzymes and DNA-damage endpoints using human cord blood; study of specific environmental genotoxins in animal perinatal models across a wide dose range; use of genetically-engineered mouse models of embryonal cancers; analysis of male-mediated and transgenerational effects in both animals and humans; and
investigation of interaction of nutrition and of non-genotoxic agents, such as endocrine disruptors, with genotoxic chemicals.
Workshop 6
Ionising radiation sensitivity
W26
The genetic basis of normal tissue reactions after radiotherapy

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Cancer patients exhibit large patient-to-patient variability in normal tissue reactions after radiotherapy. Several observations indicate that the variation in normal tissue sensitivity is influenced by genetic factors. However, little is known about the genetic variations underlying inter-individual differences when unselected cancer patients undergo radiotherapy.

To address the genetic basis of variations in radiation-induced normal tissue reactions, different lines of experiments can be pursued. We are using a 'candidate gene approach' where we analyse subsets of genetic variants in genes involved in biological pathways suspected to underlie phenotypes of interest. These variants can be either common alterations like single nucleotide polymorphisms, SNPs, or rare variants in potential susceptibility loci like ATM. In parallel, we are using microarray analysis on normal fibroblasts isolated from patients treated with radiotherapy. These data are providing a comprehensive overview of the changes in gene expression after radiation and are also used to identify genes that can predict risk of normal tissue reactions.

Together, our observations support the assumption that clinical normal tissue radiosensitivity should be regarded as a complex trait dependent on the cumulative effect of variation in several genes. Furthermore, different types of normal tissue reactions may be dependent on different sets of genetic variations. To fully address these hypothesis, carefully designed clinical studies with an accrual of very large numbers of patients have been initiated (the ESTRO GENEPI project, Radiother. Oncol. 2003, 69:121-125).

W27
DNA repair gene expression as biomarker for acute sensitivity to ionising radiation

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Ionising radiation (IR) is an important tool in cancer therapy limited by side effects. The impact of DNA repair and cell cycle control on IR-response is well established. It is however unclear how much variation within these pathways affects individual radiation sensitivity. Analysis of gene expression might help to elucidate genes involved. Previous studies in constitutive (i) and IR-induced (ii) mRNA levels identified several genes which we are investigating as potential markers for radiosensitivity. RNA was isolated from lymphocytes of 250 prostate cancer patients scheduled for radiotherapy. RNA integrity was controlled and after reverse transcription, mRNA levels were measured with quantitative real-time PCR. Corrections were made for variations in reverse transcription and PCR runs. (i) Differences in constitutive mRNA levels prior to therapy were determined for 8 repair or repair-related genes. Patients with elevated expression levels had a higher risk to develop acute side effects. (ii) Gene induction factors were measured for CDKN1A, involved in cell cycle arrest after irradiation, and PCNA, involved in DNA repair synthesis. Lymphocytes of 39 patients were irradiated in vitro. Individuals with acute side effects were only found within the "normal" range of gene induction but not among those individuals with high inducibility. Our data suggest that low mRNA levels of DNA repair and cell cycle control genes but high inducibility of CDKN1A and PCNA may protect from acute side effects of radiotherapy. Further investigations are necessary to establish specific gene expression as a biomarker for radiosensitivity.

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W28
Repair phenotype, polymorphisms in repair genes and genotoxicity in radiation exposed workers

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We studied two exposed groups of nuclear plant workers, one chronically exposed (n=28) and the other
made up seasonal cleaners acutely exposed (n=32). The third and fourth groups were controls to the first (n=19) and second (n=31) groups respectively made of office staff. Using the Comet assay, we assessed DNA damage and the DNA strand break repair phenotype. The frequencies of micronuclei were assessed by means of the in vitro micronucleus assay. Genotyping for DNA repair genes OGG1, XRCC1 and XRCC3 was performed on blood samples of the acutely exposed workers. Our data show that exposed workers repaired damage to their DNA more proficiently than their controls. Also, the exposed smokers had higher levels of DNA damage and micronuclei frequencies than non smokers. In the acutely exposed workers, a significant contribution of the OGG1 genotypes to the in vitro DNA strand break repair capacity was found. A multivariate analysis revealed that genetic polymorphisms in XRCC1 and XRCC3 polymorphisms is advised in order to assess individual susceptibility to ionising radiation. As an alternative or complement, the in vitro DNA strand break repair phenotype which integrates several repair pathways is recommended.

W29
ATM sequence variants and radiosensitivity

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The ATM (ataxia-telangiectasia (A-T) mutated) protein plays a key role in the detection and cellular response to DNA double-strand breaks. The functional consequences of the presence of heterozygous sequence variants on ATM expression and the ionising radiation (IR)-induced cellular phenotype have been examined using lymphoblastoid cell lines. A lower cell survival was found in the A-T heterozygote than in the normal cell lines with the presence of a missense compared to a truncating mutation being associated with lower cell survival after exposure to 2 GY (p=0.005). The cell lines studied carrying specific ATM SNPs and those established from A-T heterozygotes showed significantly higher mean levels of MN formation after exposure to IR compared to the group of normal lines. Within the lines carrying SNPs the group of six carrying the linked 2572 T>C (858 F>L) and 3161 C>G (1054 P>R) variants had a higher level of MN after IR exposure compared to that observed the normal cell lines. This increase was not related to the constitutive ATM mRNA level, which was similar in these and the normal cell lines. Our results indicate that alterations in the ATM gene, including the presence of heterozygous mutations and the 2572C and 3161G variant alleles are associated with increased in vitro chromosomal radiosensitivity.

W30
Association of single nucleotide polymorphisms in TGFb1 with late radiation induced damage to normal tissues in patients treated with radiotherapy for gynaecological tumors.

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Purpose: To examine the association of polymorphisms in TGFb1 (-800G>A, -509C>T, 10Leu/Pro, 25Arg/Pro) with the development of late radiotherapy (RT) reactions.

Materials and Methods: Sixty seven women with cervical or endometrial cancer treated with RT were included in the study. According to the CTCAEv3.0 scale, 24 patients showed late adverse RT reactions (grade 2 or more), 9 of these patients showed very severe adverse reactions (grade 3 or more). PCR-RFLP and PCR-SnapShot assays were performed to examine the polymorphic sites.

Results: Neither the -800A allele, nor the 25Pro allele were associated with adverse RT reactions. The -509TT and the 10ProPro genotypes were associated with the risk of developing late RT reactions. The number homoygous carriers of both the -509 and the 10 polymorphisms increased according to the severity of the RT reactions (9%, 21% and 33% for grade 0-1, grade 2+ and grade 3+ respectively). Consequently, double (variant) homozygous patients had a 4.9 times increased risk to develop severe RT reactions (p=0.16).
Conclusion: This study confirms the findings of Quarmby et al. (2003) and Andreassen et al. (2003) that homozygous carriers of the TGFβ1 -509T and the TGFβ1 10Pro polymorphisms are susceptible to fibrosis associated RT reactions.
Workshop 7
Contributions of diet to human genotoxic burden and risks
W31
Heterocyclic amines: Tissue-specific diet-derived carcinogens.

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The heterocyclic amines, formed during the cooking of meat, exhibit extreme mutagenic potency in short-term assays prompting speculation of a role in the aetiology of human cancer. Every cooked-meat heterocyclic amine examined thus far has been shown to induce tumours in laboratory animals. The most abundant cooked-meat amine, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhiP), induces tumours of the colon, breast and prostate in rats. Coincidently, human consumption of meat correlates with cancers of the colon, breast and prostate. Mechanistic studies have shown that humans consuming cooked-meat efficiently absorb and activate heterocyclic amines to their genotoxic N-hydroxy derivatives, which are then metabolically detoxified and excreted. Yet each of these steps, from exposure to excretion, varies within individuals and such variability might be expected to contribute to cancer susceptibility, if the heterocyclic amines are causal agents. With a good understanding of these molecular events, clinical trials and case-control studies have attempted to address this issue. For colonic cancer, studies of exposure have tended to support a role for the meat-derived heterocyclic amines, but studies that have used a pharmacogenetic approach have not. Thus it would appear that the case for the heterocyclic amines being causally linked to human colonic cancer is, at best, equivocal. However, the recent discovery of previously unrecognized biological activity of the heterocyclic amine PhiP suggests that this particular amine cannot yet be dismissed.

W32
Red meat and endogenous NOC production in the human gut

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To test the hypothesis that red meat would increase endogenous production of N-nitroso compounds (NOC) in the GI tract, thus accounting for the epidemiological association between red and processed meat consumption and colon cancer, we have fed increased levels of red meat and measured ATNC (Apparent Total N-nitroso Compounds) in faecal and ileostomy samples in a series of studies of volunteers maintained under controlled conditions of the Dunn Human Nutrition Unit metabolic suite. As a result of these studies we have shown a consistent dose response to red meat consumption, but no significant effects of white meat (1). We have also shown that whilst red meat diets increase faecal ATNC, the equivalent amount of protein from eggs, milk, cheese and vegetable protein has no effect. Furthermore, there appears to be a specific effect of haem whereas inorganic iron has no effect (2). Under certain conditions, haems are known to be nitrosated, and act as nitrosating agents. The formation of N-nitrosoarginine by haem enzymes under anaerobic conditions has also been demonstrated. ATNC production is also increased in the upper GI tract following processed and red meat consumption (3). The genotoxic effects of increased N-nitrosation from red and processed meat are presently being investigated. No effects of diet on genotoxicity of faecal water or ileostomy output as assessed by the Comet assay have so far been detected, although there is an individual variation (3,4). A more direct approach is to assess the presence of NOC specific adducts in colonic exfoliated cells (5,6).

References
W33
Lipid peroxidation induced DNA-damage in humans: role of fat intake and dietary antioxidants.

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Exocyclic-DNA adducts, 1,N6-ethenodeoxyadenosine (εdA), 3,N4-ethenodeoxycytidine (εdC) and malondialdehyde-deoxyguanosine(M1dG) are formed as result of oxidative stress and lipid peroxidation via reactive aldehydes such as alkenals and malondialdehyde. A pilot study in humans revealed that these promutagenic DNA-lesions are elevated in white blood cell (WBC)-DNA of female volunteers who were on a very high -6 polyunsaturated fatty acid (PUFA) diet. In a study in Japanese women urinary excretion of εdA was positively associated with PUFA intake. Studies in rats revealed higher etheno-DNA adduct levels in colon DNA after PUFA-treatment as compared to monounsaturated fat, here the adduct levels were more elevated in females than in males. A subsequent study in randomly selected female volunteers revealed a significant inverse correlation for εdA in WBC-DNA and vegetable or vitamin E consumption. However, no association was found between etheno-DNA adduct levels and PUFA intake. We concluded that elevated etheno-DNA adduct levels in WBC are not caused by PUFA intake alone but might depend on the ratio of fatty acids and antioxidants consumed in the diet. In addition, redox cycling by catechol estrogens might play a role in the induction of DNA damage by a high PUFA diet. In vitro studies and analyses of human samples for LPO-induced adducts and estradiol metabolites (in WBC-DNA and serum respectively) support the possibility of DNA damage to occur by this pathway (in part supported by EU grant QLK4-CT-2000-00286).

W34
Furan: a new food-borne carcinogen.

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On 7 May 2004, US FDA informed about the occurrence of furan in a variety of foods such as coffee, canned and jarred foods including baby foods containing meat and/or vegetables at levels up to 125 µg/kg (http://www.fda.gov/bbs/topics/news/2004/NEW01065.html). The EFSA Scientific Panel “Contaminants in the food chain” has recently delivered a scientific report on furan (http://www.efsa.eu.int/science/contam_scientific_documents/catindex_en.html). Furan, metabolised by cytochrome P450 CYP2E1 in the di-aldehyde cis-2-butene-1,4-dial, is an extremely powerful inducer of liver cholangiocarcinomas in rats, in which it also induced a dose-related increase of mononuclear leukaemia; in rats and mice (both sexes) it induced a dose-related increase of hepatocellular adenomas and carcinomas.

A key question for risk assessment and regulatory purposes is whether furan is or not a genotoxic carcinogen. Its carcinogenicity mechanism has not yet been fully elucidated and hypotheses of direct (genotoxic) or indirect (hepatocellular proliferation secondary to cytotoxicity) mode of action have been so far postulated. Evidence which favours, at least in part, a genotoxic mechanism is: i) furan induced gene mutations, chromosome aberrations and SCE in vitro and also chromosome aberrations in vivo in mouse bone-marrow cells; ii) cis-2-butene-1,4-dial induced gene mutations in S. typhimurium TA104 strain, DNA single strand breaks and DNA cross-links in vitro; and DNA adducts with deoxiribonucleotides in vitro; iii) a unique ras oncogene mutational profile in liver tumours from furan-treated mice suggests that novel mutations could have been induced by the genotoxic action of furan.

W35
Interactions of toxic metals and essential trace elements in the cellular response to DNA damage

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Nickel, cadmium, cobalt and arsenic compounds are well known carcinogens to humans and experimental animals. In addition to the induction of mainly oxidative DNA damage, they interfere with nucleotide and base excision repair at low, non-cytotoxic concentrations. As potential molecular targets, interactions with
so-called zinc finger proteins involved in DNA repair and/or DNA damage signaling such as XPA and PARP have been identified. For example, arsenite and its trivalent methylated metabolites suppress poly(ADP-ribosyl)ation at extremely low, nanomolar concentrations. A zinc binding motif is also present in p53, and water soluble as well as particulate Cd compounds convert the correctly folded “wild type” conformation almost completely into a so-called “mutant” form with an unfolded zinc binding domain. Furthermore, our experiments demonstrate changes in downstream events such as diminished transcription of p48 and XPC as well as altered cell cycle control in response to UVC irradiation and benzo[a]pyrene. Remarkably, an interaction with zinc finger structures may also occur by essential trace elements: Reducible selenium compounds deliberate zinc from XPA and provoke p53 conformational changes, presumably by oxidation of cysteines in zinc binding motifs. Thus, certain selenium compounds may exert anticarcinogenic effects at low levels but may compromise genetic stability at higher concentrations which could be reached by dietary supplements.
Workshop 8
State of the art of environmental biomonitoring – Role and perspectives for an integrated environmental risk assessment
Applications of bioindicators and biomarkers for the monitoring of aquatic environment
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With growing interest for the adoption of an ecosystem-based approach for the management of aquatic environment, new legislations are emphasising the need for biological effects of contaminants as the criteria for environmental risk assessment. Given the complexity of the functioning of coastal marine systems, there is however a need to adopt a more pragmatic approach to environmental monitoring, elucidating the cause-effect coupling for potential environmental degradation in addition to our traditional reliance on various ecotoxicological and analytical tools. In this context, the importance of applications of biomarker responses to assess the general health of the organisms is getting importance especially in the light of proposed adoption of precautionary principles. The biological responses, in particular at DNA and chromosomal levels, signal exposure to, and adverse effects of genotoxic pollutants which could also have implications for human health. Following proper validation in a range of invertebrates (bioindicators) at different trophic levels with different feeding mechanisms, we have therefore attempted to develop and implemented a range of genotoxicological and biological responses to (a) determine the relative sensitivity of key species to a range of common contaminants and (b) link and compliment observed biomarker responses to bioavailability, body burden and the site chemistry. Such a rationale could offer the opportunity to adopt preventive approach to environmental hazard and risk assessment without the need to prove the actual declines in biodiversity in years to come.

Monitoring the long term environmental impact of oil spill
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Shipment of chemicals along coastal marine route is a potential constant treat to marine life. A number of serious accidental spills of crude oil occurred all around the world. On April 1991 on board of the supertanker Haven, anchored at six miles from Arenzano coast (Ligurian sea) an explosion occurred causing the release of 10,000 thousands tons and sinking of 90,000 tons of iranian heavy crude oil. The Haven wreck at present lies on the sandy bottom at -85 m depth in front of Arenzano. An initial assessment of the genotoxic impact of Haven oil spill by the evaluation of DNA and chromosomal damage, revealed, within few weeks after the accident, severe effects in mussel transplanted in impacted sites. More than 10 years later, a biomonitoring program was carried out on native and caged molluscs (mussels and oysters), and on fish. High levels of DNA damage and micronuclei frequency were recorded in native as well in caged oysters in 5 sites inside the wreck. Genotoxic effects were already evident, although in a lesser extent, in invertebrates as well as in fish from different impacted coastal sites compared to a reference area. Significant increase of genotoxic damage, mainly as micronuclei frequency, was observed in Mullus barbatus, a higher but not significant effect was detected in a benthic species, Uranoscopus scaber and only negligible damage was appreciable in a pelagic organism such as Boops boops. The results of this biomonitoring study allow concluding that more than 10 years after the accident significant genotoxic effects are already measurable at different extent in bioindicators with different habitat and feeding behaviour.

Genotoxic impact in coastal environments
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A large proportion of anthropogenic pollutants released in the marine environment is made of potentially genotoxic and carcinogenic substances. Consequently, it is not surprising the increasing concern regarding the hazard associated with the exposure of both marine species and human populations to such contaminants. In the last decades, marine ecotoxicologists have used sentinel species, such as bivalves and fish for the evaluation of genotoxic chemicals and their risk for ecosystem functioning and human
Several biomarkers of genotoxicity have been proposed in aquatic marine organisms; many of these are based on the evaluation of the structural alteration of DNA, such as the formation of strand breaks (SB). Strand breaks may be induced directly by genotoxic compounds, or secondarily through the interaction with oxygen radicals or other reactive intermediates, or as a consequence of excision repair enzymes. Indeed the loss of DNA integrity correlates with the induction of mutations, chromosomal aberrations, birth defects, long-term effects such as cancer in vertebrates and the “genotoxic disease syndrome” in invertebrates.

Among different techniques commonly used for detecting SB, the Single Cell Gel Electrophoresis (Comet assay) has received an increasing interest in marine ecotoxicology, as very sensitive and rapid method for evaluating DNA damage associated with chemical disturbance in field investigations.

In this presentation some of the potential applications of comet assay in coastal environment biomonitoring will be shown.

W39
Contaminant health related status of UK estuaries

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The estuarine environment is a major sink for many potentially hazardous chemical pollutants emitted from industrial and domestic sources. Analytical chemistry has identified genotoxins in the sediment, water and accumulated in the tissues of aquatic organisms. However, inventory-based chemical monitoring programmes are restricted to the identification of a limited range of contaminants and provide no information on their biological significance. This has led to biological effects measurements in aquatic species being used as a way of assessing their environmental impact. A considerable amount of evidence is now available to suggest that fish living in these environments suffer effects, including cancerous pathologies and reproductive impairments. Here we describe the application of DNA adducts and histopathology in European flounder (Platichthys flesus) as part of a programme to establish the health status of UK estuaries. The analysis of DNA adducts revealed that fish at industrialized sites were exposed to a mixture of genotoxins. Histological examination of these samples revealed a range of lesions including foci of cellular alteration and benign tumours, along with non-toxicopathic alterations. While links between such pathologies and genotoxic biomarkers are not definitive the research adds to the growing database of biological effects information for UK estuaries. Continuing research is required if we are to establish the significance of these findings, and determine in full the potential ecosystem effects of chronic contaminant exposure in the marine environment.

W40
DNA damage in Mytilus edulis and intersex in Littorina littorea sampled in a Danish harbour

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The pollution of harbour sediments is common due to the painting of ships and other anthropogenic harbour activities. The active antifouling ingredients in ship bottom paints are per definition toxic for algae and other marine organisms, which grow on the surfaces of ships’ bottoms. The most effective of these biocides is TBT, the use of which has been banned (since 1987) in Denmark for small boats of less than 25m. TBT is still present in sediments and is known to be an endocrine disruptor in marine snails. PAHs and PCBs are also part of the sediment pollution in harbours and known to have genotoxic effects. In the present study we have sampled mussels (Mytilus edulis), snails (Littorina littorea) and sediments from two stations in Lynæs Harbour: the dockyard and the yacht harbour, together with a reference station without known pollution (Dragerup). TBT, DBT, MBT and heavy metals were measured on all samples of snails, mussels and sediments, whereas PAHs and PCBs were only measured in mussels and sediments. The comet assay was used to detect DNA damage in the mussels, and the snails were investigated for phenomenons as intersex index in female snails and number of penis glands in male snails. The biological effects showed significant correlations with many of the chemical parameters. In conclusion, intersex in Littorina littorea and DNA damage in Mytilus edulis are both good indicators of harbour pollution.
DNA-adduct analysis in aquatic biomonitoring

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During the last 15 years studies have been performed highlighting advantages and limitations of DNA-adduct analysis in aquatic organisms. The main advantage is the quantification of the molecular effective dose of pollutants, thus obtaining a biologically relevant indication of the presence of hazardous substances in the aquatic environment. Limitations are related to both sensitivity and specificity of the used methods.

Concerning sensitivity, pollutants undergo a relevant dilution in the aquatic environments. The use of filtering organisms has been proposed to attenuate this problem. However, these organisms are characterised by a poor metabolism limiting the amount of DNA-adduct formed as compared to more evolved organisms. Cell-proliferation rate and toxico-kinetic also contribute to determine DNA-adduct amounts in various organs, higher amounts being detectable in fish liver and intestine than in gill, blood, and brain. The same factors affect DNA-adduct decrease following exposure cessation, this decrease being faster in fish intestine than in liver and gill.

Because of the typical occurrence of multiple DNA-adducts in low amounts, the most commonly used method for DNA-adduct analysis in aquatic biomonitoring is $^{32}$P-postlabelling, able to detect multiple adducts of both endogenous and exogenous origin. Endogenous DNA-adducts are detectable in aquatic organism devoid of any exposure to pollutants, causing problems in the specificity of the results obtained. This phenomenon is more relevant in fish than in mussel and is affected by seasonal variability.

In conclusion, evaluation of DNA-adducts in aquatic organisms is a useful tool in ecogenotoxicology studies, but the limits of this approach should be considered.

Genotoxic effects and cytochrome P4501A induction in glaucous gull (Larus hyperboreus) chicks, fed environmentally contaminated gull eggs.

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Glaucous gull (Larus hyperboreus) a top-predator in the arctic marine ecosystem, has at Svalbard very high concentrations of polychlorinated biphenyls (PCBs). In the present field-laboratory study material for analysis was taken from glaucous gull chicks of the same age reared from eggs collected at Svalbard. The control group was fed with hen eggs and the exposed group was fed environmentally contaminated gull eggs. DNA adducts, in liver, were quantified by $^{32}$P-postlabeling. Cytochrome P4501A proteins were determined by Western blotting. Chromosome aberrations and DNA strand breaks were quantified from blood by cytogenetic analysis and agarose gel electrophoresis. The exposed chicks had significantly increased levels of DNA adducts relative to the controls. The level of CYP1A proteins was significantly higher in the liver of exposed male chicks than in control males. For both female and male, the fraction of damaged metaphases was higher in the exposed (not significant) than in the control groups. The differences between the control and the exposed groups were stronger when the chromosomal aberration data were treated as group totals rather than at the individual level. Consistently, in the DNA strand break analyses there was a greater median molecular length for the control than the exposed group (not significant).

The role of retinoblastoma gene in fish carcinogenesis

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Reports of isolated tumours in fish have been replaced by studies of piscine cancers to include the pathology, molecular biology, and aetiology of these lesions. The underlying molecular steps of
carcinogenesis, which are initiated by lesions to ‘cancer genes’ (oncogenes and tumour suppressor genes), are a current focus. The role of oncogenes is to promote inappropriate cell growth while tumour suppressor genes inhibit cell growth. It is well established in mammalian models that mutations in oncogenes and tumour suppressor gene lead to uncontrolled cell growth and ultimately the development of cancer. To date, two tumour suppressor genes have been characterised among fishes, p53 and retinoblastoma (Rb). P53 is conserved in fish, yet an investigation of tumours has revealed no p53 mutation, suggesting that the p53 protein has a different function in fish compared with humans.

Herein, we report on our investigation of the role of Rb in the development of cancers in fish. Medaka (Oryzias latipes) and dab (Limanda limanda) Rb homologs have been isolated using degenerate primers, RT-PCR and RACE techniques. The fish Rb sequences are highly conserved in regions of functional importance. Structural alterations have been characterised using mutation detection and sequencing. The Rb cDNA in both chemically-induced medaka and environmentally-induced dab liver tumours contains mutations. Such results suggest that the molecular aetiology of fish cancer appears to involve Rb-implicated tumourigenesis.
Workshop 9
Chromosome segregation
during cell division
**W44**

Regulation of chromosome segregation by chromosomal passenger proteins and the condensin complex

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A proteomic screen of the chromosome scaffold fraction identified 79 proteins, 30 of which had not previously been shown to be components of mitotic chromosomes. I will discuss our studies of two of these proteins. One novel component, when tagged with GFP has a distribution in cells identical to the chromosomal passenger complex of INCENP, Aurora B and survivin, which has essential regulatory roles at centromeres and the central spindle in mitosis. This protein, Borealin, is a novel member of a chromosomal passenger holo-complex. Functional studies show that the passenger holo-complex is required for regulation of kinetochore attachments to the spindle, stability of a bipolar spindle, and cytokinesis. A second protein complex found in the chromosome scaffold fraction is condensin, a complex of five polypeptides that has been reported to be required for mitotic chromosome condensation. We had previously reported a conditional knockout of the condensin component SMC2 in chicken DT40 cells. I will discuss our current studies of the role of this protein complex. These studies reveal that although the condensin complex is not required for normal levels of mitotic chromosome condensation, it is required to regulate the timing of chromosome condensation and decondensation in mitosis. Together, these studies reveal that non-histone proteins play essential roles in both the regulation of chromosomal dynamics, and the coordination of chromosomal and cytoskeletal events during mitosis.

**W45**

Understanding the inner workings of the spindle assembly checkpoint

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Our laboratory is interested in understanding the molecular mechanism of the formation of stable kinetochore-microtubule attachments during mitosis. This process is monitored by the spindle assembly checkpoint (SAC), whose components are recruited to kinetochores early in mitosis. Unattached or incorrectly attached kinetochores are the source of a signal that arrests cells in a prometaphase-like state. Metaphase, which entails the biorientation of all sister chromatid pairs, marks the end of the attachment process. This condition switches off the SAC, allowing chromosome separation and anaphase.

The SAC itself is rather complex in its molecular details. It is believed that the SAC consists of two distinct but communicating modules. One branch of the SAC is responsible for detecting absence of microtubules at the kinetochore surface (the so-called “attachment”). The SAC proteins Mad1 and Mad2 are part of this pathway. They promote the sequestration and temporary inactivation of Cdc20, whose function is required for anaphase. The second branch is believed to be able to detect lack of tension at attached chromosomes. Tension builds across the centromere of bi-oriented sisters if these attach to opposite pole. Sometimes, however, both sister kinetochores appear to attach erroneously to the same pole. This condition, known as syntelic attachment, needs to be corrected to avoid chromosome missegregation. The correction of syntelic attachment seems to involve the Aurora B kinase and its partners in the chromosome passenger complex, whose activity might help severe the faulty microtubule-kinetochore connection.

My talk will explore two mechanisms related to the regulation of the branches of the SAC described above. First, I will propose a model for the activation of Mad2 in the SAC2. This model has the potential to explain the propagation of a cytoplasmic signal from unattached kinetochores and entails a self-amplifying positive feedback loop controlling a conformational transition in the Mad2 protein3. I will also describe our recent progress on the determination of the structure of the Aurora B complex with INCENP4, another component of the chromosome passenger complex.

W46
Mouse models of aneuploidy induction in mammalian oogenesis


Aneuploidy in germ cells is an important aetiological factor in human trisomy, spontaneous abortion and genetic disease. Evidence from transgenic mouse models suggest that female meiosis is particularly susceptible to nondisjunction because checkpoints sensing prophase I failures are more permissive in female compared to male meiosis. Gender-specific susceptibility to aneugenic exposures may also relate to loss of chromosome cohesion and alterations in expression of checkpoint genes in aged oocytes. We modelled increased susceptibility to meiotic disturbances in in vitro maturing oocytes by transient knockdown of genes by siRNA technology. In vitro maturing naked mouse oocytes were used to assess hazards by aneugenic exposures and identify mode and target of aneugens. Pre-antral follicle culture, in which oocytes grow and mature in vitro up to ovulation, provided information on risks by exposures to chemicals that may affect the somatic compartment or hormonal homeostasis and indirectly or directly compromise oocyte capacity to orderly segregate chromosomes. Future approaches of expression profiling are discussed to explore the influences of aneugens in somatic cells and oocytes within follicles. In vitro models may help to restrict in vivo studies but currently it does not appear possible to extrapolate dose-response from in vitro to in vivo exposure. (Supported by EU, QLK4-CT2000-00058).

W47
Extensive DNA hypomethylation induces endoreduplication in cultured Chinese hamster cells

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Since the nuclear enzyme topoisomerase II (topo II) has been reported to be responsible for the segregation of daughter chromosomes during mitosis, in the present investigation we have studied the possible influence of the methylated state of DNA on chromosome segregation. The endpoint chosen has been the induction of endoreduplicated cells at mitosis showing diplochromosomes. Experiments were performed in the presence and absence of the cytidine analogue to assess the degree of 5-azaC-induced DNA hypomethylation, using differential cutting by restriction endonucleases Hpa II and Msp I. Using the pulsed-field gel electrophoresis (PFGE) technique, we have also observed a protective effect provided by 5-azaC treatment against DNA breakage induced by the topo II poison m-AMSA. Concentrations of 5-azaC shown as able to induce extensive DNA hypomethylation and capable to protect DNA from double-strand breaks induced by m-AMSA were used for our cytogenetic experiments to analyze chromosome segregation. Our results seem to indicate that severe hypomethylation of DNA leads to reduced chromatid decatenation that ends up in endoreduplication, most likely due to a failure in topo II function.
Workshop 10
Modulation of genotoxic and cancer risk by genetic variation
W48
Single Nucleotide Polymorphisms (SNPs) in DNA repair genes and risk for smoking-related cancers


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Lung cancer, and to a less extent head and neck cancer (HN), are common worldwide. Although most of these cancers are typically caused by smoking (together with alcohol abuse for HN cancer), variations in DNA repair activity could contribute to individual susceptibility.

The effect of SNPs in DNA repair genes on cancer risk has been typically evaluated one at a time. However, the combined effect of multiple variant alleles may be more important than the investigation of single SNPs. Our project was based on a two-stage strategy: 1) SNP discovery by sequencing 89 DNA repair genes involved in 6 main pathways in 32 healthy subjects; 2) SNP genotyping using the Illumina-chip technology in 151 lung cancer cases, 251 HN cancer cases and 171 controls.

We have characterized about 1500 SNPs. Haplotypes of each gene were statistically inferred from the observed genotypes and their estimated frequencies were compared between cases and controls by score tests.

We found significant differences in haplotype frequencies between lung cancer cases and controls for 8 genes and between HN cancer cases and controls for 6 genes. Several DNA repair pathways are concerned and the implicated genes will be described.

W49
Influence of CYP1A2 genotype on phenotype in Caucasian smokers

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The cytochrome P450 1A2 (CYP1A2) is a key enzyme in carcinogen and drug activation. The increased CYP1A2 activity was suggested to be linked to CYP1A2 inducibility by several environmental factors, especially tobacco smoking, and the occurrence of polymorphisms in CYP1A2 gene. In this study the functional significance of three CYP1A2 polymorphisms [(-3860 GÆA (allele *1C), -2467 TÆdelT (allele*1D), -163 CÆA (allele *1F)], lying in the 5'-noncoding promoter region, on CYP1A2 activity (caffeine metabolic ratio (CMR)), was studied in 95 Caucasian current smokers. Tobacco smoke intake was checked by number of cigarettes/day. We found that smokers with at least one polymorphic variant CYP1A2 –3860A and –2467 delT significantly increased CMR levels (-3860G/A versus G/G, p=0.0461; –2467delT/delT versus T/delT and T/T p= 0.0011 and p=0.0043). In a multiple regression analysis the increased CMR levels (ln values) were significantly related to the presence of -2467delT and -163A variants (p=0.036 and p=0.041), moderately to that -3860A (p=0.084), but not to the number of cigarettes/day smoked by each subject (p=0.117). In conclusion, CYP1A2 genetic polymorphism influences CYP1A2 phenotype in smokers, with -2467 TÆdelT having the main effect. The information provided here seems to be of interest for future studies in which smoke-inducible CYP1A2 genotypes are individual susceptible factors in exposure to carcinogens and drugs.

W50
Genetic polymorphisms and possible gene-gene interactions in metabolic and DNA repair genes: effects on genotoxicity markers

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We investigated the association between polymorphisms in genes coding for xenobiotic metabolizing enzymes (CYP1A1, CYP2E1, EPHX1, GSTP1, GSTM1 and GSTT1) and in DNA repair genes (XPD, XPG, XPC and XRCC1) and the levels of chromosomal aberrations (CA) and single-strand breaks (SSBs) in...
Peripheral blood lymphocytes. Main confounders did not affect either CA or SSBs in the studied population. Concerning single-genotype effect, significantly higher CA frequencies were found in individuals with low activity EPHX1 genotype compared to those with high activity genotype (P=0.010) and in individuals with XPD, exon 23 genotypes including A allele (P=0.028). SSBs were affected by XPD genotype, being the highest in individuals with the wild-type alleles (AA) and the lowest in those with homozygous (CC) genotype (P=0.033). EPHX1 activity genotype affected SSBs only in a minor extent. When gene-gene interactions were evaluated, a combination of EPHX1 activity genotypes with that of XPD, exon 23 significantly modulated CA frequency (the difference between the positive and the adverse genotype combinations being six-fold), as well as SSBs, resulting in a three-fold difference between the “protective” and the “adverse” genotype-combinations. Almost three-fold differences in both CA and SSBs were found between the positive and the negative combinations of EPHX1 activity genotype and GSTM1 and GSTT1 genotypes, respectively. In conclusion, our results suggest a relation between markers of genotoxicity and polymorphisms in genes coding for xenobiotic metabolizing and DNA repair enzymes as well as a modulating effect of combinations of these polymorphisms.

W51
Genetic susceptibility to thyroid cancer

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The purpose of this study was to investigate whether there is an association between THRA1 or BAT-40 repeats polymorphisms, located in the thyroid hormone receptor-α1 gene which is associated with thyroid cancer, and in a region of chromosome 1 which is known to be involved in thyroid cancer, respectively. Genotyping analysis was carried out in thyroid cancer patients and control individuals of a Spanish population (212 and 141 individuals for THRA1, 207 and 138 individuals for BAT-40, respectively). No significant difference in the THRA1 allele distribution between patients and controls was found, although short alleles (<128bp) might have some protective effect on thyroid cancer risk (odds ratio, 0.50; 95%CI 0.22-1.13, p=0.094). BAT40 allele distribution was significantly different between patients and controls (p=0.035). This difference was found in the genotypes involving the 111-115 bp allele range, that might be associated with a protective effect on thyroid cancer susceptibility in the studied population (odds ratio, 0.18; 95% CI 0.01-0.57, p= 0.02). Therefore, our results indicate that the BAT-40 containing region and to a less extent the thyroid hormone receptor-α1 gene are related to thyroid cancer susceptibility.

W52
Phenotypic expression of sequence variation in the DNA repair gene XPD (Lys751Gln)

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As the product of the XPD gene has function in nucleotide excision repair and in basal transcription, the association between DNA repair capacity, cancer risk and polymorphisms in this gene has been extensively studied. Several variations in the XPD sequence have been identified. The one of them, an amino acid substitution Lys751Gln, is located within the important domain and therefore could relevantly modify XPD activity.

To evaluate the impact of this genetic variation on the phenotypic expression, the response of two cell lines, HeLa (Lys/Lys, wt) and C-33A (Gln/Gln, homozygote variant) to UV irradiation was studied. Various biomarkers including cell growth activity, cytotoxicity, cumulative DNA, RNA and protein synthesis as well as the expression of P53 and P21 proteins were analysed.

A comparable inhibition of growth activity due to UV-treatment was observed in both cell lines. No differences in the viability between HeLa and C-33A cells were found immediately after irradiation; however, 24 hours after treatment a delay in cell proliferation was determined in C-33A cells compared to HeLa cells. Although the intensity of DNA, RNA and protein synthesis was significantly decreased in both UV-exposed cell lines, stronger inhibition was observed in C-33A cells.

Obtained results signalize that Lys751Gln polymorphism could have impact on XPD activity. This work was supported by Science and Technology Assistance Agency under the contract No. APVT-51-015304.
Interactive workshop 1
Teaching / training in genetic toxicology
W53
Methods for detection of clastogenic and aneugenic activity, current and future technologies

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Chromosome mutation involves change in chromosome structure and number, i.e. a change from the normal karyotype. Structural change may result from exposure to chemicals reacting with chromatin to produce breaks. Such events may be repaired so that the normal chromosome structure is reconstituted, they may be left unrepaired and be visible as breaks or fragments, or they may be misrepaired to produce structural rearrangements within or between chromosomes. Numerical change can also result from environmental factors disrupting the normal high fidelity of cell division. Although these changes may not involve direct change to DNA at the molecular level, they do involve change in DNA quantity and order which can affect gene expression.

Aberrations to chromosome structure and number are implicated as important factors in the development of somatic disease, in inherited genetic disorders and in human fertility. Currently, regulatory authorities recognise the importance of this and require the generation of data from methods that measure the frequency of chromosome aberrations both in vitro and in vivo in experimental systems. Chromosome aberrations can be detected by metaphase analysis or in interphase cells by the micronucleus assay. The techniques currently used to measure chromosome aberration can be enhanced by the addition of molecular cytogenetic techniques such as fluorescence in situ hybridisation (FISH) and confocal microscopy to perform mechanistic studies.

W54
The development, validation and application of mutagen detection systems

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Genetic Toxicology Test Systems have been developed to detect the mutagenic activity of chemicals, to quantify activity, to provide information on the consequences of genotoxin exposures and when possible to investigate mechanisms of activity. Methods developed range from the detection of exposures to the production of gene and chromosome mutations. Methods for estimating exposure include the direct measurement of DNA adducts by 32P postlabelling and indirect methods such as the assessment of chromatin damage by the Comet assay and the induction of DNA repair by the measurement of unscheduled DNA synthesis. Strategies for genotoxin assessment from the initial screening for activity in vitro followed by the verification or not of activity in vivo will be described. Recent developments include the ability to identify mutations in transgenic rodents. The identification and characterisation of germ cell effects requires the use of assays generally based upon analysis in male germ cells. However, the importance of female germ cell analysis is increasingly appreciated and suitable analytical methods are becoming available.

The UK Environmental Mutagen Society has been developing a programme that aims to provide a range of modules to provide a comprehensive education in genetic toxicology. Modules will illustrate how and why methods were developed and validated leading to our current package of basic methods. We aim to produce modules to both provide ongoing updates for current workers in Toxicology and preparatory courses for new entrants into our discipline.

W55
Biomarkers of exposure to and effects of genotoxins: definitions and predictive value for cancer and hereditary diseases

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This session will start with a global introduction on mutagens and carcinogens and early genotoxic effects, leading to a definition of biomarkers of exposure (tissue metabolites, macromolecular damage) and (early) genetic effect (comet assay, sister chromatid exchange test, micronucleus test, chromosome aberration test, and different gene mutation tests). An interactive session will follow on 1) the utility and meaning of
different types of biomarkers of exposure, 2) the predictivity of chromosome aberrations for cancer, 3) the predictivity of chromosome aberrations for the off-spring, 4) how to do biomonitoring at population and/or individual level. In part 1 of the interactive session we will focus on the mechanisms of oncogenes and suppressor genes, the correlation between frequency of chromosomal aberrations and cancer and on a theoretical calculation of increased cancer frequencies based on a doubling in the frequency of chromosome aberrations. In part 2 predictivity of chromosome aberrations for offspring will be illustrated with an example of ionizing radiation. In part 3 we will go to a more practical approach: how should one do biomonitoring at population and/or individual level? We will emphasize the importance of genetic polymorphisms, both at individual as population level, on the different biomarkers of exposure and effect. This session will end with a discussion trying to find answers to a few additional questions.

**W56**

Biotransformations in Genetic Toxicology

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The concepts of biotransformations being responsible for the activation of pro-mutagens/carcinogens and subsequent formation of genotoxic lesions will first be introduced with reference to various metabolic pathways and specifically, the oxidative activity of the cytochrome P450 cycle. The theme of in vitro genotoxic responses driven by P450 and modulated by phase II detoxification will be developed with reference to compounds such as aflatoxin B1 and the polycyclic aromatic hydrocarbons. The influence of genetic and environmental factors will be explored (including chemical inducers, inhibitors and activators) with specific reference to variances in population in the form of metabolic linked polymorphisms. Case studies using therapeutic regimens will be presented to provide examples of how such factors influence subsequent reactions. That the phase II 'detoxification' enzymes on occasions drive active lesions will be introduced as well as acknowledging the complexity of interactions that determine toxicity profiles resulting in lesions in specific tissues and organs whilst having no effect in other tissues. The application of genetically modified cells and animals to elucidate the role of biotransformation in specific toxicological pathways will be introduced culminating on the promise that humanisation in rodent models holds for future evaluation. Finally, this section of the workshop will close with a brief overview of the role of DMPK in drug development and risk assessment.

**W57**

Analysis of data from routine regulatory genotoxicity assays.

**Mitchell I. de G. Chilfrome Enterprises Limited, Dorchester, U.K**

For analysis of regulatory assays a sequence of questions has to be answered: Is the assay valid? Do treatment values exceed the negative control? If so, is the increase treatment-related? If so is the treatment-related increase biologically important? For validity the assay must conform to the appropriate guidelines, the data must be of adequate quality and the negative and positive controls must be consistent with both published and laboratory historical data. It is desirable to generate laboratory historical data from duplicate negative controls and to take the positive control concentration(s) from the steepest part of the concentration-response curve. Statistical analysis may be needed to determine if treatment values exceed the control values. Parametric or non-parametric analyses may be appropriate depending on historical data and on the data themselves (particularly if there are outliers). It is important that the assumptions underlying the chosen statistical method are consistent with the biology of the assay system. Of equal or greater importance is to correct for multiple comparisons (endpoints or tested concentrations) and to decide whether to use one- or two-tailed tests. For showing that increases are likely to be treatment-related analysis of trend can be useful but always taking into account potential artefacts such as toxicity and/or extreme conditions. Lastly, the most difficult question of biological importance must be decided. As a guide it is recommended to use some measure of variability in historical negative controls.
Experimental design and statistical analysis of genetic toxicology data

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This section of the interactive session will describe issues associated with the analysis of genotoxicity data. Statistical analysis of genotoxicity data is determined by the experimental and study design and all analyses depend upon the conduct of a valid study. The basic principles of experimental design – randomization, replication and blocking - were developed in the 1920s by R.A. Fisher but remain as relevant 80 years later. Statistical analysis of biological data is increasingly considered a modelling exercise with greater emphasis being place on the estimation of the size of effects as opposed to determining the formal statistical significance of comparisons. The session will highlight issues associated with modelling approaches for specific experimental designs, the critical concept of the experimental unit, the assumptions underlying, and the strengths and weaknesses of different statistical approaches. The relationship between statistical power, sample sizes and biologically important differences will be stressed. The potential to apply Design of Experiment (DOE) methodology, the modern extension of Fisher's factorial approach, to genetic toxicology and the opportunities to use a range of multivariate methods for the analysis of toxicogenomic data will be discussed. The statistical issues arising from observational genotoxicity studies such as biomonitoring will be contrasted with those from experimental studies.
Closing Keynote Lecture

Interindividual risk of environmental genotoxicity, mutations and cancer—
a current assessment

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Every disease has an environmental component. The two most important factors affecting one’s unique risk of an environmental disease (toxicity or cancer) are [a] exposure to the environmental agent and [b] one’s genetic makeup. Epidemiologists can calculate interindividual risk—if the exposure to environmental agents is sufficiently high and quantifiable (e.g. years of cigarette smoking, taking prescribed drugs, drinking alcohol, or exposure to radon or other radioactive material, etc.). If the dose is lower and more ambiguous (e.g. exposure to chemicals on the job, herbicides sprayed on a golf course, outdoor or indoor air pollution, living near a toxic waste dump site, etc.), however, calculations of interindividual risk become much more difficult. Highly accurate DNA tests for genetic susceptibility to toxicity and cancer have been sought, in order to identify individuals at increased risk; thus, phenotype-genotype association studies are one major goal of public health and preventive medicine programs. Yet, the task has turned out to be far more challenging than anticipated. The major stumbling block has been the difficulty in determining an unequivocal phenotype or an unequivocal genotype. We were quite optimistic 5–10 years ago that this would be easy, but now we are beginning to appreciate how difficult it is to determine an unequivocal phenotype or genotype with certainty. For many reasons set forth in this Lecture, it appears that DNA testing alone, to predict and prevent environmental disease on an individual basis, may be virtually impossible with our current knowledge and technologies, and will require novel insights before major practical applications might evolve.
Posters
1. DNA Repair
P1

Association of the repair of DNA interstrand crosslinks with the nuclear matrix.


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Using in vitro damaged plasmids in the host cell reactivation assay have shown that repair of interstrand crosslinks was carried out in this system. To study the mechanism of the repair pathway, human cells were transfected with pEGFP-1, not containing the promoter of the egfp gene, and with pEGFP-G, not containing the egfp gene. Neither of these plasmids alone was able to express the fluorescent protein. After cotransfection with the two plasmids, 1-2% of the cells developed fluorescent signal. This indicated that recombination events had taken place to create DNA molecules containing the promoter and the gene properly aligned. Crosslinking of the plasmids with Trioxsalen increased the recombination rate several fold. To identify the nuclear compartment where recombination takes place, cells were transfected with crosslinked pEGFP-N1 and the amount of plasmid DNA in the different nuclear fractions was determined by PCR. The results showed that Trioxsalen crosslinking increased the percentage of matrix-attached plasmid DNA in a dynamic and dose-dependant way. Immunoblotting experiments showed that the majority of the homologous recombination protein Rad51 was also associated with the nuclear matrix fraction. These studies suggest that repair of DNA interstrand crosslinks may take place at the nuclear matrix.

P2

The cellular repair of oxidative DNA base modification IS NOT induced by preceding DNA damage induction

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The repair of various types of DNA modification is accelerated by a preceding induction of DNA damage. For base excision repair, this adaptive response was demonstrated for bacteria, but is still uncertain for mammalian cells. We therefore analysed the repair of (i) oxidative DNA base modifications induced by the photosensitizer Ro19-8022 plus light [mostly 7,8-dihydro-8-oxoguanine (8-oxoG)] and (ii) sites of base loss (AP sites) generated by Methylmethanesulfonate (MMS), both with and without exposure to a "priming" dose of one of the damaging agents. An alkaline elution assay in combination with repair glycosylases was used to quantify the DNA modifications. The results indicate that the repair rates of the oxidative base modifications in immortalized mouse embryonic fibroblasts were unchanged or even retarded by the pre-treatment with either agent. The exposure to Ro19-8022 plus light did neither induce the expression of the repair glycosylase Ogg1 nor the activity of total protein extracts of the cells to incise at a single 8-oxoG residue in an oligonucleotide. The decline of the level of AP sites generated by MMS, which probably represent repair intermediates of alkylated bases, was also little affected by a preceding MMS treatment. We conclude that the repair of 8-oxoG is not inducible by low levels of oxidative DNA damage or MMS generated damage.

P3

Dynamic properties of nucleosomes in vivo studied by photolyase in yeast

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Folding of DNA into nucleosomes and higher order chromatin structures restricts its accessibility to proteins (regulatory proteins, polymerases, DNA-repair enzymes). Therefore structural and dynamic properties of nucleosomes play an important role in gene regulation, replication, and DNA repair. We use DNA-repair by photolyase in yeast to investigate how stable nucleosomes are in living cells and how fast DNA can be recognized in nucleosomes. Yeast photolyase is a monomeric enzyme that recognizes UV-
induced cyclobutane pyrimidine dimers (CPDs) and reverts the CPD with the energy of light to restore the native bases. Previous work established that repair of nucleosomes in yeast requires about two hours while nucleosome free regions are repaired in fifteen minutes (Suter et al. (1997) EMBO J). By overexpression of photolyase, we show now, that nucleosomes can be repaired much more rapidly (~50% in few seconds). This rapid repair was observed in the non-transcribed strand of active genes, in inactive genes, in nucleosomal promoter regions of repressed genes and in silenced chromatin. These results challenge the repressive role of nucleosomes and provide new insights in the contribution of nucleosome dynamics to the regulation of DNA accessibility in living cells.

P4
Halogen substitution protects DNA from “cleavable complex” stabilization by the topoisomerase II poison amsacrine: On the importance of the enzyme for chromosome segregation

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AA8 Chinese hamster ovary cells were treated with halogenated nucleosides analogues of thymidine, namely CldU, IdU, and BrdU, following an experimental protocol that allows a controlled halogen-substitution for thymidine into DNA. The purpose was to see whether incorporation of exogenous pyrimidine analogues into DNA could interfere with normal chromosome segregation through a possible loss of topoisomerase II function. The endpoint chosen was endoreduplication, which arises after aberrant mitosis when daughter chromatids segregation fails. Treatment with any of the halogenated nucleosides for two consecutive cell cycles resulted in endoreduplication, with a highest yield for CldU, intermediate for IdU, and lowest for BrdU. The frequency of endoreduplicated cells paralleled in all cases the level of analogue substitution into DNA. Our results seem to support that thymidine analogue substitution into DNA is responsible for the triggering of endoreduplication. Besides, all of the halogenated nucleosides tested showed as able to provide a clear protection to DNA against DNA-strand breaks induced by the topoisomerase II poison amsacrine through stabilization of the so-called “cleavable complex”. Taken as a whole, these results seem to support the idea of a fundamental role of topoisomerase II for chromosome segregation as well as the importance of a normal DNA sequence for recognition and binding of the enzyme to DNA for its function.

P5
Compromised incision of oxidized pyrimidines in liver mitochondria of mice deficient in NTH1 and OGG1 glycosylases

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Mitochondrial DNA is constantly exposed to high levels of endogenously produced reactive oxygen species, resulting in elevated levels of oxidative damaged DNA bases. A large spectrum of DNA base alterations can be detected after oxidative stress, and many of these are highly mutagenic. Thus, an efficient repair of these is necessary for survival. Some of the DNA repair pathways involved have been characterized, but others are not yet determined. A DNA repair activity for thymine glycol and other oxidized pyrimidines has been described in mammalian mitochondria, but the nature of the glycosylases involved in this pathway remains unclear. The generation of mouse strains lacking murine thymine glycol-DNA glycosylase (mNTH1) and/or murine 8-oxoguanine-DNA glycosylase (mOGG1), the two major DNA N-glycosylase/apurinic/apyrimidinic (AP) lyases involved in the repair of oxidative base damage in the nucleus, has provided very useful biological model systems for the study of the function of these and other glycosylases in mitochondrial DNA repair. In this study, mouse liver mitochondrial extracts were generated from mNTH1-, mOGG1-, and [mNTH1, mOGG1]-deficient mice to ascertain the role of each of these glycosylases in the repair of oxidized pyrimidine base damage. We also characterized for the first time the incision of various modified bases in mitochondrial extracts from a double-knock-out [mNTH1, mOGG1]-deficient mouse. We show that mNTH1 is responsible for the repair of thymine glycols in mitochondrial
DNA, whereas other glycosylase/AP lyases also participate in removing other oxidized pyrimidines, such as 5-hydroxycytosine and 5-hydroxuracil. We did not detect a backup glycosylase or glycosylase/AP lyase activity for thymine glycol in the mitochondrial mouse extracts.

P6

Lipid peroxidation product, trans-4-hydroxy-2-nonenal, forms sequence-specific adducts to p53 gene, DNA-DNA and DNA-protein cross-links

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One of the most abundant products of lipid peroxidation, trans-4-hydroxy-2-nonenal (HNE) produces propano-type or etheno-type exocyclic adducts to all four DNA bases bearing long 6-7 carbon atom side chains. We studied the sequence-specificity of HNE interaction with cDNA of human p53 gene monitoring premature chain terminations on the template damaged with HNE. A plasmid bearing full length cDNA of human p53 gene was modified with 100 mM HNE for 16 hrs, pH 5.6, 37°C, and investigated repair by human N-methylpurine-DNA-glycosylase (ANPG), E.coli mismatch uracil-DNA glycosylase (MUG), as well as HAP1 endonuclease or Fpg protein. The presence of HNE-DNA adducts in the template stopped replication by T7 DNA polymerase in the following order: dG>dC>>dAdT. HNE adducts were identified in all mutational hot-spots within the p53 gene. Moreover, in all but one hot-spot (codon 273) inhibition of DNA synthesis was observed in both DNA strands at the same site, suggesting formation of interstrand HNE-DNA crosslinks. Cross-links constituted about 5% of all identified DNA damages, as shown both for plasmid p53 gene and HNE-modified oligonucleotide. We also observed binding of HAP1-endonuclease, ANPG and Fpg proteins to HNE-damaged DNA. For HAP1, this binding involved formation of DNA-protein cross-links by the aldehyde groups of the HNE-DNA adducts. This may be a new mechanism of HAP1 inhibition in the cell.

P7

Comparison of genotoxic and cytotoxic responses of mismatch repair-proficient (HeLa) and mismatch repair-deficient (HCT116) human tumor cells to methyl nitrosourea. Possible approach to evaluation of functional activity of mismatch repair and of cancer risk.

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Deficient mismatch repair (MMR) is identified as a mutation of one of four major MMR genes and/or microsatellite instability. These genomic changes are used as markers of MMR status of the heredity nonpolyposis colorectal cancer (HNPPC) spectrum tumors – familial and sporadic tumors of colon and extracolonic cancers fulfilling Amsterdam clinical criteria II. MMR-deficiency results in mutator phenotype and resistance to geno- and cytotoxicity of alkylating agents. The main cytotoxic damage to DNA in response to chemical methylation is O6-methylguanine (O6-MeG). The secondary DNA strand breaks which are formed during the MMR functioning are proposed to be required for methylation induced cytotoxicity. We have assumed the secondary double stand breaks (DSB) upon DNA methylation are able to represent functional efficiency of MMR in cells. The purpose of the study was to test this assumption on human tumor cells differing in MMR-status and pulse-treated with methyl nitrosourea (MNU). We used 3 cell lines: HeLa (MMR-competent endometrial tumor cells), HCT116 (MMR-deficient colorectal carcinoma cells) and Colo320 (sigmoid intestine tumor cells with uncharacterized MMR status). DSBs were evaluated with neutral comet assay. Cytotoxicity/viability was evaluated with MTT-assay and apoptotic index (frequency of morphologically determined apoptotic cells). We show that (1) cytotoxic effect of MNU (250 µM) on HeLa cells was exhibited 3 days after pulse-treatment of cells with MNU; (2) DSBs occurred at 48 h after the drug treatment but prior to the onset of apoptosis of HeLa cells; (3) MMR-deficient HCT116 cells were resistant to the drug: no decreased viability, DSBs and apoptosis were observed during 3 days after cell treatment. Both cell lines exhibited high sensitivity to etoposide, classical inducer of unreparable DSBs and p53. Etoposide has been found to induce DSBs in 6-12 h which was followed by apoptosis (at 24 h). Colo320 cells exhibited intermediate between HeLa and HCT16 cell lines sensitivity to MNU according to MTT-assay and number of secondary DSBs formed in MNU-treated cells. Nevertheless in contrast to HeLa cells these breaks did not induce apoptosis in Colo320 cells. Our data suggest the assumption about case/effect relationship between functional activity of MMR in human tumor cells,
secondary DNA double strand breaks induced by monofunctional methylating agent MNU and apoptosis of treated tumor cells.

**P8**

The role of Lys63-linked polyubiquitin chains in repair and mutagenicity of benzo[a]pyrene-diol-epoxide DNA adducts.


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Benzo[a]pyrene-diol-epoxide (BPDE) derived DNA adducts are not always successfully repaired prior to DNA replication, resulting in a blocked replication fork. To alleviate this stall, cells utilize the DNA damage tolerance (DDT) systems involving error-free damage avoidance or error-prone translesion synthesis (TLS). Studies in Saccharomyces cerevisiae, suggest the involvement of Lys63-linked mult ubiquitination as a key mediator of the error-free pathway. We hypothesize that disruption of Lys63-linked multi-ubiquitination thus causing increased BPDE-induced mutagenicity. To test this hypothesis, we generated A549 cells expressing a mutant ubiquitin (UbK63R) which blocks further elongation of K63-mediated chains. 32P-Postlabeling-assessed removal of BPDE-DNA adducts showed no differences compared with cells expressing the wildtype control (UbWT), indicating that the nucleotide excision repair capacities of both cell lines are comparable. UbK63R and UbWT cells were equally sensitive to the cytotoxic effects of BPDE while significantly higher frequencies of BPDE-induced mutations at the HPRT-locus were observed. In addition, this increased mutagenesis in UbK63R cells was paralleled by enhanced Polη-FOCI formation. In conclusion, our data indicate that disruption of K63-linked multi-ubiquitination inhibits the error-free pathway of DDT, causing increased BPDE-induced mutagenicity involving recruitment of the Y-family polymerase Polη.

**P9**

A modified comet-assay to assess nucleotide excision repair


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There is an increasing need for simple and reliable approaches to phenotypically assess DNA repair capacities. Therefore, a modification of the alkaline Comet-assay was developed to determine the ability of human lymphocyte extracts to perform the initial steps of the nucleotide excision repair (NER) process, i.e. damage recognition and incision. Gel-embedded nuclei from A549 cells, pre-exposed to 0.1 µM benzo[a]pyrene-diol-epoxide (BPDE), were incubated with cell extracts from frozen or freshly isolated lymphocytes. The rate at which incisions are introduced and the subsequent increase in tail moment is indicative for the repair capacity. Freshly prepared extracts from lymphocytes of human volunteers (n=10) showed significant inter-individual variations in their DNA repair capacity, which seemed to correlate with the removal of bulky DNA lesions over a period of 48 hours determined by 32P-postlabeling. Storage of cell extracts for more than three weeks significantly reduced the capacity to incise the damaged DNA; up to 80% reduction was observed compared to freshly isolated extracts. This reduction was completely restored by adding ATP to the extracts before use, as it is required for the incision step of NER. Extracts from DNA repair deficient XPA and XPC fibroblasts will be used to further validate this assay. These first results demonstrate that this modified Comet-assay can be applied to assess inter-individual differences in NER in human cells and tissues.

**P10**

Study on the DNA repair mechanisms involved in the removal of PAHs induced lesions

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If dietary exposures to mixtures of potentially carcinogenic pollutants generating DNA reactive metabolites do indeed have a significant impact on human cancers, it should be possible to detect specific
damages in human DNA resulting from their presence in food and drink. Exposure to PAHs seems to represent one of the few available practical options relevant for the induction and prevention of diet-related cancer in humans where the results can be directly verified in vivo. Chromosomal damage (chromosomal aberrations and sister chromatid exchanges, SCEs) results from mis-repaired DNA lesions. In a series of experiments the extent of induction of chromosomal aberrations, SCEs and apoptosis induced by benzo(a)pyrene diol-epoxide (BPDE) and fluoroanthene diol-epoxide (FADE) were studied in CHO cell lines deficient in one of the different DNA repair pathways, namely, base excision repair (BER), nucleotide excision repair (NER), transcription coupled excision repair (TCR), homologous recombination (HR), non-homologous end joining (NHEJ), respectively. The results obtained suggest an important role of nucleotide excision repair, for the induction of chromosomal aberrations and SCEs by BPDE and FADE. In addition, homologous recombination also seems to play a role in cellular resistance to BPDE and FADE affecting not only the induction of chromosomal aberrations, but also apoptosis. NHEJ appears not to have any significant role for the induction of both the end-points under study.

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P11

Markers of oxidative stress in the blood of workers occupationally exposed to fine particles (PM 2.5).

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It is supposed that exposition to fine particles is associated with increased cardiovascular and pulmonary diseases mortality. The aim of study was assessment of oxidative stress markers in the blood of workers occupationally exposed to PM 2.5. The study was conducted on 56 men in the age between 23 and 43, who were workers of the same pottery production company. The blood was collected from workers three times: before first shift (after weekend), after first shift and after fourth shift. The control group consisted of 34 healthy, non-exposed men. The final results showed differences in the activities of antioxidant enzymes between PM 2.5 exposed and non-exposed workers. The activity of superoxide dismutase (SOD) in erythrocytes was higher compared to control subjects (p < 0.05) in contradiction to the activity of glutathione peroxidase (GSH-Px) in erythrocytes, which occurred to be lower (p < 0.0001). Lipid peroxidation measured by TBARS concentration was higher compared to control subjects (p < 0.0001) and was increased after fourth shift. The total antioxidant status was negatively correlated with the concentration of particles in the air (r = -0.226; p < 0.05).

In conclusion, exposition to PM 2.5 affects the antioxidant system, which may be involved in the ethiology of cardiovascular and pulmonary system diseases.

P12

DNA-PKcs-dependent recruitment of Artemis at DNA double-strand breaks

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DNA double strand breaks (DSBs) represent toxic lesions induced by ionizing radiation (IR) which are mainly repaired by non-homologous end-joining (NHEJ) in mammalian cells. Five core components of the NHEJ machinery have been identified, namely Ku70, Ku80, the protein-kinase DNA-PKcs, XRCC4 and DNA ligase IV. In humans, the deficiency in another protein, Artemis, is also responsible for a Severe Combined Immuno-Deficiency (SCID) syndrome associated with increased radiosensitivity. Although it has been suggested that Artemis could be responsible for the processing of some kinds of IR-generated DSBs, no biochemical experiments have yet substantiated this hypothesis.

Our study aimed at establishing biochemical evidence for a role of Artemis in DNA DSB-repair. With assays set up in our laboratory (1,2), we have studied, both in extracts and in cells, the recruitment of Artemis in protein complexes to DNA DSBs and the influence of the protein-kinase activity of DNA-PKcs on this recruitment. Both in cell extracts and in the cells, we observed the recruitment of Artemis as a phosphoprotein on DNA in the presence of DSBs. A fraction of Artemis existed as a complex with DNA-PKcs and we showed that DNA-PKcs was physically required for Artemis loading onto DNA. We have established that the catalytic activity of DNA-PKcs was also required for the stable association of Artemis
to damaged DNA. In conclusion, we have established that Artemis is part of the repair proteins assembled at DSB sites in damaged DNA and that DNA-PIKS is a key factor of its recruitment, both by its scaffolding and its protein-kinase functions.


P13
Effect of poly (ADP-ribose) polymerase deficiency in the spectrum of mutations induced by MNU in transgenic mice

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Poly (ADP-ribose) polymerase-1 (PARP-1) is involved in DNA repair and cell death. The objective of this work was to evaluate the effect of in vivo PARP-1 deficiency on mutagenic response to an alkylating agent. Transgenic mice harbouring a LacZ mutational target gene were crossed to PARP-1−/− mice to obtain PARP-1−/−LacZ−/− mice. Groups of 7-9 PARP-1−/−LacZ−/− and PARP-1−/−LacZ+/+ mice were injected with 30 mg/kg of N-Methyl-N-Nitrosurea (MNU) or saline and sacrificed 28 days later. The LacZ mutant frequency (MF) was determined in the mice tests and the restriction patterns of the mutant plasmids were analysed. Similar spontaneous MF were observed in PARP-1+/+LacZ+/+ and PARP-1−/−LacZ+/+ mice. MNU induced a near 1.6-fold significant increase in MF in both, PARP-1+/+LacZ+/+ and PARP-1−/−LacZ+/+ mice. Restriction pattern of the mutants analysed revealed however, a significant difference in the spectrum of MNU-induced mutations. While MNU induced mostly point mutations in PARP-1+/+LacZ mice, in PARP-1−/−LacZ mice the increase in MF was mainly due to deletions/insertions. These results indicate that PARP-1 is involved in suppressing deletion mutations in vivo.

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P14
Characterization of frequent splice variants of DNA polymerase beta in gastric cancer

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DNA polymerase beta (Pol b) is the main polymerase of base excision repair in mammals. Inactivation or overexpression of Pol b might lead to genomic instability, thus increasing cancer risk. We screened genomic DNA from normal and tumour gastric tissues for mutations in Pol b. Sequence analysis of eight DNA samples did not reveal any mutation, neither in exon sequences nor in splicing junctions. RNA analysis of samples from twenty patients showed the presence of numerous splicing variants, in both normal and tumour tissues. Among the most frequent exon lost (2, 5, 6, 9, alfa), exon 2 skipping was the predominant event, in some cases, more represented in tumour than normal tissues. Exon 2 skipping is predicted to produce a 26-aminoacids protein, likely avoided of catalytic activity. We hypothesise that Pol b splicing variants could be implicated in a regulatory mechanism of Pol b expression. To test this hypothesis we constructed a mammalian expression vector containing the exon 2-deleted Pol b c-DNA and transfected it into Pol b-defective murine fibroblasts. The vector-containing clones were characterised by RT-PCR and western blot. The exon 2 variant is transcribed in the recipient cells. Experiments are in progress to verify the translation of this variant and analyse its functional activity by appropriate in vitro assays.
P15
Preferential in vivo DNA repair of melphalan-induced damage in human genes is greatly affected by the local chromatin structure

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To investigate the mechanisms of DNA repair in humans following exposure to genotoxic agents, we studied the in vivo kinetics of melphalan-induced monoadducts and interstrand cross-links in genes with different transcriptional activity (b-actin>p53>N-ras>d-globin) from leukocytes of multiple myeloma patients following therapeutic treatment. The rate of gene-specific repair varied in the order: b-actin>p53>N-ras>d-globin and correlated with the gene transcriptional state. Following in vitro treatment of human lymphocytes with α-amanitin, a significant inhibition of the removal of melphalan-induced damage in the three active genes but not in the silent d-globin gene was found, suggesting that transcription and/or chromatin structure may play an important role in the preferential DNA repair. When the in vivo formation and repair of melphalan-induced lesions was measured in the two strands of the active genes, no strand bias was found, suggesting that the global genome repair subpathway of nucleotide excision repair with little if any contribution of transcription-coupled repair predominates. Using micrococcal nuclease digestion, we probed the chromatin structure in each gene and found that the “looseness” of the chromatin structure was in the order: b-actin>p53>N-ras>d-globin. These results indicate that the preferential in vivo DNA repair of melphalan-induced damage in humans is greatly affected by the local chromatin structure.

P16
The repair efficiency of O^6^-methylguanine is affected by the state of chromatin condensation but has a complex relationship with the cell content of O^6^-alkylguanine DNA alkyltransferase

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Following in vitro exposure of HepG2 cells with various doses of N-nitrosodimethylamine (NDMA), O^6^-methylguanine (O^6^-meG) was lost from the DNA over two phases with substantially differing repair rates. The first phase of repair was inhibited by α-amanitin, a known inhibitor of RNA polymerase II-mediated transcription, which can also induce chromatin condensation. Similar biphasic repair kinetics were also observed in four rat tissues (liver, white blood cells, lymph nodes, bone marrow) after in vivo treatment with NDMA. In this case, no dependence on transcriptional activity was found when O^6^-meG repair in specific gene sequences with different transcriptional status in rat liver was examined, suggesting that the effects of α-amanitin in HepG2 cells did not reflect inhibition of preferential repair of transcribed sequences. Surprisingly, the rates of O^6^-meG repair were similar regardless of the O^6^-alkylguanine DNA alkyltransferase (AGT) content of the different tissues. Repair was also examined in rat liver hepatocytes and non-parenchymal cells separately after in vivo administration of NDMA at non-AGT depleting doses. Within each cell-population, the repair followed single phase, first-order kinetics, with adduct loss from AGT-rich hepatocytes being significantly faster than from the relatively AGT-deficient non-parenchymal cells. However, in this case, practical complications did not allow the examination of the O^6^-meG in the early time-points where rapid repair had been observed in the other studies. To conclude, the results presented herein suggest that the repair efficiency of O^6^-meG is profoundly affected by the local chromatin structure but has a complex relationship with the cell content of the MGMT.
P17
Genetics and molecular biology of the Fanconi anaemia/BRCA genome stability pathway

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Fanconi anaemia (FA) is a rare genetic disease characterized by progressive anaemia, birth defects, chromosome fragility and a high predisposition to cancer, including leukaemia and solid tumours. The frequency of carriers is about 1/300, but it increases in consanguineous ethnic groups such as the Spanish gypsies with a carrier frequency of 1/65 (1). At least 11 genes are involved in this disease and their products interact in the so called FA/BRCA pathway of genome stability and tumour suppression. In addition, FA proteins interact with a number of proteins involved in other chromosome fragility syndromes in a network of genome stability pathways (2). These syndromes include Bloom, Seckle and Nijmegen breakage syndromes and ataxia telangiectasia. Here I will summarize our recent finding on the molecular biology of the FA/BRCA. Our biochemical, immunocytochemical and YFP-tagged FANCD2 time lapse confocal data suggest that this pathway is activated not only by interstrand cross-links but also by UVC-induced stalled replication forks in a highly regulated manner. We will also provide genetic and chromatin immunoprecipitation results suggesting that H2AX is a novel component of the FA/BRCA pathway downstream FANCD2 activation as cells deficient in H2AX or expressing a non-phosphorylable H2AX fail to relocate FANCD2 to the site of damage and show a FA like phenotype. Resembling other genome stability syndromes (3), FA cells show a telomere defect (4). Recent evidences for a novel molecular role for FANCD2 at telomeres will be presented.

(1) Callén et al., Blood, 2005
(2) Surrallés et al., Genes and Development, 2004
(3) Callén and Surrallés, Mutation Research Reviews, 2004
(4) Callén et al., Human Molecular Genetics, 2002

P18
The role of PARP-1 in DNA double strand break repair

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We examined the role of PARP-1 (poly(ADP-ribose) polymerase-1) in repair of DNA double strand breaks (DSB). DSB are repaired by nonhomologous end-joining (NHEJ) and by homologous recombination (HR). CHO-K1 wild type and xrs-6 mutant cell line (NHEJ-defective) were transfected with pLrec plasmid carrying two non-functional copies of the β-galactosidase (lacZ) gene in a tandem array. In result of recombination they give rise to a functional copy of β-galactosidase. Isolated transfected clones were used to examine the effect of ADP-ribosylation inhibition on the frequencies of spontaneous and X-ray (2 Gy) induced recombination. The cells were incubated with the PARP-1 inhibitor and recombination frequency was determined with histochemical or flow cytometry methods. The level of β-galactosidase activity (reflecting the frequency of spontaneous recombination) in transfected CHO-K1 cells was 2-3 times lower than in xrs-6 cells, whereas frequency of recombination per generation measured by flow cytometry was one order of magnitude lower. Irradiation with 2 Gy of X-rays insignificantly elevated the level of the enzyme in both cell lines. These results suggest that the defect in NHEJ-mediated DSB repair results in elevated frequency of HR. No effect of inhibition of poly(ADP-ribosylation) was observed in this experimental model.
2. Mitosis, meiosis and chromosome integrity
P19
Deregulation of the sequence of centromere separation in the metaphase anaphase transition leads to chromosome instability and aneuploidy

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In a four dimensional cascade of cyclin regulated cell cycle kinetics chromosomes have an essential role in maintaining the stability of the fluctuating genome. Human chromosomes are non randomly organized in space and more recent data shows a temporal organization of the replication, separation and segregation processes of chromosomes. Segregation of the chromosomes in anaphase is preceded by a sequential order of separation. Centromere separation is a genetically controlled process in which certain chromosomes are first to separate. In humans the first chromosome to separate and thus segregate is chromosome 18. In an ordered sequence chromosome 17, 2, 10, 12 etc. follow the separation of chromosome 18. This sequence can be deregulated in ageing cells, Alzheimer’s disease patients, various tumors, chromosome instability syndromes and by chemical induction. Peripheral blood lymphocytes of 10 healthy individuals were exposed to Cycloheximide in a dose of 10 µg/ml. To differentiate the time of premature centromere separation (PCS) in the cell cycle we used fluorescent in situ hybridization (FISH) for the α-centromeric region of the 18 chromosome. In parallel of estimating the phase of the cell cycle in which PCS occurs we also investigated any alteration of the sequence of centromere separation and for the detection of aneuploidy we used the CB- micronucleus test in vitro. Our results show that PCS can occur much earlier than previously known (metaphase), i.e. in the interphase of the cell cycle, immediately after the replication, but Cycloheximide does not alter the sequence of centromere separation. We argue that the phenomenon of premature centromere separation can be perceived as a deregulated spatial and temporal control of replication, separation and segregation of human centromers which may lead to genome instability and aneuploidy.

P20
Cytogenetic analysis by CGH and Q-banding of three human colon adenocarcinoma cell lines in two and three dimensional culture systems


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Multicellular tumor spheroids (MCTS), a three dimensional culture system (3D), are providing new insights into tumour biology. MCTS are an “organ-like in vitro model” of intermediate complexity between monolayer cultures in vitro (2D) and tumours in vivo. The aim of the present study was to investigate in three human colon adenocarcinoma cell lines (LoVo WT, Ht-29 and HCT116 E6) whether chromosome alterations in the 2D and 3D culture systems were present and could provide guidelines for evaluation of different growth in the two different cell cultures systems.

In MCTS the cell cycle, after 7 days culture, arrests in G1 phase, making impossible to analyze the karyotype. We therefore analyzed the three cell lines by using CGH to evaluate possible differences regarding chromosomal imbalances. Our results did not show any specific rearrangements in the MCTS vs. the monolayer cultures, suggesting that in vitro growth differences between the two models may be attributed to mechanisms different from genomic rearrangement.
3. Molecular and cellular mechanisms of mutagenesis: Metabolism, DNA damage responses
**P21**

Evaluation of indirubin as endogenous ligand of the aryl hydrocarbon receptor

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The aryl hydrocarbon receptor (AhR) plays an important role in the activation of many xenobiotics including chemical carcinogens. Binding of the ligand like β-naphthoflavone to AhR results in its translocation into the nucleus and expression of several genes responsible for activation and detoxification of carcinogens, regulation of the cell cycle or apoptosis.

Despite numerous studies on the AhR, this protein is still recognized as an orphan receptor, e.g. receptor whose physiological ligand remains unknown. Recently indirubin was reported to activate the AhR in yeast cell bioassay system and was postulated to be its physiological ligand. To verify this hypothesis, in this study mice were treated i.p. with different doses of indirubin or β-naphthoflavone. The activity and expression of cytochrome P450 1A1 and NADPH:quinone oxidoreductase, enzymes regulated by AhR, was evaluated in hepatic microsomes. Indirubin showed up to be much weaker inducer of AhR dependent enzymes than β-naphthoflavone in this in vivo system. Thus, although indirubin can be considered as AhR agonist, is not rather its physiological ligand.

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**P22**

Genotoxic stress activation of GADD45a in human cells is dependent on the integrity of a p53 response element in the third intron.

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Gadd45a protein mediates key processes required to maintain genomic stability in the cellular response to genotoxic stress, including cell cycle arrest, DNA damage repair, and apoptosis. Gadd45a may also play a role in anti-tumorigenesis. The regulation of GADD45a transcription is complex, involving a number of key Tumour suppressors and oncogenes including BRCA1, p53 and c-Myc.

The contribution of the GADD45a proximal promoter to gene regulation has been well studied. The lack of appropriate molecular tools has hampered investigation of other regulatory pathways. In this work a number of novel GADD45a reporters based in the human lymphoblastoid cell lines have been used to analyse the role of the third intron in the transcriptional regulation. The integrity of the p53 response element was found to be essential for genotoxic dependent gene induction. The reporters created in this work represent a powerful tool for studying the molecular biology of the cellular response to genotoxic stress. The simple assay methods that can be used with these reporters also give them the potential for exploitation in pre-regulatory genotoxicity screening. This presentation will provide data demonstrating how the genotoxic signal is enhanced by exploitation of a combination of regulatory features. It will also provide data from assay following exposure to a number of key compounds of interest to genetic toxicologists.

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**P23**

Telomeric length as modulating factor of genetic damage in newborns

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Telomeres are structures placed at the ends of each chromosome arm constituted by a short nucleotide sequence tandemly repeated. Its function is to avoid the end to end chromosome fusions, protect the chromosome from different injuries and to maintain the genomic stability. It is known that different proteins associated to the telomeric D-loop structures are involved in repair, which reinforces the hypothesis linking telomere integrity and genomic stability.

This work plans to correlate individual telomeric length with the levels of genetic damage, both basal and induced by genotoxic agents, to determine whether telomeric length can be considered as an individual
factor of genotoxic risk. To validate this hypothesis we have used lymphocytes from cord blood samples from 70 newborns. In these samples, the genetic damage has been measured by using the micronucleus assay both spontaneously and after treatment with mitomycin C. Telomeric length has been measured by using the TRF-Southern technique.

This experiment has been carried out by duplicated (35+35). Results from the first replicate indicate that those subgroups constituted by individuals with shorter (9 cases) and longer (11 cases) telomeres have basal (5.88 and 2.45, respectively) and induced (142.11 and 100.64, respectively) levels of micronuclei significantly different. Donors with short telomeres show higher frequency of genetic damage. These results would reinforce the hypothesis indicating that telomeric length can be considered as an individual susceptibility factor.

This work has been carried out in the frame of the Childrengenonetwork (QLK4-CT-2002-02198)

P24


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p53 is found mutated in about 50% of all tumor types. Since p53 plays a crucial role in cell cycle arrest, DNA repair and apoptosis, the question of which functions are affected and which are retained by any p53 mutant protein is an important issue in terms of the significance of p53 mutations in cancer. Our aim is to investigate, in lung cancer cells, the role of wild type and mutant p53 in the activation of some known p53 target genes and to make a correlation with apoptosis induction.

In A549 (p53wt), LX1 (p53R273H), and SKMes1 (p53R280K) cell lines we determined i) the kinetics of p53-binding and histone acetylation at the promoter of p21, mdm2, bax and PUMA genes by Q-ChIP and ii) the expression levels of the same genes by RT-PCR. In LX1 and SKMes1, mut p53 was unable to bind p21, bax, mdm2, and PUMA promoters after UV. However, the mRNA expression of the same genes did not always correlate with p53 binding ability. After UV, both A549 and LX1 cells were undergoing apoptosis and accumulated in S-phase, while the SKMes1 cell line was refractory to apoptosis as well as to cell cycle arrest. The possibility to reactivate the mutant p53 is under investigation.

P25

Toxicity of benzo[a]pyrene is associated with production of reactive oxygen species, p53 induction and caspase-3-like protease activity in MCF-7 cells.

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Benzo[a]pyrene (BaP), a genotoxic compound, is a potential human carcinogen. Reactive BaP metabolites, e.g. benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), bind to DNA producing DNA adducts. In addition, increased production of reactive oxygen species (ROS) may also be involved in BaP-induced DNA damage. To further clarify mechanisms of BaP-induced toxicity, we studied the effects of BaP on ROS production, p53 protein levels, cell viability and caspase activity in human MCF-7 breast adenocarcinoma cells. Cells were exposed to BaP (0, 0.1, 1 and 10 µM), and at various time points (24, 48 and 72 h) ROS production and cell viability were determined by using fluorescent probes dichlorodihydrofluorescein diacetate and propidium iodide, respectively. BaP increased ROS production and cytotoxicity within 72 h dose- and time-dependently. In accordance with our earlier studies, immunoblotting of p53 revealed that the amount of this tumor suppressor protein increased in cells exposed to BaP. Caspase-3-like protease activity, a marker of apoptosis, increased in cells exposed to 10 µM BaP as determined by measuring the cleavage of a fluorogenic caspase substrate (Ac-DEVD-AMC). Our results do not rule out the possibility that increased ROS production, in addition to BPDE-DNA adducts, is involved in BaP-induced toxicity, e.g. in DNA damage. Since MCF-7 cells are known to be caspase-3-deficient, our results suggest that other caspases of p53-dependent apoptotic pathway are activated by BaP in these cells.
P26
Comparative analysis of the effect of in vivo PARP-1 deficiency and PARP inhibition on radiation-induced chromosome instability

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The in vivo effect of chemical inhibition of poly (ADP-ribose) polymerase (PARP) and of PARP-1 inactivation on the clastogenicity of ionizing radiation was compared. Groups of PARP-1+/+ C57Bl/6 mice were injected with either PBS or a PARP-inhibitor, 3-aminobenzamide (3-AB) or 5-aminoisoquinolinone (5-AIQ), and irradiated with 3 Gy gamma-radiation. A group of PARP-1−/− mice was submitted to the same radiation dose. Micronucleated polychromatic erythrocytes (MN-PCE) were analysed 48h after irradiation. The level of PARP inhibition was assessed by measuring PARP activity in spleen of PARP +/+ mice, and was found to be significantly stimulated following gamma-irradiation. Neither 3-AB nor 5-AIQ displayed significant inhibitory effects in the absence of the genotoxic insult, but 3-AB modulated the rise in PARP activity induced by radiation. Both inhibitors failed to affect the spontaneous or radiation-induced MN-PCE levels. In contrast, following irradiation, PARP-1−/− mice revealed a significant increase in MN-PCE comparatively to PARP+/+ mice. Our data shows that the total ablation of PARP-1 activity in vivo amplifies the level of radiation-induced chromosome breakage, most likely due to an impairment of DNA repair, which could not be compensated by the other PARP-related proteins. On the other hand, inhibition of PARP proteins at the levels achieved by inhibitors treatment was not able to influence the normal cellular clastogenic response to radiation.

P27
Role of Brca2 in the formation of Mitomycin C-induced chromosomal aberrations and sisterchromatid exchanges

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The XRCC11 mutant V79 Chinese hamster cell line V-C8 is a compound heterozygote for Brca2 mutations and displays a.o. a) enhanced spontaneous as well as Mitomycin C-induced chromosomal aberrations (CA), b) lack of sce induction by Mitomycin C and c) abnormal centrosome functioning (Kraakman-van der Zwet et al. 2002). In order to analyze a possible effect of Brca2 haploinsufficiency, we recently generated V-C8 revertants with one wild type and one disrupted Brca2 allele. Studies on chromosomal aberrations and SCEs indicated:
1. Clear partial correction for the revertants of spontaneous and induced structural CA, indicating haploinsufficiency.
2. No further correction for CA upon introduction of human #13 carrying the BRCA2 gene.
3. Partial correction of centrosome functioning in the revertants, which was paralleled by the degree of aneuploidy.
4. Full revertant correction of sce formation indicating no strict correlation between sensitivity for sce’s and CA.
5. Linear dose response kinetics for chromosomal exchanges in all cell lines suggesting either single hit events being responsible for exchanges or lesion non-lesion interactions.


P28
Biomethylated arsenicals interfere with poly(ADP-ribosyl)ation in cultured human cells.

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Arsenic is known as one major health problem in many parts of the world. Thus over 100 million people are exposed against arsenic via drinking water, which causes a multitude of diseases including different types of cancer. In the last two years numerous studies provided evidence that biomethylated arsenic metabolites contribute to inorganic arsenic induced genotoxicity.
In the present work we compared the impact of different arsenic compounds on poly(ADP-ribosyl)ation in HeLa S3 cells. Poly(ADP-ribosyl)ation is predominantly mediated by PARP-1, a zinc finger protein involved in DNA repair. Poly(ADP-ribosyl)ation was measured by detecting poly(ADP-ribose) immunologically with fluorescence-coupled antibodies and quantified by fluorescence microscopy. All trivalent arsenicals inhibited H2O2-induced poly(ADP-ribosyl)ation at nanomolar concentrations with the methylated metabolites inhibiting poly(ADP-ribosyl)ation at 10 times lower concentrations as compared to arsenite. Pentavalent metabolites had no effect on poly(ADP-ribosyl)ation even at cytotoxic doses. Further experiments applying Real-Time RT-PCR demonstrated that these effects were not due to changes in gene expression of PARP-1. One potential reason for inhibition of poly(ADP-ribosyl)ation consists in the interaction of trivalent arsenicals with the zinc finger domains of PARP-1. Since zinc finger proteins are involved in many processes essential for maintaining genomic stability, their inactivation could contribute to genotoxic and carcinogenic effects of arsenic.
4. Mutagen / carcinogen-induced cell death
P29
Evaluation of the nuclear DNA Diffusion assay to detect apoptosis and necrosis

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We applied the nuclear DNA Diffusion assay, described as an accurate estimation of apoptotic and necrotic cells, to tobacco root and leaf cells. In the Diffusion assay, isolated nuclei are embedded in an agarose microgel on a microscope slide and low molecular weight DNA fragments diffuse into the microgel. Hydrogen peroxide exposure to roots significantly increased the averaged nuclear area of isolated nuclei. After 4 and 24 h of recovery, all DNA damage was repaired. The data clearly demonstrate that the manifestation of diffused nuclei by hydrogen peroxide are not the result of non-repairable apoptotic or necrotic DNA fragmentation, but are repairable genotoxin-induced DNA damage. In contrast, treatment with the alkylating agent ethyl methanesulphonate (EMS) and an additional 24 h of recovery produced a significant increase in the averaged nuclear area. Heat treatment of leaves at 50°C for 1 to 15 min leading to necrosis, and treatment of isolated nuclei with DNase-I that digests DNA to nucleosomal-sized fragments as during apoptosis, also led to a dose dependent increase in the nuclear area. The use of different fluorochromes (ethidium bromide, DAPI or YOYO-1) for staining DNA yielded similar results in the Diffusion assay. As all types and sizes of diffused nuclei were observed after hydrogen peroxide and EMS treatments, we were unable to differentiate, according to the structure of the nuclei, between apoptotic or necrotic DNA fragmentation and other types of genotoxin-induced DNA damage.

P30
Possible role of apoptotic and anti-apoptotic signals for the carcinogenic effects of PAH

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Qualitative as well as the quantitative characteristics of DNA-damage determines it’s potential to be properly repaired, result in apoptosis or cause mutations. Apoptosis is considered to have an anti-carcinogenic function by removing cells with extensive DNA-damage and by balancing cell proliferation. Here we show that B[a]P cause apoptosis in Hepa1c1c7 cells. B[a]P exposure resulted in an accumulation of p53 as measured by Western blotting. No changes were observed in the protein levels of Bax and Bcl-2, whereas the anti-apoptotic Bcl-xl protein was down-regulated. Fluorescence microscopic analysis revealed a translocation of p53 to the nucleus and of Bax to the mitochondria. Use of inhibitors suggested that p53 was pro-apoptotic whereas ERK1/2 seemed to be anti-apoptotic. Although B[a]P markedly increased the phosphorylation of p38 and JNK, these MAPK’s were apparently not important for the induced apoptosis. The results with the reactive metabolite B[a]PDE supported the above findings. Interestingly, the parent compound B[a]P increased anti-apoptotic signals (phospho-Akt and phospho-Bad) independently of the formation of reactive metabolites. In conclusion, DNA-damage induced by reactive B[a]P-metabolite(s) resulted in apoptotic as well as anti-apoptotic signals, whereas the parent compound seemed to give anti-apoptotic signals. An implication of this could be that cells having B[a]P-induced DNA damage that normally would result in apoptosis nevertheless may survive, but with an increased risk of having mutations.

P31
Molecular markers involved in methylating agent-induced cell death in an O6-methylguanine- DNA-methyltransferase inducible HeLa cell line

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Recent studies have shown an extensive involvement of RNA-binding proteins of the post-transcriptional machinery in apoptotic processes induced in human cell lines. To investigate the type and extent of alterations occurring on RNA binding proteins during methylating agent –induced apoptosis and assess their possible use as markers of tumor resistance/sensitivity to chemotherapy, we employed N-methyl-N-
nitrosourea (MNU) on a O6-methylguanine-DNA-methyltransferase (MGMT)-inducible HeLa Tet-On cell line. Cells were treated with different concentrations of MNU under varying expression levels of MGMT (0 - 30 fmol/ug DNA). Equimolar cytotoxic doses for each condition were determined through clonal survival assay. Cells completely lacking MGMT were far more sensitive to MNU treatment than those with even low levels of the DNA repair enzyme. Further on, FACS analysis after double DIOC6/PI staining confirmed the protection provided by MGMT against MNU-induced apoptotic death. Molecular analysis through Western blotting revealed PARP cleavage and DNA fragmentation as late events, prominent 72 hours following MNU treatment. Interestingly, no caspase-3 cleavage was observed under the same conditions in which PARP cleavage was observed. Concerning protein species of the post-transcriptional apparatus, we have identified poly(A) polymerase (PAP) cleavage uniquely occurring in cells lacking MGMT which was initiated at 48 hours after treatment while no modifications related to hnRNP A1 and A2/B1 proteins were observed. We are currently investigating the molecular apoptotic pathway activated and additional RNA binding proteins are being examined for possible involvement in the MNU-induced apoptotic pathway.

P32
Assessment of Nuclear Factor Kappa B (NF-κB) involvement in apoptosis induced by selected proapoptotic compounds

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The results of numerous studies provide evidence that after inhibition of NF-κB activity the level of apoptosis in some cancer cells increases. However, the role of NF-κB in the induction of apoptosis is still unclear and it likely depends on the cell type and the type of inducer. The aim of our study was to investigate the role of NF-κB in apoptosis induced by etoposide and arsenite. Cytotoxicity of selected compounds on Jurkat cells was assessed with MTT reduction assay. Sulindac and pirrolidine dithiocarbamate (PDTC) were used as inhibitors and phorbol-myristate acetate (PMA) was used as an activator of NF-κB.

We observed that cytotoxicity of the compounds in the presence of sulindac (0.5 mM) was significantly increased. In TUNEL staining significant increase in the number of apoptotic cells was shown (control cells 2±0.9%, sulindac 2±0.5%, arsenite 24±8%, arsenite+sulindac 93±5%, etoposide 30±12%, etoposide+sulindac 41±9%). The changes were associated with increased activity of caspase-3 in the exposed cells. PDTC in non-cytotoxic concentration of 10 μM had no effect. PMA (100 ng/ml) showed clear protection against cytotoxicity of the chemicals on Jurkat cells. It significantly decreased the number of apoptotic cells (control cell 2±0.4%, PMA 1±0.8%, arsenite 16±6%, arsenite+PMA 5±7%, etoposide 29±9%, etoposide+PMA 16±6%), and caspase-3 activity.

The results suggest that activation of NF-κB may protect Jurkat cells against proapoptotic effects of the chemicals while its inhibition may enhance the cytotoxicity.
5. Toxicogenomics
P33  
Clonogenicity and gene expression modulation in the bone marrow of mice chronically exposed to arsenic and atrazine.

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The clonogenicity of myeloid progenitors (CFU-GM) and the modulation of gene expression of 1185 cancer-related genes by DNA-microarrays in bone marrow were used to investigate in male and female mice the combined effects of continuous exposure to arsenate and atrazine in drinking water. In male mice, the exposure to arsenate or to atrazine alone and the combined exposure did not change the clonogenicity of the progenitors. In females the percentage of CFU-GM decreased significantly after atrazine exposure, did not change with arsenic treatment, but dramatically increased after the combined exposure to the two chemicals. Results from microarrays indicate that atrazine alone didn’t stimulate the expression of any of the cancer genes analyzed in both male and female. Arsenic induced gene expression modulation only in female and had no effects on male. Major significant changes on the gene expression in bone marrow cells resulted following the co-exposure to arsenic and atrazine in both male and female. THESE RESULTS INDICATE THAT CO-EXPOSURE OF MICE TO ATRAZINE AND ARSENATE INDUCES SIGNIFICANT EFFECTS AT THE LEVEL OF TRANSCRIPTIONAL ACTIVATION OF GENES IN BONE MARROW CELLS, AS WELL AS STIMULATING THE MYELOID PROGENITORS TO PROLIFERATE, PARTICULARLY WHEN CO-ADMINISTERED IN DRINKING WATER TO FEMALE MICE.

P34  
Microarray analysis of the effect of benzo(a)pyrene exposure on MCF-7 and HepG2 cells

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Microarray technology is a tool for identifying gene expression changes in response to chemical exposure. Microarrays of 22K human genome-wide cDNAs were used to measure gene expression changes in MCF-7 and HepG2 cells following exposure to the carcinogen benzo(a)pyrene (BaP), or its non-carcinogenic isomer benzo(e)pyrene (BeP), in order to identify genes/pathways involved in the carcinogenic process. Treatment concentrations of BaP (0.25-2.50 nM) were selected that gave no cell cytotoxicity and a dose-response in DNA adduct formation up to 48h. In MCF-7 cells, BaP caused a dose- and time-dependent increase (>1.5-fold) in gene expression for 48 genes and a > 1.5-fold decrease for 61 genes. In HepG2 cells 27 genes were up-regulated and 23 down-regulated by at least 1.5-fold. Functions of genes with altered expression included those for xenobiotic metabolism, cell cycle regulation, oxidative stress response and chromatin remodelling. Sixteen genes whose expression was altered in both cell lines included NQO1, AKR1C3 and TXNRD1. BeP did not form DNA adducts or induce any consistent gene expression changes at equimolar concentrations. Analysis of expression profiles by microarray technology can identify gene expression changes induced by carcinogens at levels relevant to carcinogenesis, and may provide mechanistic insight into how cells respond to such compounds.

P35  
Gene expression changes of PAHs in vitro in relation to DNA adduct formation and carcinogenicity

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To improve carcinogenic risk assessment for Polycyclic Aromatic Hydrocarbons (PAHs), we investigated gene expression modulation in HepG2 cells and rat liver slices after exposure (6 or 24 hours) to six ambient PAHs, namely benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), fluoranthene (FA), dibenzo[a,h]anthracene (DBa,h)A), 1-methylphenanthrene (1-MPA) or dibenzo[a,l]pyrene (DBa,lP). RNA was hybridized on a human cDNA array (600 genes) or a rat oligonucleotide array (5800 genes).
Modulated genes were identified using ImaGene and GeneSight. DNA adducts were measured by $^{32}$P post-labeling. All PAHs, except 1-MPA in HepG2 cells, modulated gene expression. Each compound induced similar gene expression changes at all concentrations. The six PAHs can be divided into 3 groups, which correspond with carcinogenic potency; namely FA, 1-MPA → B[a]P, B[b]F, DB[a,h]A → DB[a,l]P. High to low DNA-adduct formation for the PAHs was for HepG2, B[a]P ≥ DB[a,l]P ≥ B[b]F ≥ DB[a,h]A ≥ 1-MPA ≥ FA, and for liver slices, DB[a,l]P ≥ B[a]P ≥ DB[a,h]A ≥ B[b]F ≥ FA ≥ 1-MPA. Correlation of gene expression with DNA adduct formation, carcinogenicity potency or Ah-receptor antagonicity for HepG2 data, gives high correlations for several genes, but these differ between parameters. So, no single gene is a perfect indicator for carcinogenic potency of PAHs. However, the gene expression profiles can separate PAHs with different carcinogenic potency, and therefore gene expression changes could be a classifier for the carcinogenicity of a PAH. This project is funded by EU-AMBIPAH.

P36
Differential gene expression in human blood induced by cigarette smoke

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Toxicogenomics allows to study the molecular basis of the relationship between xenobiotic exposure and (early) human response at the genome level, i.e. that of altered expression for large numbers of genes simultaneously. The aim of the study was to investigate differential gene expression after exposure to chemical carcinogens in human surrogate cells from blood, using cDNA microarray technology. Our research focused on the response induced by cigarette smoke and related agents. First an in vitro study was conducted in human peripheral blood mononuclear cells. Cigarette smoke condensate and a selection of its carcinogenic constituents were found to significantly induce differential gene expression, even at a low dose exposure. Subsequently, gene expression was analyzed in vivo in blood from monozygotic twins that were discordant for cigarette smoking, searching for genes discriminating smokers and non-smokers and gene expressions correlating with DNA adduct levels. In both studies Phase1 Human Tox 600 cDNA microarrays were used, containing 600 toxicologically relevant genes in quadruplicate. The most relevant data were verified with real-time PCR. Both the in vitro and in vivo study showed significant differential gene expression induced by cigarette smoke. Regarding these differentially expressed genes, the study provides a basis for the selection of candidate genes for the biomarker. However, additional data will be needed to substantiate these results.
6. Biomarkers and molecular epidemiology
**P37**

**In Vitro Chemical Exposures in Mother and Baby Pairs**

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The present study has investigated differences in susceptibility between mothers and their babies (using venous cord blood), from the West Yorkshire area of the UK. After appropriate ethical approval was obtained, hospitalised mothers signed a consent form and completed a questionnaire. Samples were coded with a unique number. A second questionnaire completed by the midwife provided baby information. Standard protocols were used for assays.

Twenty four mother and baby pairs were examined in the Comet assay using ethyl methanesulfonate (EMS). No statistically significant differences were observed between mothers and babies using a Mann-Whitney analysis, although mothers tended to show greater levels of DNA damage than the babies.

Eighteen mother and baby pairs were also examined in the Comet assay using sodium nitrite and hydrogen peroxide showing similar results.

Twelve mother and baby pairs were studied in the micronucleus assay using EMS and showed no significant differences. Preliminary investigations of SCEs between mothers and babies produced a low frequency of basal SCEs, although a high incidence of label switches (WHO/IPCS Guidelines, 2000, suggest discounting these anomalies). Using Doxorubicin, a further SCE study is ongoing, and a FISH study with the Chromosome 1 probe.

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**P38**

**Gene expression in large biobanks**

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Biobanks are promising tools for studying gene-environment interactions and disease. Peripheral blood is typically collected and stored. The Norwegian Mother and Child Biobank comprises 50,000 births today and will hold 100,000 when complete. We have investigated the logistics and technologies for collection, transportation and long-term storage of blood samples that preserve RNA, allowing future gene expression analysis.

Two commercial RNA preservation collection tubes were used, PAXgene® Blood RNA System (Qiagen) and Tempus™ Blood RNA Tubes (Applied Biosystems). Adult and cord blood were collected in Norwegian hospitals, transported by regular mail, and frozen at -80°C. Other samples were collected from volunteers, stored for 0 – 7 days, then frozen. After thawing, RNA was extracted following the manufacturers’ recommended protocols. DNase1 was included. We measured amounts and purity of RNA (NanoDrop ND-1000) and RNA integrity (2100 Bioanalyzer, Agilent). RNA of high purity and integrity was obtained from both types of collection tubes. Typical yields per ml blood were 1-6 micrograms (adult blood) and 5-17 micrograms (cord blood). The expression of 4 different genes, measured by quantitative RT-PCR was shown to be stable after storage of the blood at room temperature for up to 7 days. RNA extracted from blood collected in EDTA tubes showed satisfactory purity, yields and apparent integrity, but gene expression was highly unstable. We conclude that gene expression analysis of biobank blood samples is feasible, but that special collection tubes are necessary.

This work was supported by the EU Commission (Children Genotoxicity Network, QLRT-2001-02198).

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**P39**

**Does Occupational Exposure to Crystalline Silica-Containing Dust Induce Cytokinesis Blocked Micronucleus Frequency?**

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Biomarkers and molecular epidemiology

The International Agency for Research on Cancer (IARC, 1997) reclassified respirable crystalline silica (CS) (quartz and cristobalite) inhaled from occupational sources as a human carcinogen, based on epidemiological and animal studies. There are few data about the genotoxic effect of CS in occupational environments. The aim of our study was to evaluate the genotoxicity of CS-containing dust exposure in the workplace by performing the cytokinesis blocked micronucleus test (CB-MN) in peripheral blood lymphocytes. The study was carried out among 50 male workers from 7 different workplaces, mainly involved in grinding, mixing, bagging and sandblasting and 29 healthy male officials matched for age and smoking status as non exposed control group. Workers were exposed to dust for between 4 months and 28 years with an average duration of 7 years. The respirable total dust and the respirable CS concentrations of the workplaces were between 2.3-24.21mg/m³ and 0.23-3.07mg/m³, respectively. The mean±SD frequencies (%) of MN of workers and control group were 1.25±0.42 and 0.56±0.29, respectively. Analysis of variance indicated a significant effect of occupational exposure on MN induction in workers compared with control group after adjustment for age (p=0.000). No effect of smoking was observed. The MN frequencies were similar for the sandblasters (1.22±0.29) and the remaining workers (1.27±0.49) when grouped according to job title (p>0.05). The MN frequency of workers exposed to dust for at least 5 years (1.30±0.48) was not significantly different from those exposed for less than 5 years (1.18±0.32) (p>0.05). Although the peripheral lymphocytes are not primary target cells for respiratory particulate toxicants, an evident increase in MN frequencies in this surrogate tissue may supply an index of the accumulated genetic damage associated with silica exposure.

This study was supported by Gazi University Scientific Research Fund (project No. 02/2003-04) and Ministry of Labour and Social Security.

P40
Response to challenging dose of X-rays as biomarker of susceptibility in molecular epidemiology

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Elevated frequencies of chromosome aberrations are recognisable biomarkers of hazardous genotoxic effects. Results of previous studies on cytogenetic damage in lymphocytes have shown an association with adverse health outcomes, and on the other hand a dependence on the exposure and lifestyle related factors. However, a fast and reliable method for monitoring the individual susceptibility to the induction of critical damage is still needed. This review is based on studies in which radiation was applied as a challenging dose and DNA damage induced and unrepaired was analyzed with the use of the single cell gel electrophoresis (SCGE) assay. Comparison between induced DNA damage measured by SCGE assay and cytogenetic methods revealed a strong correlation between the two assays. Results from studies on repair competence carried out in various groups of exposed workers, controls, and cancer patients (more than 700 donors including people exposed to pesticide, mercury ions or PAHs) show variability between donors both in a response to challenging treatment and in the efficiency of repair process. Influences of the occupational exposures and other factors depending on genotypes or lifestyle on cellular capacities have been observed. Results suggest that application of ionizing radiation as a challenging treatment and SCGE as the method for the DNA damage measures in a combination with testing a repair competence might be used as a fast and reliable biomarker. This phenotype related biomarker in the molecular epidemiology or preclinical studies could help to predict a cellular susceptibility to various genotoxins and exposures (environmental, occupational or therapeutic).

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P41
The radiation-induced frequencies of micronuclei and nucleoplasmic bridges in head and neck cancer patients and healthy subjects

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The micronucleus (MN) assay has been used to evaluate the radiation sensitivity and to predict cancer risk of human subjects. In the last decade, the micronucleus assay has been evaluated as a multi-endpoint genotoxicity assay. In the present study, we have investigated the chromosomal radiosensitivity of patients of squamous cell carcinoma of head and neck (SCCHN) (n=40) and normal healthy controls (C) (n=22) by measuring micronucleus frequencies as an end-point for chromosome breakage and loss and nucleoplasmic bridges (NPBs) which are thought to be a measure of chromosome rearrangement. For the micronucleus assay, blood samples were exposed in vitro to 2 Gy γ rays (60Co) at a dose rate of 0.62 Gy/min. The mean ± SD frequencies (%) of radiation-induced MN were 16.31 ± 4.53 and 14.82 ± 4.67 in SCCHN patients and C subjects, not different significantly. Baseline MN frequencies of SCCHN patients (2.81 ± 1.04) were significantly higher than C subjects (0.82 ± 0.64) (p=0.00). Baseline NPB frequencies of SCCHN patients (0.42 ± 0.44) were significantly higher than C subjects (0.023 ± 0.075) (p= 0.000). The mean ± SD frequencies (%) of radiation-induced NPBs were (0.5 ± 0.56) and (0.47 ± 0.38) in SCCHN patients and C subjects (p>0.05). Using cut-off value for micronucleus frequencies at the 90th percentile (20.37 MN per 100 cells), the proportion of sensitive patients and healthy subjects were 17.5% and 10%, respectively. For the NPBs, using the cut of point at the 90th percentile (1.00 NPB per 100 cells) the proportion of sensitive patients and healthy subjects were 12.5% and 4.5%, respectively. MN assay seems to be useful for the categorization of normal subjects and cancer patients to their radiation sensitivity.
This study was supported by Gazi University Scientific Research Fund (Project No. 02/2004-17)

P42
Assessment of sensitivity to ionizing radiation in subjects with familiarity for gastric cancer

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Variations in the incidence of gastric cancer (GC) were observed in different populations, implicating a role of environmental factors in the aetiology of this cancer. However, increasing evidences suggest that some gastric cancers are caused by inherited predisposition. Since DNA damage is a common result of different aetiological factors of GC, variability in DNA repair capacity (DRC) could influence individual susceptibility to GC and partially explain its inherited component. In this study, individual DRC was evaluated by the mutagen sensitivity assay, measuring the amount of cytogenetic damage (micronuclei) induced in G0 by 2 Gy γ-rays. The investigation was performed on 41 first grade relatives to GC cases and 41 controls. A significant effect of gender and age on the spontaneous level of micronuclei as well as on the radiosensitivity was observed, suggesting a decline of DRC with age. To obtain some insight into the molecular mechanisms of individual radiosensitivity, the expression profile of 1800 cancer related genes was evaluated after gamma-irradiation by the use of commercial microarrays (MWG-Biotech) and correlated with the cytogenetic effects induced. Principal component analysis identified nine components explaining ~70% of the variance observed. GC familiarity was correlated to a minor component explaining 5% of total variance (p=0.03), while no factor was related to the radiosensitivity.
P43
Technology-driven molecular pathology and epidemiology, new concepts and methods; Emanating advances in disease-knowledge acquisition and intervention not otherwise possible.

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Concepts and methods for the identification of disease, health threatening abnormalities, and the delivery of high quality of medicine and health care are briefly introduced as serving the central aims of traditional Pathology and Epidemiology. There follows identification and brief discussions of: 1) the principal focus of Pathology on the individual patient, 2) the principal focus of Epidemiology on a specified population of patients, 3) the distinct types of data sets and tools used in each specialty and 4) the complementary role of the two specialties as well as their significant advances. An exception, discussed in detail, is a tool-type used by both specialties that had serious impact on traditional pathology and epidemiology and ultimately limited further advancement. There follows a tutorial presentation of novel concepts and methods appropriate to the nanoscale level of molecular events, successively addressing cases not otherwise possible. These concepts and methods involve the fusion of selected elements, relevant to Pathology and Epidemiology, from system-approach concepts and methods formulated, used and taught over several decades, that include: 1) Computational Intelligence technologies, 2) Medical Informatics and Decision making and 3) selected elements of recently formulated and used post-Genomic emerging Bioinformatics concepts and methods. The tutorial concludes with the presentation of two illustrative applications involving actual data. Applications and evaluations of presented approaches and methodologies received partial support over the years from ARP/Armed Forces Institute of Pathology, CSC, M.D.Anderson Cancer Center, MIEMS, NASA, NIH, NIST, NSF.

P44
Biomonitoring of asbestos exposed workers: frequency micronuclei, protein carbonyls, gene p53 mRNA, Epidermal Growth Factor, and Basic Fibroblast Growth Factor

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Environmental and occupational exposures to chemicals appear to alter proteins involved in cellular growth signal transduction. Exposure to asbestos has been shown to be mutagenic both in vitro and in vivo experiments. However, the effect of asbestos exposure on proteins has not been investigated. Thus, the frequency of micronucleus formation (FMN) in circulating lymphocytes, RT-mRNA of gene p 53, plasma basic fibroblast Growth Factor (bFGF) and Epidermal Growth Factors (EGF) and protein carbonyls (CP) were measured in the blood of 40 asbestos exposed workers versus 20 controls. Although non of the of the pesticide workers showed x-rays signs of neither cancer nor fibrosis, yet there has been an increase in levels in the FMN (6.5 ± 4 vs. 2 ± 1.1 %, t=2.14, p<0.05), bFGF (7.3 ± 4.9 vs. 1.9 ± 1.36 ng/ml, p< 0.001) and EGF (5.2 ± 1.5 vs. 1.02 ± 0.2 ng/ml, p<0.0001), and increased expression of mRNA as shown by gel-electro-horesis and PC (0.9 ± 0.4 vs. 0.54 ± 0.2, p<0.0001). It can be concluded that occupational exposure to asbestos induces an oxidative stress that can consequently induce genotoxicity. Growth factors such as EGF and bFGF are expressed as a consequence of the elicited oxidative stress. Although the significant increase in both growth factors could be an adaptive and/or repair response induced as a consequence of asbestos exposure, the presence of such growth factors circulating in blood, jeopardize such workers to malignant transformation. These results show that growth factors and protein carbonyls can be reliable biomarkers in monitoring early effects of pesticides as well as the previously established frequency of micronuclei.
P45
Comparison of chromosome aberration frequency and spontaneous abortions in female populations occupationally exposed to ionizing radiation.

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In order to investigate consequences of X-ray and internal radioisotopes radiation exposure on pregnancy outcome in childbearing age women we performed follow up study using chromosome aberration assay and databank of miscarriage register. Two cohorts of Croatian health professional women employed in the radiation field during their child bearing years were identified over a period of fifteen years. Information concerning their pregnancy outcomes (as well as other individual factors, including smoking) was collected via standardized questionnaire forms. 172 women were occupationally exposed to X-ray at radiological hospital departments and 60 in the nuclear medicine and biochemical hospital departments. The miscarriage rates detected in the two groups were compared to each other, and to that of the concerned general Croatian population. Conventional chromosomal aberrations were measured in both groups. The study findings showed an increase in spontaneous abortions in the X-ray exposed group and a significant increase in the radioisotope exposed group compared to Croatian women control. Women exposed to radioisotopes experienced a threefold higher rate of spontaneous abortions than those exposed to X-ray (RR=2.9, 95%CI=1.74-4.85, p<0.0001). Significantly higher frequencies of chromosomal aberrations were detected in both groups compared to referent values but there was no significant difference in chromosomal aberration frequency between exposed groups. For exposure levels within standard recommended guidelines, radioisotopes are far more efficient at producing spontaneous abortions than X-rays. Such biological effect is not followed by deviations in chromosomal aberration assay, a recommended biomarker for populations exposed to ionizing radiation.

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P46
Evaluation of biomarkers associated with cellular radiosensitivity in cervical cancer patients.

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Purpose: The present pilot study is designed to find a variety of biomarkers associated with individual response to radiotherapy among cervical cancer patients. We wanted to determine whether there is any correlation among the level of initial DNA damage, the kinetics of DNA strand break rejoining, the level of residual DNA damage, genetic variation of DNA repair genes and radiosensitivity/radioresistance of cancer patients.

Materials and Methods: The alkaline comet assay has been used to analyze radiation-induced DNA damage and DNA repair in peripheral blood lymphocytes (PBL) of 10 cervical cancer patients, stages IIB-IVA.

Results: A lower level of residual DNA damage 30 min after the treatment and a faster DNA strand break rejoining (shorter half-time of rejoining) were assessed in PBL of two patients. According to the clinical outcome these patients were resistant to radiotherapy. Genetic polymorphism of various DNA repair genes (XRRC1, XRCC3, XRCC4, hOGG1, XPD) was analyzed by RFLP-PCR assay.

Conclusions: These preliminary data support the hypothesis that the initial DNA damage and DNA repair kinetics could correlate with individual response to radiotherapy.

This study was supported by the project 2003 SP 51 028 08 00/028 08 01 from the national program Use the Cancer Genomics to Improve the Human Population Health.
P47
Gene-environment and gene-gene interactions dictate the effects of DNA repair enzyme polymorphisms on lymphocyte DNA adducts in subjects exposed to air pollution

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Little is known about the impact of genetic variation on DNA damage induced by urban air pollution. In the context of the "Aulis" project, the levels of bulky DNA adducts were measured in lymphocytes of 194 non-smoking students living in the city of Athens, and the rural region of Halkida, Greece once in the winter and again in the following summer. In these individuals personal exposure to PAH was also measured. Furthermore, genetic polymorphisms were examined in a variety of phase I and Phase II genes as well as polymorphisms coding for the DNA repair enzymes XPD (Asp312Asn, Lys751Gln) and XRCC1 (Arg399Gly). The results indicated that the levels of DNA adducts were marginally higher in subjects which were homozygotic for the XRCC1 polymorphism (Gly399Gly) relative to the wild-type carriers (1.53±0.96 and 1.17±0.77 /10^8 nucleotides respectively; p=0.05) all sampled during the winter period, while the polymorphisms of the XPD gene did not have any effect on the DNA adduct level. Samples were then stratified according to the personal exposure to B[a]P as negligibly, intermediate and highly exposed individuals. The effect of the XRCC1 polymorphism on the DNA adduct levels was enhanced stepwise in the order negligible < intermediate < high exposure to B[a]P. In the latter subgroup, individuals with the combined XRCC1(Gly399Gly)/GSTM1*0 and XRCC1(Gly399Gly)/“slow” mEH (His139His) genotype had higher adduct levels than the XRCC1(Gly399Gly)/ GSTM1*1 and XRCC1(Gly399Gly)/mEH (“fast” His139Arg or Arg139Arg) genotype respectively (2.04±1.32 and 2.36±1.66 relative to 1.24±0.69 and 1.24±0.88 /10^8 nucleotides respectively; both p<0.05). Thus, compelling interactions of the XRCC1 and GSTM1 or mEH polymorphisms were observed but only for individuals highly exposed to urban air pollution. No significant effects were observed for the summer samples. However, this is explicable because the exposure to B[a]P during this season is very low and never reaches the high levels encountered during the winter period.

P48
DNA repair, apoptosis and necrosis in patients with pancreas cancer

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The DNA damage caused by ethanol and H2O2 as well as the DNA repair capacity in a population of patients suffering from cancer of pancreas was estimated with the comet assay using an image analysis system.

Apoptosis and necrosis was also estimated, by studying the morphology of the lymphocytes after the effect of ethanol and H2O2 with the stains Acridine Orange and Ethidium Bromide.

The results obtained indicate that pancreas cancer patients have increased endogenous DNA damage in comparison to the controls. They are more sensitive to the effects of ethanol, and hydrogen peroxide, from the control population. DNA damage increases with increased concentration of the agents and that their repair capacity was not able to reverse their damage to the control population levels.

Ethanol and H2O2 were found to promote apoptosis. In particular pancreas cancer patients have been found with increased apoptosis if compared to the controls.

88
P49
Mutagen sensitivity of patients with cancer of head and Neck

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The bleomycin (BLM) sensitivity assay is believed to measure individual risk of environment-related cancers and, thus, of HNC as well. Previously we have shown that this method is only moderately suitable in Hungary for the identification of individuals at high risk for developing HNC. We evaluated whether the method is more sensitive when site-specific character of the risk is measured. 278 untreated patients with HNC at 4 different tumour sites, and 356 frequency-matched controls were studied. Significant difference in BLM-induced break/cell (b/c) values was found between patients (1.11 b/c) and controls (0.97 b/c), however, the overall cancer risk showed that only 58% of patients and 43% of controls were mutagen sensitive. When HNC patients with different cancer sites were analysed, mutagen sensitivity of patients with tumours of the oral cavity, oropharynx, and hypopharynx was significantly higher than that of the frequency-matched controls (1.12-1.14 b/c vs. 1.00 b/c), while no significant difference was found from controls (1.00 b/c) in patients with laryngeal tumours (1.05 b/c). An association between BLM sensitivity and the risk of HNC was observed in patients with tumours of the oral cavity and oropharynx (OR = 1.97 and OR = 1.90), while no association was found in patients with hypopharyngeal or laryngeal tumours. Though, the mutagen sensitivity decreased from the oral cavity down to the larynx, indicating that the site-specific risks may differ, this biomarker may not allow the accurate determination of individual risks at specific sites of HNC either.

P50
GSTM1 and GSTT1 gene polymorphisms and larynx cancer risk in a Turkish population.

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Genes coding for GSTM1 and GSTT1 proteins are polymorphic in humans and these genes are absent in 50% and 20% in Caucasians, respectively. PCR was used to detect the presence or absence of the GSTM1 and GSTT1 genes in this study. Genomic DNA was isolated from 52 patients with larynx cancer and 75 controls without any cancer. The frequency of GSTM1 null genotype was 73.07 % in cases and 50.7 % in controls with an OR of 2.643. The frequency of GSTT1 null genotype was 17.30 % in cases and 20.0% in controls (OR: 1.194). This study evaluates the influence of genetic polymorphism at GSTM1, GSTT1 gene loci on larynx cancer among Turkish patients. Our preliminary results suggest that the GSTM1 null genotype may be risk factor for development of larynx cancer. No significant association was observed between cases and controls in the frequencies of the GSTT1 null genotype and larynx cancer risk. Although carcinogenesis is a multistep process, the genetic variability of metabolic enzymes constitutes a major factor in the increased risk of cancer development. (Supported by Ankara University, Institute of Biotechnology, Project No: 2001-K-120-240)

P51
DNA repair in young lung cancer patients measured with the alkaline Comet assay

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Several studies showed an elevated risk for lung cancer in miners who were exposed to underground radon. We hypothesized that radiation sensitivity determined by genetic predisposition in DNA damage
Biomarkers and molecular epidemiology

detection and repair processes might interfere with the development of lung cancer. Therefore, patients with lung cancer are expected to show deficiencies in DNA repair capacity more likely as supposed by cellular response to in vitro radiation exposure.

In an ongoing case control study, 62 patients with lung cancer up to the age of 50 are compared with 30 healthy controls. DNA damage/repair capability was measured with the alkaline comet assay. Preliminary results show significant differences in DNA repair kinetic and repair capacity between lung cancer patients and controls using Tail Olive moment (TOM) as parameter. In the mean of controls, 96% of DNA damage of a cell is repaired 60 min after radiation. Cells of lung cancer patients are only able to repair 92% of DNA damage within the same time. This difference is significant by p=0.01 (Wilcoxon’s Rang-Sum-Test). However, this preliminary result is still dependent on the choice of the main outcome parameter. Looking at the winsorized mean of percentage of DNA in tail, 79% and respectively 72% of DNA was repaired. This difference remains non-significant (p=0.12).

P52
Assessment of DNA damage in glue sniffers by use of the alkaline comet assay

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Toluene is used widely, not only in industry, but also in households where toluene exposure and abuse can occur. To estimate the genotoxic risk of toluene exposure, DNA damage was determined in peripheral lymphocytes of 20 glue sniffers and 20 age-matched controls by use of the alkaline comet assay. Urinary hippuric acid and o-cresol excretion rates, which are used as a marker for toluene exposure, were also measured in sniffers and compared with historical control values. The increase in genetic damage in sniffers was statistically significant as compared to control subjects (P < 0.0001). The mean values of the hippuric acid and o-cresol excretion rate for glue sniffers was 73- and 1582-fold higher, respectively, than in controls and confirms the putative exposure. Education of the general public and efforts to keep adolescents away from volatile solvent-based products, which may lead to a desire of sniffing in the future, would be advisable.

P53
Induction of DNA damage and its repair in lymphocytes of children environmentally exposed to lead.

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Using standard and modified comet assay we examined the steady-state level of SSB and FPG-sensitive sites in DNA of children environmentally exposed to lead. 92 nine years old children, living in the polluted region of Poland (Silesia) and 49 matched control children were examined. Exposure to lead was assessed by determination the level of lead in blood (PbB) using atomic absorption spectroscopy. Environmental exposure to lead resulted in significantly increased levels of Pb in blood (5.29 ±2.09 µg/dl in exposed group and 3.45 ±1.20 µg/dl in controls). In spite of difference in PbB no difference was found in steady state level of SSB (12.9 ± 6.6 and 12.8 ± 6.5), TCR mutation (12.1 ± 11.1 and 12.5 ± 7.8) and GPA mutation (32.9 ± 43.0 and 17.0 ± 24.4) between exposed and unexposed group, respectively. The only difference between exposed and unexposed group was found in the steady-state level of FPG sites (75.5 ± 25.6 and 53.2 ± 21.3).
Positive correlation was found between the level of lead in blood and SSB for exposed group (r=0.201, p=0.021) and FPG-sensitive sites for whole population (r=0.229, p=0.009).
P54
DNA damage-repair in lung cancer patients’ lymphocytes

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In this study using the Comet Assay technique, we estimated the DNA damage caused by ethanol, γ-irradiation and H2O2 as well as the DNA repair capacity in lung cancer individuals. By studying the morphology of the lymphocytes after the effect of γ-irradiation, ethanol and H2O2 we measured apoptosis and necrosis with the stains Acridine Orange and Ethidium Bromide.

In conclusion in this study we demonstrated that lung cancer patients have increased endogenous DNA damage in comparison to the controls. They are also more sensitive to the effects of external factors (H2O2, ethanol and gamma irradiation) from the control population with their repair capacity not been able to reverse their damage to the control population levels.

Finally lung cancer patients have been found with increased apoptosis if compared to the controls.

P55
European network on children’s susceptibility and exposure to environmental genotoxicants (CHILDRENGENONETWORK) QLK4-CT-2002-02198

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The Concerted Action, Children Genotoxicity Network, explores gene-environment interactions during the foetal, neonatal and infancy developmental periods, concentrating on genotoxic exposures and environmental factors with focus on air pollution (traffic and tobacco). Work-package (WP) 1 has evaluated several studies analysing the effects of children’s exposure to environmental pollutants with biomarkers of genetic damage. A number of biomarkers resulted to be consistently increased in children (MN, CA, DNA and protein adducts). MN show increasing frequencies with age even in children. WP2 identified several mother/child cohorts within Europe with biobanks enabling follow up studies with biomonitoring. WP3 has initiated pilot studies of placental transfer. A pilot family study has been performed in WP4, with samples from 24 families, including mothers and two siblings from two different areas in Czech Republic, as well as feasibility studies in Belgium, The Netherlands, Hungary, UK, Norway, Spain and Greece to explore adult vs child/newborn gene expression, DNA repair and susceptibility. WP5 aims to identify the critical health effects suitable for risk assessment in children and use of biomarkers. State of the art studies with focus on ethical issues in pre- and post-natal studies on children have been summarized in WP6. These issues are related to incentives to participate, data protection, data storage and dissemination. Discussions of maturity age and assent/consent from children are also important.

www.pubhealth.ku.dk/cgn.
P56
Apoptosis and DNA damage-repair in obstructive sleep apnea patients’ lymphocytes

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In the present study with the Comet Assay technique, we examined the DNA damage caused by external factors such as ethanol, γ-irradiation and H2O2 as well as the DNA repair capacity in a population of patients with Obstructive Sleep Apnea (OSA) by measuring the percentage of DNA in tail using an image analysis system connected to a computer with Kinetic Analysis System. Furthermore, by studying the morphology of the lymphocytes after the effect of γ-irradiation, ethanol and H2O2 we measured apoptosis and necrosis with the stains Acridine Orange and Ethidium Bromide. Analysis of our results indicates that Obstructive Sleep Apnea patients have increased endogenous DNA damage in comparison to the controls. They are also more sensitive to the effects of external factors, ethanol, hydrogen peroxide and γ-irradiation, from the control population. DNA damage increases with increased concentration of the agent and that their repair capacity was not able to reverse their damage to the control population levels. Finally it was found that H2O2, ethanol and γ-irradiation promote apoptosis. In particular patients with Obstructive Sleep Apnea Syndrome have increased apoptosis if compared to the controls.

P57
Urinary excretion of guanine nucleotide damage products and risk of lung cancer

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Oxidation of guanine in DNA or in the nucleotide pool may give rise to 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) for urinary excretion. 7-Methylguanine (m7Gua) is a product of base excision repair of lesions in DNA and a metabolite from RNA.

We examined associations between urinary excretion of 8-oxodG or m7Gua and risk of lung cancer as well as potential interaction with the OGG1 Ser326Cys polymorphism prospectively in a population-based cohort of 25,717 men and 27,972 women. We included 260 cases with lung cancer and a sub-cohort of 263 individuals matched on sex, age and smoking duration. The excretion of 8-oxodG and m7Gua was higher in current smokers. Overall the incidence rate ratio (IRR) (95% confidence interval) of lung cancer was 0.99 (0.80-1.22) per doubling of 8-oxodG excretion, whereas among never-smokers the IRR was 11.8 (1.21-115) per doubling. There was no interaction with OGG1 genotype. In unadjusted analysis the IRR of lung cancer was 1.20 (1.00-1.43) per doubling of m7Gua excretion and 1.08 (0.89-1.31) after full adjustment for smoking.

The data suggest that oxidative damage to DNA nucleotides is important among never smokers, whereas methylation of guanine may be important among smokers.
Occupational exposure to arsenic compounds: biomonitoring of genotoxic effects

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This bio-monitoring study aimed at evaluating the frequencies of sister chromatid exchanges (SCE), high frequency cells (HFC), micronuclei (MNCB and MNMC) and chromosomal aberrations (CA) in 19 protected workers regularly exposed to arsenic compounds (Group 1), 16 non-protected workers accidentally exposed to arsenic (Group 2) and a third group of 21 matched office employees as controls. Considering an exposure above TLV-TWA level, the workers of Group 1 were equipped with a NIOSH level B protection. The assessment of urinary arsenic levels did not reveal a higher level in Group 1 as compared to Group 2. However, comparison between the two exposed groups (Mann-Whitney U) showed increased SCE, HFC and MNMC (but not CAs) frequencies in workers from Group 1 as compared to workers from Group 2. Additionally, exposed workers from Group 1 showed significantly higher SCE and HFC frequencies as compared to controls. A significant increase in the percentage of total CA was observed in exposed smokers as compared to exposed non-smokers. Multivariate analysis adjusting for confounding factors indicated that workers from Group 1 had increased frequencies of SCE, HFC, MNMC and total CA. We concluded that workers from Group 1 may be at higher risk for increased genotoxic effects and thus need continuous medical surveillance in the future.

Baseline micronuclei frequency in children: estimates from meta- and pooled analyses

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The number of studies evaluating the effect of environmental exposure to genotoxic agents in children has rapidly increased in the last few years. The frequency of micronuclei (MN) in peripheral blood lymphocytes determined with the cytokinesis block assay is among the most popular biomarkers used for this purpose, although a large inter- and intra-laboratory variability of this endpoint has been observed in population studies. The availability of reference measures is therefore necessary to laboratories to validate protocols and analytical procedures, and to molecular epidemiologists as well, to estimate the statistical power of studies and to assess the quality of data.

In the framework of the project European Network on children’s susceptibility and exposure to environmental genotoxicants (CHILDRENGENONETWORK), estimates of the baseline frequency of MN in children were provided. Thirteen papers reporting on field studies were selected after a MedLine/PubMed search for a meta-analysis. Individual data from 12 laboratories (available from published studies and from the HUMN database) were used for a pooled analysis. An overall mean of 4.48 (95% CI=3.35-5.98) and 5.70 (95% CI=4.29-7.56) MN per 1000 binucleated cells were estimated by the meta- and pooled analysis, respectively. A clear effect of age was detected, even within the restricted range of pediatric age considered, with significantly lower frequency values in newborns. No influence of gender was found.

The study showed the advantage of using data from large collaborative studies, and suggested a synergistic use of meta- and pooled analysis.
P60
Children’s exposure to environmental pollutants and biomarkers of genetic damage: review and meta-analysis

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In the framework of the project European Network on children’s susceptibility and exposure to environmental genotoxicants (CHILDRENGENONETWORK), a review was performed to identify molecular epidemiology studies conducted among children exposed to chemical environmental pollutants incorporating biomarkers of genetic damage.

After an extensive MedLine/PubMed search (time period 1980-2004), 178 publications were retrieved; 10 additional papers were manually identified.

Among the cytogenetic biomarkers, chromosome aberrations and micronuclei but not sister chromatid exchanges (confirmed in a meta-analysis in newborns) were found consistently increased in exposed children.

The effect of exposure to airborne urban pollutants was consistently reported by field studies measuring DNA, albumin and haemoglobin adducts. Meta-analyses performed in children exposed to ETS and in newborns exposed in utero to maternal smoke showed 1.3 and 7 times higher levels of haemoglobin adducts compared to referents, respectively.

The limited number of studies measuring DNA fragmentation (Comet assay), HPRT and GPA mutation frequency precluded a meaningful evaluation of the usefulness of these assays.

Higher levels of PAH-DNA adducts were found in fetal than in maternal tissue, suggesting a specific susceptibility of the fetus to these pollutants.

In conclusion, future research and biomonitoring programs on children would greatly benefit from the inclusion of selected biomarkers that could provide biologically based evidence for the identification of intervention priorities in environmental health.

P61
Searching PubMed for molecular epidemiology subjects: the case of Chromosome Aberrations

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More than twenty years have passed since the first studies integrating molecular biology into traditional epidemiological research were published. However, the available tools for searching literature on molecular epidemiology subjects remain largely unsatisfying. An example of these difficulties is reported.

In the framework of the project European Network on children’s susceptibility and exposure to environmental genotoxicants (CHILDRENGENONETWORK), a review was performed to identify molecular epidemiologic studies conducted in children exposed to chemical environmental pollutants (including exposure in utero) and measuring biomarkers of DNA damage.

After an extensive MedLine/PubMed search (time period 1980-2004), 178 publications were retrieved. The MeSH keyword “Epidemiology, molecular” (introduced in 1994) appeared only in two articles, “Biological markers” (introduced in 1989) in 30. A satisfactory search strategy required the use of specific terms indicating different biomarkers (MeSH keywords or free text terms).

An unexpected problem rose with the MeSH keyword Chromosome Aberrations (CA), i.e., 44 out of the 78 retrieved articles (56.4%) were non-pertinent. For example, this keyword indexed both studies where the frequency of CA in lymphocytes was measured as a biomarker of early biological effect, and studies where CA referred to spontaneous abortions or congenital abnormalities (n=2378, 29.5%).

In conclusion, a better standardization of indexing procedures is clearly needed. The introduction of a new MeSH keyword, able to distinguish the biomarker CA, would definitely help, while existing MeSH keywords related to molecular epidemiology should be more widely used.
P62
K-Ras codon 12 mutations detected in plasma DNA is not an indicator of disease in NSCLC patients.

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Overexpression or mutational activation of K-ras gene has been found in 30-80% of non-small cell lung cancer (NSCLC). We analysed K-Ras mutations at codon 12 in plasma of NSCLC patients by two different methods; compared data from plasma and the respective cancer tissue; evaluated the presence of K-ras mutations in plasma from hospital controls. An overall number of 48 NSCLC patients and 40 hospital controls participated in the study. In 12 patients K-ras mutations in cancer tissue showed no correlation with the respective plasma, either extracted by the Qiagen or by a modified Guanidine/Promega Resin method. Codon 12 K-Ras mutations were present in two tissue samples out of 12, whereas no mutation or two mutations different from those identified in the corresponding tumor tissue were detected in plasma with Qiagen and Guanidine/Promega respectively. In addition, DNA isolated (Qiagen) from 76 further plasma samples was amplified by enriched PCR. Codon 12 K-Ras mutations were detected in plasma from 15 out of 36 NSCLC patients (41.7%) as well as from 12/40 controls (30.0%). Our results do not support the detection of codon 12 K-ras mutations in plasma DNA as a biomarker for tumor diagnosis.

P63
Evaluation of DNA damage and repair in autonomous thyroid nodules and peripheral blood lymphocytes in patients receiving 131-Iodine therapy


Radioiodine treatment of patients with autonomous thyroid nodule leads to cellular DNA damage not only in thyreocytes but also in peripheral blood lymphocytes (PBL). The purpose of this study was to evaluate DNA breakage and base damage in thyreocytes and PBL in patients treated with 131-Iodine. In all the patients, thyroid scintigram was performed using 131-I. Damage to DNA was estimated by comet assay. Samples were taken three times: before radioiodine treatment, and 14 and 56 days after. Preliminary results indicate a high diversity in the level of DNA damage among the patients. In lymphocytes after 56 days the level of DNA damage was even lower than in the control. In contrast, in “hot nodule” cells DNA damage persisted until the 56th day after 131-I treatment. Differences in the type of damage between thyreocytes and lymphocytes were also observed. In lymphocytes there was more base damage while in thyreocytes single strand DNA breaks prevailed. The comet assay can be a valuable tool for monitoring radioiodine treated patients. The differences in the type and persistence of DNA damage in lymphocytes and thyreocytes might indicate different mechanisms of DNA damage induction and/or DNA repair mechanisms.
P64
Assessment of genotoxic damage by alkaline comet assay in nurses occupationally exposed to anaesthetic gases or antineoplastic drugs

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In a hospital routine, several mutagenic and carcinogenic agents are used, either for diagnosis or treatment of patients. The possibilities of a potential mutagenic/carcinogenic action of waste anaesthetic gases and antineoplastic drugs on human populations who are exposed occupationally have been previously reported in several studies. The objective of the present study was to assess the exposure of nurses who are handling antineoplastic drugs or who are exposed to waste anaesthetic gases during their normal work routines, by using the highly sensitive method for the evaluation of DNA damage; alkaline comet assay. Nineteen nurse anaesthetists and 19 nurses handling antineoplastic drugs currently employed at the different hospitals under similar working condition in Ankara were compared with 19 non-exposed controls. Significant differences were detected between control (108.63 ± 4.72) and exposed groups (anaesthesia nurses: 121.84 ± 5.17; antineoplastic handlers: 119.78 ± 5.06) in terms of DNA damage (p<0.001). Furthermore, it seems that cigarette smoking had no significant effect on the grade of DNA damage. The study supports the existence of high risk for mutagenesis and/or carcinogenesis in nurses than the unexposed population and no statistical difference were observed in terms of total comet scores between anaesthesia and oncology nurses (p>0.05).

P65
Preliminary study on the genotoxic effects of exposure to Prestige oil during cleaning tasks

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In November, 2002, the oil tanker Prestige broke up in front of the coasts of Galicia, spilling more than 73,000 tons of crude oil all over the coastal area. The oil was composed basically of volatile organic compounds (VOC), polycyclic aromatic hydrocarbons and resins. From the beginning, a lot of people worked together in cleaning tasks. Among them, there were volunteers that collaborated for one week (V), hired manual workers (MW) and high-pressure cleaner workers (HPW). The aim of this work was to evaluate the potential risk that oil exposure could exert on the human health. Environmental exposure to VOC was evaluated by means of passive dosimeters and a chromatographic methodology. Two genotoxicity tests were applied on human leukocytes: comet assay and sister-chromatid exchanges (SCE). Since conjugation with glutathione is one of the main metabolic pathways for both benzene and benzopyrenes (the most mutagenic oil components), we have determined the effect of genetic polymorphisms in GSTM1 and GSTT1 on the genotoxic parameters evaluated. Increased DNA damage was observed both in MW and HPW groups, and increased SCE in HPW group, regarding to controls. This was consistent with the environmental determination of COV that showed higher exposure levels in the HPW group. No effect of aging was detected, but smokers showed higher DNA damage and SCE frequency than non-smokers. Finally, increased DNA damage was observed in individuals null for GSTM1, and no effect of GSTT1 was detected.
P66
DNA damage–repair and apoptosis in stomach cancer patients’ lymphocytes

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With the Comet Assay technique, we examined the DNA damage caused by ethanol and \(\text{H}_2\text{O}_2\) as well as the DNA repair capacity in a stomach cancer population of patients by measuring the percentage of DNA in tail using an image analysis system.

We also measured apoptosis and necrosis, by studying the morphology of the lymphocytes after the effect of ethanol and \(\text{H}_2\text{O}_2\) with the stains Acridine Orange and Ethidium Bromide.

Our results indicate that stomach cancer patients have increased endogenous DNA damage in comparison to the controls. They are also more sensitive to the effects of ethanol, and hydrogen peroxide, from the control population. DNA damage increases with increased concentration of the agents and that their repair capacity was not able to reverse their damage to the control population levels.

\(\text{H}_2\text{O}_2\) and ethanol promote apoptosis. In particular stomach cancer patients have been found with increased apoptosis if compared to the controls.

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P67
Anti-B[a]PDE-DNA adducts in environmentally low-exposed humans

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Within a cross-sectional study we are currently evaluating anti-B[a]PDE-DNA adduct levels (formed by the ultimate carcinogen of B[a]P) in lympho-monocytes of humans environmentally exposed to polycyclic aromatic hydrocarbons (PAHs) by HPLC/fluorescence analysis. Aims of work are to validate this bioindicator of effective dose on a surrogate tissue and to determine high risk individuals for their genetic characteristics. The study design (October 2002-July 2005) involves: a) recruitment of 600 Padova municipal workers within their periodic check-ups at our Preventive Medicine Ambulatory; b) a signed informed consent by each participant; c) collection by questionnaire data regarding PAH (B[a]P) exposure by tobacco smoke, diet (grilled meat or pizza baked in wood-burning oven), indoor exposure (fireplace and/or coal or wood-stove lighted in house, hobbies with PAH introduction, exposure to passive smoke), residence place (town/suburbs and rural), traffic around residence (intense or moderate/absent), outdoor exposure to traffic pollution for least 4 hours/day; d) blood (15ml) and urine (200ml) sample collection from each participant in the morning. To date 438 subjects were examined (age 20-62 years, males (52%). Smokers (23%) have significantly higher adduct levels than non smokers (\(p<0.001\)). Non smokers with \(\geq 52\) (N=142, 42%) than those with < 52 times/year of PAH-rich meals increase significantly adduct levels (\(p<0.01\)). Our current results indicate that anti-B[a]PDE-DNA adduct formation is modulated by PAH(BaP) exposure with smoke and dietary habits in environmentally low-exposed humans.
P68
Micronuclei in families exposed to air pollution. A pilot study in the Czech Republic.


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The frequency of micronuclei (MN) in lymphocytes was used to assess the cytogenetic effects of 24 families, including mothers and two siblings. Teplice, a former mining district, was selected for investigation of the effects in a population exposed to air pollution and compared with a population from rural Prachatic. Significant increased MN frequency was found in children and mothers from the Teplice area as compared with those from the reference area. Significant increased MN level was found in girls as compared to boys. Significant effect of age was found in children. Higher MN frequency in families living close to traffic and in families suspected to be exposed to adverse indoor emissions of PM indicate a potential impact of these sources on the MN frequencies. No significant effect of environmental tobacco smoke was found. The family pilot study indicates that MN is a valuable and sensitive biomarker for early biological effect associated with environmental exposure in children and adults.

The pilot project was financed by the CHILDRENGENONETWORK (QLK4-CT-2002-02198).

P69
DNA damage-repair after the effects of H2O2, γ-irradiation and ethanol in child, adult and old age human populations

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In this study lymphocytes from individuals of three different age groups (1-5, 40-50 and 70-80 years old) were exposed to increased concentrations of ethanol, γ-irradiation and H2O2. The amount of endogenous DNA damage, the DNA damage caused by the above agents and the repair efficiency were estimated with the comet assay technique.

Our results show that the lymphocytes from the young age group have smaller levels of basal DNA damage and smaller sensitivity to the effects of external factors. Difference in the efficiency of DNA repair was also observed among these age groups.

P70
Molecular mass determination of two new cis-benzo[a]pyrene adducts by the use of LC-fluorescence and mass spectrometry.

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The aim of the present study was to identify two new benzo[a]pyrene adducts found in plasma proteins from benzo[a]pyrene exposed rats. Male Wistar rats were intraperitoneal injected with benzo[a]pyrene, and serum albumin was isolated and subjected to acid hydrolysis at 70 °C for 3h. The hydrolysate was
subjected to LC separation and fractions of the two unknown adducts were collected. The molecular masses of the two unknown compounds were identical with tetrols as judged by LC electrospray mass spectrometry. The two unknown compounds were shown to have similar chromatographic properties and fluorescence characteristics as the standard tetrols. In addition, they behaved similar to the four standard tetrols in both dose response experiments and time course experiments. The in vivo findings of the two unknown adducts are probably the result of the formation of the less carcinogenic diol epoxide, BPDE III in the metabolism of benzo[a]pyrene. For practical reasons they have been named BP-7,8 cis tetrol 1 and BP- 7,8 cis tetrol 2. These results give supporting evidence of BP-7,8 cis tetrol 1 and BP- 7,8 cis tetrol 2 as two new tetrols with the C7-OH and C8-OH groups in a cis position. We have also identified one acetylcysteine conjugate of BPDE III in the rat urine, which give rise to BP-7,8 cis tetrol I/2 when isolated and subjected to acid hydrolysis. The quantification of protein-benzo[a]pyrene adducts represent a more sensitive method than quantification of BP-DNA adducts. As a biomarker these new tetrols are important mostly do to the high cytotoxic effect of BPDE III.

P71
Assessment of DNA damage in postmenopausal women under hormone replacement therapy

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To evaluate the possible DNA damage in peripheral blood leukocytes of postmenopausal women under different hormone replacement therapies (HRT), comet assay, a standard method for assessing genotoxicity has been used. 46 women were categorized in three groups—Group A: 15 surgical menopausal women who underwent surgery for benign conditions, receiving conjugated equine estrogen, 0.625 mg/day (CEE) for 2.3 ± 1.5 years, Group B: 16 spontaneous menopausal women receiving conjugated equine estrogen, 0.625 mg/day plus medroxyprogesterone acetate, 5 mg/day (CEE + MPA) for 2.4 ± 1.0 years and Group C: 15 spontaneous menopausal women receiving tibolone, 2.5 mg/day for 2.4 ± 1.3 years. Control group consisted of 15 spontaneous menopausal women who never had HRT. Significant differences in terms of DNA damage were observed between Group A and B with controls as mean total comet scores 23.93 ± 5.84, 19.44 ± 6.19 and 10.07 ± 2.40, but no significance (P > 0.05) were detected between Group C and controls as mean total comet scores 12.07 ± 3.65 and 10.07 ± 2.40, respectively. Reduced DNA damage was observed with tibolone compared to CEE or CEE + MPA therapy. Studies of this approach are needed.

P72
Aromatic DNA adducts as a sensitive biomarker of ambient genotoxic exposure of children.


Genotoxic hazard of environmental exposure of children and related biomonitoring is a priority topic in environmental health research. The aim of our study was to explore the potential of DNA adducts as a biomarker of exposure of children to air pollution. The research was based on a former environmental epidemiological study of children, 7 to 9 years old, in three industrialised and three urban background settlements in Hungary. Traffic and environmental tobacco-smoke exposure was assessed by questionnaires. DNA was isolated from frozen whole blood samples from 90 subjects stratified into various exposure categories. DNA samples were analysed for aromatic DNA adducts by 32P-postlabelling using electronic autoradiography for detection. Many samples exhibited one major and one weaker distinct spot with characteristic Rf. The detection limit of those single spots was 0.02 adducts/10^8 nucleotides. Total adduct levels were up to 1/10^5 order of magnitude. In the highest-exposure group (n=18) 83% of the samples presented the major spot and 27% the secondary spot, whereas in the lowest-exposure group (n=29) 35% and 14%, respectively. The odds ratio for the major spot was 2.42 (95% CI 0.90-6.53) (p=0.089). The results open further perspectives for the use of DNA adducts for biomonitoring of ambient genotoxic exposure of children.
Biomarkers and molecular epidemiology

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P73
Somatic mutagenesis at glycophorin A locus in residents of radiation contaminated territories of Orel region

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Actual problem of radiation biology and medicine is study of effect of low dose radiation exposure on somatic cell genome. One of the methods for somatic mutagenesis evaluation is assessment of frequency of cells with glycophorin A (GPA) variant/mutant phenotype.

Purpose of this work was to compare the GPA (NO)-mutant cell frequencies in unexposed control persons and residents of four districts of Orel region contaminated with radionuclides as a result of Chernobyl accident. Mean $^{137}$Cs density in the districts varies from 22 to 113 kBq/m². The GPA assay was performed separately in persons with thyroid nodules (n=47) and without this pathology (n=58) 15 years after the accident. Mean frequency of the GPA-mutant cells in persons without thyroid nodules did not differ from control level (p>0.05). On the contrary, mean GPA-mutant frequency in group with nodules was significantly higher than in control group: (45.8±5.1)$ \times 10^{-6}$ vs (22.7±4.7)$ \times 10^{-6}$ correspondingly (p=0.01). Dependence of the GPA-mutant frequency on cumulative dose and level of region contamination is discussed.

P74
Effect of vitamins intake on spontaneous and in vitro induced mutagenesis in human lymphocytes

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The aim of this study was to assess the level of spontaneous and in vitro induced chromosome aberrations in peripheral blood lymphocytes of healthy donors, receiving vitamins. The evaluation was conducted before and after 14 and 30 days of regular everyday oral intake of the vitamin complex containing vitamins-antioxidants, group B vitamins in doses exceeding recommended daily consumption and others.

Whole blood samples of 15 healthy donors were cultivated during 54 hours using the standard procedure. Mutagens dioxidine (0.03 and 0.1 mg/ml) and cadmium chloride (0.02 mg/ml) were added to cultures 4 hours prior to fixation. At each variant of the experiment 300-400 metaphases were analyzed (total 57,000).

The results obtained demonstrate that vitamin complex intake significantly increases the blood levels of vitamins C, B2, beta-carotene and total carotenoids, does not influence the level of spontaneous mutagenesis, and increases the tolerance of peripheral blood lymphocytes to the damaging action of dioxidine and cadmium chloride. The antimutagenic effect is more evident after 30-day vitamin intake. The level of spontaneous mutations is inversely proportional to the concentration of vitamin C. The level of the chemically induced chromosome aberrations is inversely proportional to the concentrations of beta-carotene and total carotenoids and exponentially depends on the concentrations of vitamins E and B2.

The study was performed under a partial funding of RFBR № 03-04-48591.
P75
The extent of damage and repair in the p53 gene after treatment of myeloma patients with melphalan is individualized and may predict clinical outcome

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The purpose of the present study was to quantitate the individual levels of melphalan-induced DNA damage formation and repair in vivo, and to search for possible correlations with clinical outcome in patients with multiple myeloma. Thus, the formation and subsequent repair of DNA damage (monoadducts and interstrand cross-links) in the p53 tumor suppressor gene, the protooncogene N-ras, and the housekeeping gene b-actin during the first 24 h after treatment with high-dose melphalan (HDM, 200 mg/m²) and supported by autologous blood stem cell transplantation, was measured in blood leukocytes of 26 patients with multiple myeloma. The levels of gene-specific DNA damage formation and the individual repair capacity varied up to 16-fold among patients, indicating that the melphalan-induced biological effect in vivo is highly individualized. A significantly greater DNA damage (peak monoadducts: 15.2±1.5 versus 12.4±1.6 adducts/10⁶ nucleotides, p=0.002; peak interstrand cross-links: 1.7±0.4 versus 1.2±0.5 adducts/10⁶ nucleotides, p=0.04) and a slower rate of repair (monoadducts: 35.9±5.6% versus 49.9±8.1% decrease of maximal levels during the 2-24 h time-period, p=0.001; interstrand cross-links: 30.0±6.5% versus 45.9±10.7% decrease of maximal levels during the 8-24 h time-period, p=0.001) in the p53 gene were found in patients who achieved tumor reduction, compared to non-responding patients. Furthermore, longer progression-free survival correlated with increased peak monoadduct levels in the p53 gene (p=0.032). To conclude, increased DNA damage and slower repair capacity in the p53 gene from leukocytes following HDM correlate with improved outcome of patients with multiple myeloma who undergo autologous blood stem cell transplantation, suggesting that quantitation of such biological endpoints may identify patients more likely to benefit from this procedure.

P76
Chromosome aberrations, micronuclei and DNA primary damage in nurses occupationally exposed to antineoplastic agents. Influence of genetic polymorphisms

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The widespread use of chemotherapy raises concern about the high health risks for care personnel. In fact they are handling large amounts of these compounds, which can be adsorbed despite appropriate protective measures being taken. We investigated whether occupational exposure to antineoplastic agents resulted in genetic damage, possibly indicative of adverse health effects in the long term. A cytogenetic investigation (chromosomal aberrations, micronuclei and DNA primary damage was carried on a group of 83 workers (mean age = 36 years) of oncology units and on 96 subjects of a control group matched for gender and age. Furthermore, as specific polymorphisms in the metabolic or DNA repair genes can modulate the individual response to mutagens and carcinogens, we studied four gene polymorphisms (GSTM1, GSTT1, XRCC1, XRCC3).

With regard to the cytogenetic assessment, the exposed group showed a significantly higher frequency of genetic damage when compared to the control group considering all the cytogenetic parameters evaluated (p<0.0001 for chromosome/chromatid-type aberrations frequencies, total of chromosome aberrations and micronuclei; p<0.0002 for DNA primary damage). These results are indicative of a potential genotoxic risk and corroborate the need to maintain safety measures to avoid exposure.

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P77
Biomonitoring of occupational exposure to styrene

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Styrene is a commercially important chemical widely used in the manufacture of synthetic rubber, resins, polyesters, and plastics. The highest levels of human exposure to styrene occur in occupational settings, especially during the production of reinforced plastic products, which involve manual lay-up or spray-up operations.

The objective of this work was to study occupational exposure to styrene in a multistage approach, in order to integrate the following end-points studied: styrene in workplace air, mandelic and phenylglyoxylic acids in urine, haemoglobin adducts, SCE and genotypes of polymorphic genes of some xenobiotic-metabolising enzymes. Seventy-five workers from a fibreglass-reinforced plastics factory and seventy-seven unexposed controls took part in the study.

A fairly strong correlation was observed between styrene concentration in the breathing zone and the MA+PGA in urine of workers (r=0.85, P<0.001). The levels of Hb adducts and SCE in exposed workers were significantly increased as compared with controls. Concerning the effect of the genetic polymorphisms on the different exposure and effect biomarkers studied, we observed the effect of microsomal epoxide hydrolase activity on Hb adducts of highly exposed individuals and on the levels of SCE of exposed workers.

The present results suggest the importance of individual susceptibility factors in modulating genotoxicity, although cautious interpretations are required since the size of the study population limits the power of many of the analyses.

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P78
Long-term biomonitoring of breast cancer patients under adjuvant chemotherapy, with the comet assay

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Breast cancer patients receiving CMF adjuvant chemotherapy were biomonitorized along the treatment using the comet assay, with peripheral blood lymphocytes. Samples were collected before the start and at days 1 and 8 of each chemotherapy cycle. Different haematological and biochemical parameters, the genotypes for GSTM1 and GSTT1 genes, and some characteristics of the tumours were considered in the analysis.

Results of 21 patients show a large interindividual variability of basal DNA damage and a possible relationship between haematological toxicity and low damage level. In addition, patients are classified in two groups depending on the treatment effect: A) no detectable induced DNA strand breaks; B) treatment increases DNA strand break levels. The B group can also be divided considering the responses at the end of the treatment. Ex vivo incubation of lymphocytes with MMS reveals that lack of response, or even decreases, could be related to cross-links in DNA.

These results confirm that GSTM1 null genotype present high basal DNA damage, and indicate a relationship between the tumour stage and the basal DNA damage. No effect of confounding factors (age, seasonal variation, smoking) is found.

All together, this work supports the use of the comet assay in long-term biomonitoring studies.
7. Environmental mutagenesis and carcinogenesis
P79
Genotoxic activity of standard cigarette condensate on Swiss3T3 cells

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The Cigarette Smoke Condensate (CSC) is a complex mixture of chemicals that produce a wide spectrum of biological effects. Our previous results showed that CSC induced cellular death in Swiss 3T3 cell line after 24hs of treatment with 25-50-100-150 µg/ml (IC50=130 µg/ml) following both apoptosis and necrosis pathways. Moreover, cells lose their ability of forming colonies starting from 100 µg/ml, and giant multinucleated cells were observed at 25 and 50µg/ml. The nature of the biological effects of CSC was further investigated by performing the micronucleus assay after 24hs of treatment with 30 µg/ml of CSC. The observations were made immediately after the treatment and after 24-48-72-96-120hs of growth in medium CSC-free. Preliminary results showed that CSC induced micronuclei at subsequent cell divisions to exposure; in fact, the frequency of micronucleated cells increased 72hs after the treatment. The indirect immunofluorescence assay with β-tubulin antibody showed that mitotic spindles were well assembled and no lagging chromosome was seen in treated metaphase cells. Therefore the delayed appearance of micronuclei could be explained by CSC capability of producing DNA single strand breaks, which, replicated during successive S-phase, lead to double strand breaks. In fact, an increase of DNA strand breaks after 90min and 3hs of treatment with CSC, starting from 100µg/ml, was detected by comet assay.

P80
Hydrochlorothiazide enhances micronucleus formation and affects chromosome segregation in cultured human lymphocytes

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Hypertension is the most common cardiovascular disease. Hydrochlorothiazide (HCTZ), a diuretic, is used against hypertension. In vitro genotoxicity of HCTZ is equivocal. The aim of this study is to evaluate HCTZ genotoxicity, using the Cytokinesis Blocked Micronucleus (CBMN) assay in human lymphocyte cultures in vitro. MN generation was analyzed by Fluorescence In Situ Hybridization (FISH) with α-satellite DNA centromeric probe and thus clastogenic and aneugenic effects were discriminated. Double-color centromeric FISH was applied to assess the involvement of chromosome X, Y and 8 in micronuclei and non-disjunction. The influence of centromere organization and spatial distribution on HCTZ genotoxicity was also evaluated. Thirty-two healthy adults were enrolled in the study. Two HCTZ concentrations, 5µg/ml and 40µg/ml were studied. Age, gender and smoking habit were taken into account in the analysis. We found that HCTZ induced increased frequencies of MN and chromosome malsegregation. The generation of HCTZ induced MN is provoked mainly through chromosome delay without excluding chromosome breakage. HCTZ increased the inclusion of chromosomes X, Y and 8 in MN. Non-disjunction of chromosomes X, Y and 8 was enhanced after HCTZ treatment. Age was shown to positively affect the response of lymphocytes to HCTZ. HCTZ genetic activity is not related with centromeric spatial organization of the interphase nucleus.

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P81
DNA damage in cultured human bronchial epithelial cells by polycyclic aromatic hydrocarbons and alpha irradiation

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Polycyclic aromatic hydrocarbons (PAHs) and radon are known to increase the risk of lung cancer in human. The aim of the study was to investigate the interaction of environmental PAH and radon exposure
in human bronchial epithelial cells. BEAS-2B cells were treated separately and in combinations with alpha irradiation and benzo[a]pyrene, benzo[a]anthracene and chrysene. PAH-DNA adduct levels were determined by \(^{32}\)P-postlabelling. DNA strand breaks were measured by Comet-assay. The DNA adduct-forming potential was benzo[a]pyrene >> benzo[a]anthracene ≈ chrysene in a dose range of 0.1-4 µM, at 37°C for 24 hours. DNA adduct formation by the three PAHs was synergistic as compared to the single compounds. Alpha irradiation (10 mGy) prior to treatment of the cells by the PAHs substantially decreased the adduct level. Alpha irradiation significantly induced DNA strand breaks, whereas the PAHs at 0.2 µM did not have measurable effect by the Comet assay. In combination of alpha irradiation and the PAHs, only benzo[a]pyrene had a modifying, ie. additive effect to alpha irradiation. The results obtained by the two end-points may suggest the impairment of the PAH activation mechanisms and direct increase of the DNA strand breaks by the alpha particles.

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P82
The air pollutant and potent mutagen 3-nitrobenzanthrone forms DNA adducts after reduction by NQO1 and conjugation by acetyltransferases and sulfotransferases in human hepatic cytosols.


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3-Nitrobenzanthrone (3-NBA) is a potent mutagen and suspected human carcinogen identified in diesel exhaust and air pollution. We compared the ability of human hepatic cytosolic samples to catalyse DNA adduct formation by 3-NBA. Using the \(^{32}\)P-postlabelling method, we found that 12/12 hepatic cytosols activated 3-NBA to form multiple DNA adducts similar to those formed in vivo in rodents. By comparing 3-NBA-DNA adduct formation in the presence of cofactors of NAD(P)H:quinone oxidoreductase (NQO1) and xanthine oxidase most of the reductive activation of 3-NBA in human hepatic cytosols was attributed to NQO1, and was confirmed by inhibition with dicoumarol and by using human recombinant NQO1. When cofactors of N,O-acetyltransferases (NATs) and sulfotransferases (SULTs) were added to cytosolic samples, 3-NBA-DNA adduct formation increased 10- to 35-fold. Using human recombinant NQO1 and NATs or SULTs, we found that mainly NAT2, followed by SULT1A2, NAT1 and, to a lesser extent, SULT1A1 activate 3-NBA. We showed that 3-NBA is activated by NADPH:cytochrome P450 oxidoreductase (POR) in human hepatic microsomes. Therefore, we evaluated the role of hepatic POR in the activation of 3-NBA in vivo by treating hepatic POR-null mice and wild-type littermates ip. with 3-NBA (0.2 or 2 mg/kg body weight). No difference in DNA binding was found in any tissue examined (liver, lung, kidney, bladder, colon) between null and wild-type mice, indicating that 3-NBA is predominantly activated by cytosolic nitroreductases rather than microsomal POR. These results demonstrate the role of human hepatic NQO1 to reduce 3-NBA to species being further activated by NATs and SULTs.

P83
In-vivo Combined Effect of Cypermethrin and Diode Laser Irradiation: Comet Assay Measurements of DNA Damage in Rat Liver Cells

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In the present work, the in-vivo combined effect of cypermethrin and 650 nm diode laser irradiation was considered. The DNA damages were detected after exposure to different combined treatments of diode laser (640-660 nm) and pesticide (cypermethrin). The combination of cypermethrin treatment with laser irradiation showed a synergistic effects of both low dose of level cypermethrin, with low dose of diode laser (p<0.0001), and high dose level of cypermethrin, with high dose of diode laser. The produced combined effect might occur via enhanced mechanism. Such combined exposure may serve as environmental risk factor. Finally, an interaction between the laser irradiation and cypermethrin might occur. This implies the storage of the pesticide in dark containers. In addition to the need for permanent bio-monitoring of subjects
Genetic and acquired susceptibility

Occupationally exposed to various mixtures of different environmental agents, in order to detect early genetic biomarkers of exposure and to prevent further induction of DNA legends, which could include neoplastic growth of damaged somatic cells.

P84
FISH analysis in children and mothers exposed to air pollution.
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A family pilot study was conducted in Czech Republic to test the hypothesis that genotoxic exposure to airborn particulate matter (PM) in children results in detectable effects in a number of biomarkers of exposure and early effects. The FISH chromosome painting analysis in peripheral blood lymphocytes was used to assess cytogenetic effects of 39 children from 20 families (mean age 8±1.6 years). Mean age of parents was 36±6.4 years. The subjects were selected from two different regions: Teplice, the mining district of Northern Bohemia with high air pollution, and Prachatice, the rural area in Southern Bohemia. Significant difference of FG/100 (genomic frequency of translocations) was found between parents and children (1.31 vs 0.16, p< 0.001) in Prachatice and between all parents and all children from both regions (0.86 vs 0.25, p<0.01). No increase in FG/100 was occurred in adults or children from Teplice in compare with those from Prachatice. Conclusion: no difference between two regions was found, that could be caused by the small number of subjects. The effect of age on the FG/100 was proved even in this small group.

This study was supported by Czech Government.

P85
The effects of exposure to different clastogens on the pattern of chromosomal aberrations detected by FISH
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The pattern of chromosomal aberrations (CA) was studied by FISH technique (whole chromosome painting) in workers occupationally exposed to following conditions: acrylonitrile (ACN), ethyl benzene (EB), carcinogenic polycyclic aromatic hydrocarbons (c-PAHs), and irradiation in nuclear power plants (NPP), respectively. A decrease in the relative frequency of translocations was observed in EB group, and an increase in reciprocal translocations in ACN and NPP-exposed groups. An increase in a relative number of insertions was registered under all four conditions. Significant differences in the percentage of lymphocytes with aberrations on chromosome #1 (58.8±32.7 %, vs. 73.8±33.6 % in the controls, P<0.05), and chromosome # 4 (47.0±34.1 %, vs. 29.4±32.2 %, P<0.01) were found in workers exposed to ACN. Similarly, a decrease in the proportion of cells with aberration on chromosome #1 (61.0±24.0%, vs. 73.8±33.6 %, P<0.05) and an increase on chromosome #4 (45.6±24.6 %, vs. 29.4±32.2 %, P<0.05) were observed in workers exposed to EB. % AB.C. as well as FG/100 increased with age (P<0.01). Aging also increased the percentage of translocations and reciprocal translocations (P<0.05), but decreased the relative number of acentric fragments (P<0.01). Smoking led to significantly increased FG/100 (P<0.05), but did not affect the pattern of chromosomal aberrations. Our results seem to indicate that different carcinogens may induce a different pattern of chromosomal aberrations.

P86
Nickel affects poly(ADP-ribose)polymerase-mediated DNA repair in normal and cancer cells
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Nickel(II) can be genotoxic, but the mechanism of its genotoxicity is not fully understood and the process of DNA repair may be considered as its potential target. We studied the effect of nickel chloride on the poly(ADP-ribose) polymerase (PARP)-mediated repair of DNA damaged by γ-radiation and the anticancer
Genetic and acquired susceptibility

drug idarubicin with the alkaline comet assay. We also performed MTT assay in order to evaluate the
cytotoxic potential of the singly compounds and their combined treatments. Three types of human cells:
normal lymphocytes, HeLa cervical cancer cells and K562 leukemic cells were employed. In the cells pre-
incubated with nickel chloride at 1 µM or the specific PARP inhibitor 3-aminobenzamide (3-AB) at 200 µM,
we observed an increase of DNA damage evoked by idarubicin or γ-radiation during repair incubation. In
the case of the lymphocytes treated with γ-radiation, we observed an increase of DNA damage only after
pre-incubation with nickel chloride alone and co-pre-incubation with nickel chloride and 3-AB. Our results
indicate that nickel chloride at very low, non-cytotoxic concentration 1 µM, can affect PARP-mediated DNA
repair of lesions evoked by idarubicin and γ-radiation in normal and cancer cells. We also suggest that in
the quiescent lymphocytes treated with γ-radiation, nickel(II) could interfere with DNA repair process
independent of PARP.

P87
The effect of radon, asbestos and glass fibers on DNA

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Our purposes were to analyze the DNA damaging effect of physical carcinogens i.e. alpha particle,
asbestos, glass fibers (biosoluble-bgf and non-biosoluble-nbgf) separately and in combination.
Human, mammalian lung cell lines and primary cultures of rat pneumocytes and macrophages were
exposed to alpha-particle (2, 10 mGy) and treated with asbestos or glass fiber. (0-10 mcg/cm²). Survival
and DNA damage were measured by colony forming and comet assay, respectively.
Asbestos alone induced significant cell death in case of high concentrations. The cell survival decreased
as the dose increased and the combination of the carcinogen agents further enhanced cell death. 2 and 10
mGy alpha irradiation and the low concentration of asbestos alone were not enough to increase the DNA
fragmentation. Enhanced asbestos concentration resulted in a higher DNA fragmentation. Combination of
nbgf and alpha particle induced a lower DNA damage than nbgf alone. The combined effect of bgf and
alpha particle resulted in a higher DNA damage, initially. The rejoining of the breaks started after 20
minutes repair and was almost completed at the end of 4th hours.
The fibres induced DNA damage through oxidant mediated mechanism and low dose alpha particles
enhanced the initial DNA damage caused by asbestos, glass fibre and also modified the kinetics of repair.
This work was supported by NKFP-1B/047/2004.

P88
Antagonistic effects of Acrylamide on clastogenicity of VP16

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We investigated on the Acrylamide (AA) capability of influencing the clastogenic effects of VP16, the
topoisomerase II targeting drug, by performing sequential treatments in V79 Chinese hamster cells. The
VP16 cytotoxicity resulted almost completely antagonized by preincubating cells with nontoxic
concentrations of AA, as inferred by statistically significant differences versus response with VP16 alone.
Moreover, the severe clastogenic effect of VP16, evidenced by the presence of complex structural
chromosome aberrations and by high frequencies of micronulei and sister chromatid exchanges, was
reduced by AA in a dose-dependent manner. For example, the frequency of micronucleated cells induced
by VP16 was 24.9%, and it became 11.2% or 6.3% when cells were pretreated with 1mM or 5mM AA,
respectively.
These findings let us to suppose that AA interacts with topoisomerase II. To address this question, we
performed the kDNA decatenation assay and found that nuclear extracts from cells treated with either
VP16 or AA had reduced topoisomerase II activity, as demonstrated by an inability to convert kDNA from
the catenated to the decatenated form; on the contrary, nuclear extracts from cells pretreated with AA and
then with VP16 were able to convert kDNA to the decatenated form.
Taken together, these results show that acrylamide can efficiently minimize the clastogenicity of VP16 and
suggest that it plays a role in the inhibition of topoisomerase II.
P89
Cytogenetic Effects of Arsenic Compounds in Peripheral Human Lymphocytes

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This study focuses on the analysis of genotoxic effects on human peripheral lymphocytes in vitro exposed to arsenic compounds by means of micronucleus assay (MN). Blood from three healthy non-smoker males were challenged with six arsenic compounds. Sodium arsenite (As III) and sodium arsenate (As V) were tested among inorganic As salts. Among organic salts, MMAs III, MMAs V, DMAs V and TMAO were chosen. For As III and As V at the concentration of 4 µM and 32 µM respectively an increase of MN frequency was observed. MMAs III and MMAs V show instead effects which vary widely. Statistical significance is in fact present, with a wide range, form 2 µM till 500 µM respectively. MMAs III showed also a significant increase in mitotic index suggesting a possible mitotic spindle poison effect. Moreover the FISH analysis, by pancentromeric probe, of the micronuclei reveals that the genotoxicity effect of MMAs III is of aneuploidogenic source. For DMAs V no sub-toxic concentration was identified, where a significant increase of MN was observable although a citotoxic effect was observed. Finally for TMAO no toxicity was observed up to 1 mM.

P90
Low dose radiation induced bystander effect and genomic instability in cadmium toxicity

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Low dose alpha particles derived from radon induce non-DNA targeted effects, such as bystander phenomenon, adaptive response or genomic instability, which may increase or decrease the risk of lung cancer.

Our study was designed to test the hypothesis whether these effects contribute in the toxic effect of cadmium. Human lung fibroblast cells (HFL1) were treated with Cd-chloride (0,001-10 mM) alone and in combination with alpha irradiation (10-500 mGy). Then they were assayed for cytotoxicity (MTT-assay); DNA damage and repair (COMET-assay). To see the genomic instability, the plating efficiency and micronucleus induction was followed through 40 generation of cell proliferation.

The results showed that the radioadaptive response induced by 10 mGy alpha particles was diminished by Cd (24-48 h) incubation. Cd (0,01 mM) enhanced the radiosensitivity of cells. Bystander cells found to be more sensitive to Cd, then direct irradiated ones. In the presence of Cd the rejoining of the radiation induced DNA breaks slowed down. The data on proliferation and micronuclei induction indicated that the genetic changes were detected in the progeny of irradiated and Cd treated cells.

We can conclude that the non-targeted effects of radiation should take into consideration in estimating the health risk from complex environmental exposures.

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P91
DNA-adducts, mutations and SCEs following benzo(a)pyrene exposure: A review of quantitative aspects

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To investigate the impact of benzo(a)pyrene on health, we are conducting a study of literature concerning quantitative aspects of the formation of DNA-adducts and the induction of sister chromatid exchanges and mutations following BaP exposure. We included in vitro and in vivo studies and human and animal experiments. BaP-DNA-adduct data can be compared to quantitative data concerning DNA-adducts that are formed endogenously (De Bont and van Larebeke, 2004). From studies in vitro that measured BaP-
DNA-adducts as a result of BaP exposure, it could be concluded that low doses showed a higher amount of BaP-DNA-adducts per dose-unit than higher doses. We also considered studies that quantify BPDE-deoxyguanosine adducts following Bap or BPDE exposure. BPDE is a highly mutagenic metabolite of BaP. In order to include BPDE data in comparisons, we converted these data. We assume, for our calculations, that BPDE adducts represent 100% of BaP adducts and we calculated that a factor 18.58 must be taken into account to convert BPDE exposure to BaP exposure. The data obtained after conversions showed the same trends. We plotted different in vivo animal mutation studies and could again observe the same trend: low doses of BaP result in a higher mutation frequency per dose-unit than higher doses. Per dose-unit more mean SCE’s are induced at low doses than at higher doses.

P92
Chemical and toxicological characteristics of ambient particulate matter

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The chemical composition and toxicity of particulate air pollution may vary considerably depending on its origin. The presence of transition metals and aromatic organic compounds may contribute to adverse health effects through generation of reactive oxygen species. In order to establish the relationship between chemical composition of PM, radical generating capacity and genotoxicity, we sampled total suspended particulate (TSP), PM_{10} and PM_{2.5} at 6 locations with pronounced differences in traffic intensity. DNA reactivity (DNA-adduct formation and induction of oxidative DNA damage) and the mutagenic capacity were analyzed as well as levels of polycyclic aromatic hydrocarbon (PAH), elemental composition, and radical generating capacity. We found pronounced differences in the genotoxicity and chemical characteristics of PM, although we could not establish a correlation between traffic intensity and any of these characteristics for any of the PM size fractions. A positive correlation was found between the concentration of total (carcinogenic) PAHs and the radical-generating capacity, direct and S9-mediated mutagenicity, and DNA-reactivity. The interaction between total PAHs and transition metals correlated positively with DNA-adduct formation. Radical-generating capacity was not associated with one specific PM size fraction, but mutagenicity, and S-9-mediated DNA reactivity were relatively high in PM_{10} and PM_{2.5} as compared to TSP. These data demonstrate that the assessment toxicological characteristics into account may be more efficient and valuable for reducing the health risks associated with PM exposure.

P93
Effects of nitrogen mustard analogues on mitotic spindle of C2C12 mouse cell line

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Nitrogen Mustards are among the oldest anticancer drugs currently used for the treatment of various human cancers. Recent findings have shown that the nitrogen mustard analogue, phenylacetic acid, chlorambucil’s active metabolite, induced increased rate of chromosome delay in human lymphocyte cultures. The correct chromosome segregation during mitosis depends on the formation of mitotic spindle, a dynamic bipolar array of microtubules. The centrosomes, which constitute the major microtubule organizing centers, play an important role during mitosis and they contribute to control spindle bipolarity, spindle positioning and cytokinesis. In the present study an effort was made to elucidate the mechanism by which phenylacetic acid induced chromosome delay. Additionally chlorambucil and melphalan were also studied as two nitrogen mustard’s analogues, which are currently used in clinical cancer chemotherapy. Hence the effect of the above mentioned compounds was investigated on the organization of mitotic apparatus. The analysis was performed in C2C12 mouse cell line using double immunofluorescence for the α- and γ- tubulin, which constitutes basic components of mitotic spindle. We found that the studied compounds affected the organization of microtubules and induced the formation of abnormal mesophase and mitotic cells with increased centrosome number, which resulted in enhanced frequency of multipolar metaphases. They also provoked micronucleus frequency and decreased mitotic index.
P94
The role of genotoxic and non-genotoxic mechanisms in tissue specificity of aromatic hydrocarbons

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The purpose of this study was to evaluate the role of genotoxic and non-genotoxic mechanisms in tissue specificity of 7H-dibenzo[c,g]carbazole and its derivatives, N-methylidenbenzo[c,g]carbazole (MeDBC), tissue specific sarcomagen, and 5,9-dibenzo[c,g]carbazole (diMeDBC), strict hepatocarcinogen. Various end-points such as DNA adduct formation, gene mutations and micronuclei (MNi), inhibition of gap junctional intercellular communication (GJIC) and induction of AhR activity were evaluated in mammalian cells exposed to tissue specific DBC derivatives. Significant differences in both genotoxic and non-genotoxic effects were detected among DBC derivatives. The total DNA-adduct level induced by DBC in mouse embryo cells was approximately 10-fold that of MeDBC. Activation of DBC and MeDBC via CYP1A1 and CYP1A2 resulted in MN formation, gene mutations and several stable ^32P-postlabelled DNA adducts. DiMeDBC, the liver carcinogen, was devoid of any activity in the primary mouse embryo fibroblasts and V79Mzh1A1 cells. Although diMeDBC induced significant levels of gene mutations and MNi in V79Mzh1A2 cells no stable DNA-adducts were found using a similar treatment conditions. In rat liver epithelial progenitor cells, DBC was the most efficient GJIC inhibitor, while diMeDBC manifested the strongest AhR inducing activity in both liver progenitor and hepatoma cells. The inhibitory effect of DBC on GJIC was not transient but it proceeded up to 24 hours. In contrast, MeDBC, a tissue specific sarcomagen, was a weak inhibitor of GJIC and inducer of AhR.

Based on these results we suppose that both genotoxic and non-genotoxic mechanisms play important role in tissue specificity of these aromatic hydrocarbons.

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P95
Combined cytogenetic action of hydroquinone and ionising radiation as analysed in metaphase and G2-phase peripheral blood lymphocytes.

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Hydroquinone [HQ], a metabolite of benzene, is a widely used chemical and a potential hazard to humans. Exposure may occur through the natural (diet, tobacco smoke, hair dyes) and occupational environment. Radiologists and X-ray technologists may be exposed both to ionising radiation and HQ. At present, there is a controversy concerning the mutagenicity of HQ, in particular when combined with ionising radiation, and only limited data are available.

The objectives of the present study were to investigate in vitro the clastogenic and mutagenic effect of HQ, the possible action of HQ as a radiosensitizer, and the potential synergistic effect of the simultaneous HQ and ionising radiation exposure. The endpoints used are chromosome aberrations and sister chromatid exchanges (SCE). We applied conventional cytogenetics, premature chromosome condensation (PCC) and the G2-chromosome radiosensitivity assay to analyse the combined genotoxic action of HQ and ionising radiation in peripheral blood lymphocytes at metaphase as well as directly in G2-phase using PCC. Moreover, flow cytometric analysis was applied to investigate the possible effect of HQ on cell cycle kinetics.

The results obtained suggest an additive effect of HQ when combined with ionising radiation in terms of SCEs, and a possible synergistic effect when chromatid breaks are analysed after G2-phase irradiation.
P96
Micronuclei in peripheral blood lymphocytes and buccal epithelial cells in Polish children environmentally exposed to lead.

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In this study we investigated whether an environmental exposure to lead in children produced a significant increase of micronuclei (MN) in both peripheral blood lymphocytes (PBL) and buccal epithelial cells. Examined population was composed of 92 nine year old children, living in the region where non-ferrous ores are extracted and processed and 49 control children of the same age. Exposure to lead was assessed by a lead in blood (PbB) determination by atomic absorption spectroscopy (AAS). The frequency of MN was determined by cytokinesis-block micronucleus assay. Environmental exposure to lead resulted in significantly increased levels of PbB (5.29±2.09 µg/dl vs. 3.45±1.20 µg/dl in controls), although the average level was much below the value of the biological exposure limit = 10 µg/dl. The results showed a significant difference (p<0.001) in the level of MN detected in buccal cells between the exposed and control group (6.13±5.64 vs. 3.08±4.19, respectively). The level of MN in PBL was slightly higher in exposed children (1.36±1.49 vs. 1.27±1.65 in the controls), but the difference was not statistically significant. In conclusion, our results indicate that although environmental exposure to lead was not high, it resulted in measurable biological effects in examined children [MN]. Higher level of micronuclei detected in buccal epithelial cells suggests that they are more sensitive than lymphocytes.

P97
Subway particles are more genotoxic than street particles and induce oxidative stress in cultured human lung cells

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Epidemiological studies have shown an association between airborne particles and a wide range of adverse health effects. The mechanisms behind these effects include oxidative stress and inflammation. There is a lack of knowledge regarding how particles from different urban sub-environments differ in toxicity. By using the comet assay and cultured human lung cells we show in this study that particles generated in a subway system were approximately eight times more genotoxic than particles from a nearby urban street. Analysis of the oxidative DNA lesion 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoG) showed that the subway particles also induced oxidative stress. The oxidative stress was likely generated by redox-active iron on the subway particles. Further, analysis of the atomic composition showed that the subway particles to dominating degree (atomic %) consisted of iron, mainly in the form of magnetite (Fe₃O₄). By using electron microscopy, the interaction between the particles and the lung cells was shown. The in vitro reactivity of the subway particles in combination with the high particle levels in subway systems give cause of concern due to the high number of people that are exposed to subway particles on a daily basis.

P98
The mutagenic potency of 5 polycyclic aromatic hydrocarbons in Mutamouse™) parallels their carcinogenic potency in the A/J mouse lung carcinogenesis system

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Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous environmental pollutants and powerful carcinogens which may play a role in human carcinogenesis. In the context of a project examining the
tumour initiating and promoting properties of atmospheric PAHs individually and their mixtures, the mutagenic potency (in the lung and liver) of selected PAHs in the A/J transgenic mouse model (Mutamouse™) was examined. The five PAHs examined were benzo[a]pyrene (B[a]P), benzo[b]fluoranthene (B[b]F), dibenzo[a,l]pyrene (DB[a,l]P), dibenzo[a,h]anthracene (DB[a,h]A) and fluoranthene (FLA).

Groups of male mice were treated i.p. with 3 different doses of each of the above agents and sacrificed 28 days later for mutation analysis. Clear dose-related increases in mutant frequencies occurred for all PAHs except FLA, with similar absolute and fold-increases being observed in the two tissues. Based on the mutagenic potencies observed, the five PAHs can be divided into 3 groups: a) DB[a,l]P and DB[a,h]A, with similar potencies and about 30-40 times more mutagenic than B[a]P; b) B[a]P, about 3 times more mutagenic than B[b]F; c) FLA, which is almost non-mutagenic although statistically significant mutation frequency increases were recorded after multiple dosing of very high doses (3x1000mg/kg). This relative mutagenic potency is strikingly parallel to the lung carcinogenic potency observed for the same PAHs in the A/J carcinogenesis system.

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**P99**

**Comparison of somatic mutational spectra in populations from Russia, Sweden and USA**


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Comparison of mutation spectra at the hypoxanthine-phosphoribosyl transferase (HPRT) gene of peripheral blood T lymphocytes may provide insight into the aetiology of somatic mutation contributing to carcinogenesis and other diseases. To increase knowledge of mutation spectra in healthy people, we have analysed HPRT mutant T-cells of 50 healthy Russians. Reverse transcriptase polymerase chain reactions and DNA sequencing identified 161 independent mutations among 176 thioguanine resistant mutants. 94 of these were single base substitutions, including 19 which had not previously been reported in human T-cells. Comparison of this base substitution spectrum (Noori et al, Carcinogenesis, in press) with mutation spectra in a USA (Burkhart-Schultz et al. Carcinogenesis 17, 1996, 1871) and two Swedish populations (Podlutsky et al, Carcinogenesis 19, 1998, 557, Mutation Res. 431, 1999, 325) revealed similarity in the type, frequency and distribution of mutations in the four spectra, consistent with aetologies inherent in human metabolism. Statistical analyses showed that the Russian spectrum was different from both Swedish spectra (P=0.007, 0.002), but not different from the USA spectrum (P=0.07) when Bonferroni correction for multiple comparisons was made. Age and smoking did not account for these differences. Other factors causing mutational differences need to be explored.

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**P100**

**Assessment of the genotoxic hazard posed by oral exposure to vanadium tetravalent**


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In the frame of the safety assessment of inorganic contaminants present in the water destined to human consumption, compounds of both V⁴⁺ and V⁶⁺ were assayed for in vivo genotoxicity. Micronuclei and DNA lesions detectable by comet assay have been measured in mice receiving tetravalent vanadium in drinking water along a five weeks period. Groups of at least eight male CD-1 mice were treated with a wide range of VOSO₄·5H₂O concentrations, corresponding to approximately 100, 50, 10 and 1 mg/kg b.w of vanadyl sulfate. Micronuclei were analysed both in blood reticulocytes and in bone marrow PCEs, whereas comet assay was performed on bone marrow and testicular cells. Toxicity parameters, i.e. body weight variation, as well as individual water and food intake were also registered. Kidney, liver, spleen, bone, stomach, small intestine and testis were removed at the end of treatment to evaluate vanadium uptake by atomic absorption spectrometry. Our results showed no dose related DNA damage, although an increase of
micronucleated reticulocytes frequency and tail moment values in bone marrow cells suggested a genotoxic effect at low concentrations of V$^{4+}$. Spectrometric measures of vanadium absorption showed a substantial metallic uptake only at higher doses of treatment. Further work is ongoing to confirm these preliminary findings.

This work was partially supported by ACOSET (Azienda Consorziale Servizi Etni).

P101
Genotoxic effects after combined exposure to arsenic, cadmium and benzo(a)pyrene in C57BL/6J/Han mice.

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Co-exposure to arsenic, cadmium and polycyclic aromatic hydrocarbons is widespread in the environment. All these agents are reasonably anticipated to be human carcinogens, but the molecular mechanisms of their interactions remain unclear. To provide more details on interactions of these common environmental pollutants the assessment of micronuclei frequency after combined exposure to Cd(II) or As(III) and BaP was performed.

In the study male C57BL/6J/Han mice were used. The animals were pretreated with 100 mgCd/l or 50 mgAs/l in drinking water for 7 days and then exposed by intraperitoneal injection to single dose of BaP. Since our previous experiments have shown that the dose of 15 mg BaP/kg b.w. did not induce any effect in animals pretreated with Cd(II), the higher dose i.e. 200 mg BaP/kg b.w. was applied. The mice were killed after 12, 24, 48 and 72 hours after BaP administration. The chromosome damage was assessed in bone marrow using micronucleus test.

The analysis of cytogenetic effect in mice exposed to Cd(II) and BaP showed statistically higher frequency of MN in samples at 48 h after BaP administration in comparison to control animals treated with Cd only (14.2±7.5 vs 5.4±1.6, respectively). The MN frequency in animals treated with Cd(II) + BaP did not differ from those administered only with BaP in adequate time points studied. Similarly, the level of chromosomal damage observed in mice treated with Cd(II) and in those given water only was not statistically different. In animals exposed to As and BaP the significantly elevated level of MN in polychromatic erythrocytes was shown in samples studied at 12, 24 and 48h after BaP administration comparing to animals given BaP only. Statistically higher MN frequency was found also in bone marrow of animals exposed only to As(III) comparing to controls receiving water (9.2±2.6 vs 3.8±1.3).

P102
Placental toxicokinetics of benzo(a)pyrene.

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Benzo(a)pyrene (BP) is a chemical carcinogen present e.g. in cigarette smoke and smoked and grilled foods. The ATP-binding cassette transporters may play a role in the toxicokinetics of BP. Several ABC-transporters are expressed in human placenta. Putatively, they protect fetus from foreign chemicals. The aim of this study was to clarify placental transfer and DNA-binding of benzo(a)pyrene using a dual recirculating human placental perfusion method. For placental transfer, 5 placentas were perfused for 6 hours with 0.1 µM BP. In order to study the effects of p-glycoprotein inhibition on BP transfer, four placentas were perfused with 0.1µM BP and 1 µM of verapamil. Also, 2 perfusions were done using 1 µM BP and 2 control perfusions without BP or verapamil. Antipyrine was used in all perfusions as a reference substance. $[^{3}H]$,BP concentrations were quantified by liquid scintillation counting and BPDE-DNA adducts by synchronous fluorescence spectrophotometry and $^{32}$P-postlabeling. BP crossed the placenta slowly the average fetato-maternal ratio being 0.78 at 6 hours (range 0.27-1.14). Verapamil decreased the transfer of BP to fetal circulation (average fetato-maternal ratio 0.54; range 0.21-0.78). The effect was most pronounced during first 3 hours of perfusions. BPDE-DNA adducts were produced in significant amounts in one of the perfusions with 1 µM BP. In conclusion, BP crosses placenta slowly but the fetal exposure is significant taking into account the chronic nature of BP exposure in humans. In contrast to hypothesis, verapamil inhibited BP transfer suggesting that the vascular effects of verapamil are more important than inhibition of efflux transporters.
P103
Somatic allelic deletion of GSTM1 and GSTT1 in leukoplakia as possible event in oral carcinogenesis

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Genetic instability and allelic imbalances constitute hallmark of oral cancer progression. In an earlier study we observed a high prevalence of GSTM1 and GSTT1 null genotypes as risk factors for oral leukoplakia using DNA extracted from oral leukoplakia biopsies and buccal exfoliated cells (Nair et al. Carcinogenesis, 1999, 20:743). Although somatic mutation in metabolic genes have not been reported in pathologies of oral cancer, the variants in these biopsies could be a consequence of allele loss, a possibility that has also been suggested in colon cancer. Buccal cells have been used as blood surrogate for genomic DNA but nothing is known about leukoplakia tissue. To address these queries, 33 paired blood and oral leukoplakia biopsies were collected from Indian cases, DNA extracted and genotyped at the GSTM1, GSTT1 and GSTP1 loci by multiplex PCR or fluorescence-based melting curve analysis (Lightcycler). GSTM1 and GSTT1 were concordant in 88% and 91% of the matched blood-leukoplakia DNA pairs. However, in 24% of the paired samples a clear difference was observed, with the biopsies showing GSTM1 (n=6) or GSTT1 (n=3) allelic loss or GSTP1 (n=3) variant. Our data, to be confirmed by DNA sequencing and extended in a larger study, suggests that somatic allelic deletion in oral tissue could be a possible event in oral carcinogenesis in a subset of oral leukoplakias.

P104
Formation of adducts in the reaction of acrolein with 2´-deoxyadenosine and calf thymus DNA

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Acrolein, a genotoxic α,β-unsaturated aldehyde, is found ubiquitously in the environment. It is produced as a result of the incomplete combustion of organic material and thus present in tobacco smoke and exhaust gases from automobiles. It is formed also endogenously during the metabolic oxidation of polyamines1 and is a product of lipid peroxidation2,3. The mutagenic properties of acrolein have been explored in prokaryotic and eukaryotic cells4,5. The mutagenicity of acrolein is thought to be due to its ability to form adducts to DNA. It has been shown that acrolein reacts with deoxyguanosine bases in DNA to form DNA adducts in vitro6. The objective of our work was to investigate the reactivity of acrolein with 2´-deoxyadenosine and calf thymus DNA. Besides 9-hydroxy-1,N4-propano-2´-deoxyadenosine, identified previously by Smith et al.7, the reaction with 2´-deoxyadenosine gave three novel adducts. They were all isolated by liquid chromatography and structurally characterised by mass spectrometry and NMR spectroscopy. The major adduct was formed by addition of two acrolein units to the adenine base incorporated as fused rings between N-1 and N-6. The minor adducts were identified as 3-(N4-deoxyadenosinyl)-propanal and 9-deoxyribosyl-6(3-formyl-1,2,5,6-tetrahydropyridyl)purine. The identification of acrolein adducts with both double and single stranded calf thymus DNA is under investigation.

P105
Interactions of polycyclic aromatic hydrocarbons in the rat liver

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The risk assessment procedures for mixtures of polycyclic aromatic hydrocarbons (PAHs) assume that these do not interact. However, interactions between mixture components could occur and carcinogenic potency of PAH mixtures may differ from that estimated for individual compounds. In this study, specific PAHs have been chosen based on their abundance in ambient air and carcinogenicity, and the aim is to evaluate the effects of these PAHs on cellular pathways relevant to tumour-initiation. Precision-cut liver slices were used to determine the induction by individual and mixtures of PAHs of enzymes catalysing the metabolism of PAHs. The model PAH was benzo(a)pyrene and ethoxyresorufin O-dealkylase was used to monitor CYP1A1 activity. Optimum CYP1A1 induction occurred following incubation for 24 hours, and the effect was concentration-dependent. Benzo(b)fluoranthene and dibenzo[a,h]anthracene were also potent inducers of CYP1A1, while dibenzo[a,l]pyrene, fluoranthene were weak inducers and 1-methylphenanthrene had no effect. Epoxide hydrolase and glutathione-S-transferase activities were also inducible by the PAHs, but to a much lower extent compared with CYP1A1. Epoxide hydrolase activity was inducible only by benzo[a]pyrene and dibenzo[a,h]anthracene, while there was a modest induction of GST activity solely by benzo[a]pyrene. Present studies reveal that the inductive potential of benzo[a]pyrene is influenced by the presence of other PAHs, both synergistic and antagonistic interactions being observed, that may influence their carcinogenic activity.

P106
Treatment of C2C12 mouse cell line with hydrochlorothiazide revealed microtubule disorganization and abnormal mitotic figures

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Modifications of the microtubule network (α- and β-tubulin) and microtubule organizing centers (MTOCs, γ-tubulin) may cause severe damage of mitotic apparatus, disturbing the segregation of chromosomes and resulting to aneuploid cells. Hydrochlorothiazide (HCTZ) is widely used for the treatment of hypertension and has been found to induce non-disjunction in Aspergillus nidulans and chromosome missegregation in cultured human lymphocytes. To elucidate the mechanism by which HCTZ affects chromosome segregation, we investigated the effect of HCTZ on the organization of mitotic apparatus of C2C12 mouse cell line. Cells were treated with HCTZ for one or two cell cycles. Combined application of double immunofluorescence staining assay, for the visualization of microtubules (α- and β-tubulin), centrosomes (γ-tubulin) and kinetochore proteins (CREST autoantibodies) was performed. HCTZ increased the percentage of metaphases and decreased the percentage of ana-telophases indicating a metaphase arrest. HCTZ induced disorganization of the microtubule network and also provoked high frequency of abnormal metaphases with various γ-tubulin signals. Enhanced frequency of micronuclei with CREST signals was observed in mesophase cells as well as in anaphases and telophases. These findings confirmed previous results according to which HCTZ, in human lymphocyte cultures treated in vitro, affected chromosome segregation leading to aneugenic phenomena. This work was partially supported by the "K. Katheodori" grant 1930, Research Committee of Patras University

P107
The impact of different concentrations of lead on inversion polymorphism in Drosophila subobscura

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Environmental pollution by heavy metals, especially by lead as the most widespread pollutant, mostly results from human activity. Natural populations evolve resistance, or increased tolerance when exposed
to high concentration of heavy metals. Incrising evidence suggest that genetic polymorphism of many species is related with adaptation to these specific environmental changes.

In this study we analyzed the changes of chromosome inversion polymorphism enteties in fruitfly Drosophila subobscura maintained on media with different concentrations of lead. The effects of lead were observed by cytological analysis of gene arrangements on all of the five acrocentric chromosomes and of karyotypes on the four autosomes. Frequencies of some gene arrangement on all of the five acrocentric chromosomes of Drosophila subobscura maintained on polluted media change significantly. The length of exposure to different lead concentrations results in the significant change of a few gene arrangements on the two autosomes. However, different concentrations of lead, as well as the length of exposure, do not affect parameters of inversion polymorphism (Index of Free Recombination and degree of heterozygosity). The monitoring of microevolutionary change as a way of studying the effect of global change seems promising by using Drosophila subobscura and its inversion polymorphism as a model system for further investigation.

P108
In vitro genotoxicity of organic extract from urban air particles

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Acellular assay of calf thymus DNA +/− rat liver S9 fraction coupled with 32P- postlabelling was used to study the genotoxicity of organic compounds bound onto PM10 particles collected in 3 European cities (Prague, Kosice and Sofia). Bioactivation using S9 fraction caused 2- to 7-fold increase of DNA adduct levels compared to −S9 samples indicating a crucial role of indirectly acting genotoxic EOM components. We have demonstrated a significant positive correlation between B[a]P content in EOMs and total DNA adduct levels detected in the EOM treated samples (R = 0.83; p = 0.04) suggesting that B[a]P content in EOM is an important factor for the total genotoxicity of EOM and/or B[a]P is a good indicator of the presence of other genotoxic compounds causing DNA adducts. Even stronger correlation was found between the content of eight c-PAHs in EOMs and total DNA adduct levels detected (R = 0.94; p = 0.005). Our findings support hypothesis that relatively limited number of EOM components is responsible for major part of its genotoxicity.

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P109
Chromosome aberrations in residents exposed to uranium mines waste

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The present study was conducted to evaluate the existence of potential genetic risks among long-term residents in the vicinity of uranium mines and its open-air tailings. Inhabitants of the same region but out of the influence of mines or uranium ores were selected as controls. Each group included 30 non-smoking males, aged 45 – 65 years. FISH was performed using whole chromosome paints for chromosomes 1, 2 and 4. For each individual, 2000 metaphases from PBLs were scored for spontaneous aberrations while 700 metaphases were analysed to determine the frequency of aberrations after a challenging dose (2 Gy) of gamma-radiation. Preliminary data of the frequencies of translocations and unstable aberrations involving painted chromosomes, obtained in a subset of 15 individuals from each group, are presented. Statistical comparison of the mean frequency of translocations failed to detect significant differences between the two groups. However, analysis of gamma-rays-induced chromosome aberrations showed a significant excess of translocations and acentric fragments in the control comparatively to the study group, suggesting the existence of an adaptive response in the lymphocytes of those individuals exposed to the influence of the mines waste.
P110
Carcinogenic effects in C57Bl/6J mice exposed to arsenate in drinking water and modulation of the effects by low selenium diet

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In order to assess carcinogenic effects in mice chronically exposed to arsenate, groups of ten C57Bl/6J mice were given As in drinking water at 50, 200, 500 µgAs/l for 24 months. To assess modulating role of selenium in development of carcinogenic effects of arsenic, animals were fed with specially prepared low selenium diet and were supplemented with sodium selenite (0.2 ppm) in drinking water (supplemented groups) or were without Se supplementation (non-supplemented groups).

Measurements of glutathione peroxidase activity in erythrocytes and plasma as well as selenium concentration in plasma performed after 3, 6, 12 and 18 months showed 30-40% decrease of all values in animals from non-Se supplemented groups in comparison to Se supplemented groups.

Incidence of malignant neoplasms in mice exposed to 0, 50, 200, 500 µgAs/l and supplemented with Se was 11% (9/83), 17% (15/90), 19% (16/85) and 32% (29/91, p<0.005), respectively, while in mice not supplemented with Se the incidence was 16% (14/88), 15% (13/89), 20% (19/89) and 28% (24/87, p<0.05), respectively. Malignant neoplasms of the lymphopoetic system were the most frequent neoplasms observed. Their incidence in mice exposed to 0, 50, 200, 500 µgAs/l and supplemented with Se was 8% (7/83), 10% (9/90), 16% (14/85) and 24% (22/91, p<0.01), respectively, while in mice not supplemented with Se the incidence was 14% (12/88), 12% (11/89), 17% (16/93) and 26% (23/87, p<0.05), respectively.

The statistical analysis did not show any influence of the diet and selenium supplementation on the carcinogenic effects of arsenic and survival of the animals.

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P111
Evaluation of decontamination products of industrial wastes by polyorgan micronucleus test

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The assessment of cytogenetic and cytotoxic effects of three kinds of products of a decontamination of industrial wastes is carried out by polyorgan micronucleus test (Sycheva, 2002). Bone marrow polychromatic erythrocytes (PCE), lung cells and epithelial cells of urinary bladder were studied. Micronuclei, protrusions (broken eggs or nuclear buds), binucleated cells were counted up at analysis 1000 cells in each organ. Products of neutralisation β-chlorovinyldichloroarsine (PNC; oral administration to mice for 8 days in doses 1,56; 7,8 and 39 mg/kg); sulphur mustard gas (PNS; p.o. introduction to mice within 6 days in doses 3,6; 18 and 92 mg/kg), and sovto1 (oral administration to the rats for 3 months in doses 0,005; 0,05; 0,5 mg/kg) were investigated. A range of doses - from 1/1250 up to 1/10 LD50. PNC induced micronuclei in PCE (1,56 and 7,8 mg/kg); protrusions and binucleated cells in lung (1,56 mg/kg); micronuclei, protrusions (39 mg/kg) and binucleated cells (1,56-39 mg/kg) in urinary bladder of mice. PNS induced protrusions in lung cells (3,6 mg/kg and 18 mg/kg), micronuclei, protrusions (92 mg/kg) and binucleated cells (3,6 and 92 mg/kg) in urinary bladder of mice. Sovto1 did not induce cytogenetic or cytotoxic effect in rats. Results were used at a complex assessment of efficacy of neutralisation of this wastes and hygienic substantiation of technology their processing.
Absence of genotoxic effects of radiofrequency GSM/Basic 935 and 1800 MHz on human blood cells

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The aim of this study was to evaluate the potential genotoxic effect of radiofrequency (RF) alone or in combination with X-rays (1Gy) on human blood cells. Three different conventional and molecular cytogenetic tests: chromosome aberrations, micronuclei and alkaline comet assay were applied. Heparinized whole blood taken with informed consent from healthy non-smoker donors (10 for 935 MHz, 1 W/Kg and 5 for 1800 MHz, 1W/Kg) was exposed to RF for 24 h either before or after X-rays together with concurrent appropriate sham exposures. RF exposures were done at 37°C in waveguides installed in tissue culture incubators. Immediately after treatment, blood aliquots were collected to be processed by the comet assay. Slides were analysed by a computerised image analysis system. Chromosome aberration and micronucleus assays were performed using whole blood cultures for 48 or 72h respectively as suggested by standard protocols. Proliferation index was also evaluated. Results did not show any significant difference between radiofrequency exposed and sham samples for each cytogenetic endpoint analyzed. Similarly, the combined exposure failed to indicate the presence of any synergistic effect between radiofrequency and X-rays. Proliferation indices did not indicate any differences between samples.

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Aluminum genotoxicity and human risk assessment from surrogate aluminum food

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Aluminum (Al) utensils are considered as potential source of dietary Al. Report suggests that acidic food cooked or stored in presence of Al foil contain high concentrations of Al. The extent of leaching mainly depends on the impurities present in the metal, pH, duration of the boiling and presence of some ions like fluoride.

Present work deals with systematic study on F induced leaching of Al from certain size plate (area 100 cm²) made up of known Al alloy( the foil contents Fe - 0.3%, Cu – 0.06%, Zn - 0.03%, Ni – 0.005% , Si - 0.04%, Ni -0.02%, Al- 99.545% ) under action of ascorbic and sulphic acid at different pH. Higher concentrations of F- and lower pH enhance Al leaching to a great extent.

Evidence was obtained showing that after a 2–days exposure at room temperature in presence of NaF, aluminum foil liberated nearly 1.00 mg/l of aluminum, compared with less than 0.041 mg/l in absence of fluoride. Experiments with boiling of solutions also resulted the maximum Al leaching at 10 mg/l F, while minimum Al leached in the absence of F. The results clearly indicate, that after a 60- minute boiling in the presence of Al foil 55.7% of the initial amount of ascorbic acid in the solution was destroyed, while in the presence 1 mg/l F the respective data constituted 42.95%.There is reason to believe that in the experiments with ascorbic acid NaF prevents the oxidation of ascorbic acid, when the level of NaF is 1 mg/l.

Much interest over Al in food, was finding possible genotoxic and immunotoxic effects in plants as a result of aluminum action.

We hypothesized that even low concentrations Al-F may be factors of chromosomal aberrations in plants growing on acid soil.

The leaching experiment demonstrates the effect of fluoride on mobilization Al in soils. The different soil samples were percolated under laboratory conditions with solutions containing halides (NaF, KaF NaCl). The leaching effects were compared with those of distilled H₂O. Fluoride – containing solutions induce substantial losses of Al especially from contaminated acid soil.

The phyto- and genotoxical effects of Al-F combination in water solutions and soil extracts were investigated. Samples of 10 roots of germinated seeds reaching the length of 5mm were fixed in the ethanol-acetic acid system (3:1) at 4°C for 2-12 h. Squashed samples were obtained from barleys apical root meristem and stained with acetic orcein.

After processing the barley sprouts with aluminum salts in presence of 1.5-100 mg/l F– the different types
Genetic and acquired susceptibility

of structural chromosomal aberrations (genome mutations, chromatid and chromosomal aberration) were established.

P114

Studies of the genotoxic effects of some phthalates on germ cells of the laboratory male mice

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The di-esters of o-phthalic acid (1,2-benzene dicarboxylic acid) are among the most extensive used industrial chemicals and are widely distributed in the environmental due to their extensive use as industrial solvents and plasticizers in manufacturing of plastics. Many of them have toxic potential in gonads by induction of changes in germ cells, and also impacting fertility.

In these studies have been used: di-n-butyl phthalate (DBP) and butyl benzyl phthalate (BBP). The mice indicated that 8 weeks exposure to higher doses of DBP as well as BBP increased testes weight and the number of abnormal spermatozoa as well as diminished sperm motility and concentration. Both DBP and BBP phthalates slightly increased the level of single strand breaks in haploid germ cells.

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P115

Induction of xenobiotic metabolising enzymes in precision-cut lung slices by polycyclic aromatic hydrocarbons.

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The lung is a target organ for many xenobiotics including the polycyclic aromatic hydrocarbons (PAHs), an ubiquitous class of chemical carcinogens encountered in the environment including ambient air. PAHs are indirect-acting carcinogens that require metabolism to reactive intermediates in order to manifest their carcinogenicity. The enzymes catalysing their bioactivation are cytochromes P450 (CYP1A1, 1B1) and epoxide hydrolase, and the principal ultimate carcinogens are dihydrodiol epoxides. In the lung both CYP1A1 and CYP1B1 are poorly expressed but are, however, inducible. In the present study we investigated the induction of these enzymes by PAHs, as well as of epoxide hydrolase and glutathione S-transferase, following their incubation with precision-cut lung slices.

Lung slices incubated in the presence of benzo(a)pyrene showed a concentration and time-dependent induction of CYP1A1 and epoxide hydrolase. Maximum induction of CYP1A1 was observed at a concentration of 1µM, following 48 hours of incubation. Maximum induction of epoxide hydrolase occurred at 24 hours of incubation, at the same concentration of benzo(a)pyrene. These conditions were employed to assess the inductive potential of various other PAHs, namely dibenzo(a,h)anthracene, fluoranthene, benzo(b)fluoranthene, dibenzo(a,l)pyrene and 1-methylphenanthrene. Marked differences in the induction of these enzyme systems by PAHs were noted.
P116
In vivo genotoxicity of model particulate matter (PM) in rat lung

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Inhaled particulate matter (PM) consists of a complex mixture of compounds that can contribute to genotoxic reactions in lung tissue. In this study, genotoxic effects of particle constituents and their contribution to PM-induced mutagenicity were investigated. Transgenic lacI-rats were treated intratracheally in a chronic study with ultrafine carbon black (CB) and CB reconstituted with benzo-(a)-pyrene (CB+BaP) with or without iron sulphate (FS). Bronchoalveolar lavage was performed and analysed for lung toxicity and inflammation. Mutation frequencies, mutation spectra, and pre-mutagenic DNA lesions were investigated in lung tissue. All particle treatments caused a marked neutrophilic inflammation, but this effect was significantly lower for the CB+BaP and the CB+BaP+FS group. Interestingly, the inflammatory effects were found not to correlate with an induction of oxidative DNA-damage, whereas the particle-associated BaP was found to induce significantly higher BPDE-DNA adducts than (free) BaP, instilled at the same dose. The estimation of mutation frequencies revealed differences between particle and control groups. Moreover, the molecular analysis of mutations showed significant differences in mutation spectra between particle groups and control groups, indicating a modulatory effect of particles on molecular effects of mutagenesis. In conclusion, our data show that the carbonaceous particle core may have considerable impact on the availability of mutagens as well as on the molecular events of mutagenesis in the respiratory tract.

P117
Exposure and Biological Effects of Ultra Fine Particles – A factorial study on healthy, Danish non-smokers


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Exposure to ultra fine particles (UFP) from vehicle exhaust has been related to the risk of cancer as well as cardiovascular and pulmonary disease. Exercise may augment adverse effects through increased exposure and deposition. Using a 2 x 2 factor design we studied the effects of UFP exposure and exercise on 30 healthy young adults. Each person was his/her own control and studied in four periods of 24 hours confined in a room with air pumped in from one of Copenhagen's main traffic arteries. On two occasions the volunteers were asked to cycle for 180 min during rush hours. On two occasions all particles were removed from the air by a filter. The particles were completely characterised by number and size distribution, mass and chemical composition in fraction.

Using blood and urine samples collected on each experimental 24-hr period we assessed oxidative damage to DNA, protein and lipids; analysed red blood cell and platelet counts, concentrations of haemoglobin and fibrinogen and markers of inflammation and gene expression. Furthermore, biomarkers of lung and endothelial function were also included. Finally, lung deposition measurements were taken on all volunteers.

Preliminary results indicate that both UFPs and exercise have a significant effect on endothelial function, which is important for development of cardiovascular disease. No gender differences are observed. In our remaining analyses we expect to see correlations between exposure and oxidative stress and related damage and hope that results will be useful in relation to risk assessment and possible intervention programs.
P118
Cytotoxic and genotoxic effects of aromatic hydrocarbons and its binary mixtures induced in human hepatoma cell line HepG2.

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The aim of the study was to evaluate the biological effects of 7H-dibeno[c,g]carbazole (DBC), benzo[a]pyrene (BaP) and its binary mixtures in metabolically competent human hepatoma cell line HepG2.

Cytotoxicity of aromatic hydrocarbons and its binary mixtures was determined by MTT assay, genotoxicity by the micronucleus assay. The immunofluorescent staining was used to characterize the origin of induced micronuclei (MNi) in treated cells. The interactions of DBC and BaP in binary mixtures (DBC:BaP - 1:1; 0.1:1; 1:10; 0.1:10 microM) were calculated according to the formula of Surralles et al. (Mut. Res.1995; 342: 43-59).

No substantial cytotoxic effect was determined after 2 h cell exposure to DBC, BaP and its binary mixtures. Both BaP and DBC significantly increased MNi levels in comparison to control; DBC was more potent genotoxin than BaP. The level of MNi induced by binary mixtures was comparable or even lower to those induced by DBC or BaP. Immunofluorescent staining revealed the clastogenic activity of both DBC and BaP, and its binary mixtures.

We suppose that the antagonism (cf < 1) determined in the binary mixtures could be caused by the saturation of the drug metabolizing enzymes involved in biotransformation of both genotoxins.

This study was supported by the Agency of the Slovak Republic (VEGA grant 2/3092).

P119
Effect of hypothermia on micronucleus frequency and growth properties of cultured mouse lymphoma L5178Y cells

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Several in vivo studies have reported the association between hypothermia and micronucleus induction in rodents. One in vitro report (CHL cells) is available.

To investigate whether the effect of hypothermia on micronucleus induction could be confirmed in L5178Y cells, we conducted a micronucleus test under hypothermic conditions (incubation at 25-33°C for 24 or 48 hours). Only incubation at 25°C for 48 hours resulted in an increased frequency of micronucleated L5178Y cells. Under these hypothermic conditions, no increase in apoptotic or necrotic cells was observed. Cell growth was strongly reduced and the number of mitotic cells was increased, indicating that the cell cycle is prolonged and/or arrested. Flow cytometric cell cycle analysis revealed that L5178Y cells had accumulated in S and G2/M phase.

To investigate whether hypothermia increases the sensitivity of the in vitro micronucleus test for compound testing, L5178Y were treated with nocodazole (0.01 and 0.13 µg/ml) for 24 hours both at standard (37°C) and hypothermic conditions (29°C). The results showed that nocodazole and hypothermia act synergistically on micronucleus induction in L5178Y cells.

In conclusion, hypothermia can lead to micronucleus induction even under conditions where the growth rate is strongly reduced.

P120
Fractionation of organic matter extracted from airborne particles in Mexico City, and their mutagenicity

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A year long sampling and 24 h analysis of airborne particles ≤ 10 µm (PM10) were conducted in SW Mexico City in 1998. The extractable organic matter (EOM) adsorbed to the particles was accumulated
Genetic and acquired susceptibility

monthly. Values found for PM10 and EOM were highly correlated (r = 0.973, p< 0.01). Mutagenicity of EOM was tested through the Ames assay of the strains TA98 with and without the S9 mixtures of mammalian microsomal enzymes, and YG1021 (a nitroreductase overproducer). Mutagenic potency (revertants/m²) was the slope in the regression line of the complex mixture response as well as of the lines reflecting the four organic fractions (F1-F4) obtained in chromatographic columns with increasing polarity: hexane, hexane-dichloromethane, dichloromethane and methanol. EOM and its fractions were analyzed by gas-chromatography-mass spectrometry (GC-MS) to determine polycyclic aromatic hydrocarbons (PAH) and nitro-PAH, two of the most active mutagens and carcinogens. Mutagenicity in both strains was higher, in at least one fraction, than in the respective EOM. The combined fractions maintained in the same concentrations as in the EOM produced different mutagenic potencies; however, they induced the same mutagenicity when mixed in equal proportions. A dilution factor was perhaps involved in the effects observed. Work supported by CONACyT-34340-T and PAPIIT-UNAM-IN213498 and -IN213100.

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P121
Metabolism of 4,4’-Methylene-bis(2-chloroaniline) Associated with the Formation of 8-Hydroxy-2’-Deoxyguanosine

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4,4’-Methylene-bis(2-chloroaniline) (MOCA), an aromatic amine, is mutagen and animal carcinogen and used in the production of polyurethane foams, urethane elastomers and as epoxy resin hardeners. A higher incidence of bladder cancer was reported among workers exposed to MOCA. Although the mechanism of MOCA carcinogenesis is not clear, the formation of DNA adducts is considered as an early event in the carcinogenic process. The objective of this study was to study the association of MOCA exposures and the formation 8-hydroxy-2’-deoxyguanosine (8-OHdG). Urine samples were cleaned up by using solid-phase extraction, urinary MOCA, acetyl-MOCA, and 8-OHdG were analyzed using our newly-developed LC/MS/MS methods. There was no significant correlation between MOCA and 8-OHdG and acetyl-MOCA and 8-OHdG prior to and after the shift, respectively. However, 8-OHdG levels after the shift were significantly greater than those prior to the shift (P < 0.05). The levels of 8-OHdG were linearly correlated with the ratios of acetyl-MOCA/MOCA (R² = 0.61). In conclusion, MOCA exposures would cause the increase in the formation 8-OHdG. The levels of 8-OHdG were associated with the metabolic rate from MOCA to acetyl-MOCA. These results suggest that the leakage of electron during metabolism of MOCA may play an important role in MOCA carcinogenesis.

P122
Effects on DNA of the urban air pollutants 2- and 3-Nitrobenzanthrone in human lung cells

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3-Nitrobenzanthrone (3-NBA) is a highly genotoxic urban air pollutant mainly originating from diesel exhaust, while its isomer 2-nitrobenzanthrene (2-NBA) recently has been found at much higher concentrations compared to 3-NBA in ambient urban air. To compare effects of 2-NBA and 3-NBA, human A-549 lung cell line was exposed to either of the two compounds or blank carrier and analyzed for DNA adducts by 32P-postlabeling with HPLC separation and online radioactivity detection (32P-HPLC), for the common oxidative lesion 8-oxo-2’-deoxyguanosine (8-oxo-dG) by HPLC separation with electrochemical and ultraviolet absorption detection (HPLC-EC/UV), and for general but mainly oxidative DNA damage by single cell gel electrophoresis with formamidopyrimidine DNA glycosylase (FPG-SCGE, Comet assay with FPG). Both substances caused significant levels of DNA adducts and oxidative DNA damage, with 3-NBA being 3-4 times more potent, while neither caused any significant level of the specific oxidative lesion 8-oxo-dG. These results show that the so far poorly investigated 2-NBA should be of concern with regards to genotoxicity in urban air, and also raises questions about the oxidative pathway causing DNA damage from 2- and 3-NBA.
8. Genetic and acquired susceptibility
P123
Low-dose radiation in occupational settings: variability within DNA repair genes and radiosensitivity.

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The aim of the present study was to investigate the influence of genetic polymorphisms (SNPs) in key DNA repair genes on the possible induction of an altered genotoxic sensitivity, or adaptive response (AR), in lymphocytes of radiological workers after a challenging dose of bleomycin (BLM) in vitro. The study group comprised 21 radiological workers and 49 unexposed individuals enrolled as controls. The micronuclei (MN) assay was applied to assess genetic damage. SNPs in the DNA repair genes XRCC1, XRCC3 and XPD were determined by RFLP-PCR. Both the incidence of baseline MN and the yield of BLM-induced MN were significantly higher in the exposed group compared with controls [6.27±2.69 and 13.6±4.81 vs 8.62±2.80 and 18.0±2.78; p=0.001 and p=0.0002 respectively]. The large inter-individual difference in sensitivity to BLM observed both in controls and radiological workers was modulated by XRCC3 and XPD polymorphisms, whereas the influence of XRCC1 was not clear. This work shows a clear genotoxic effect associated to ionizing radiation occupational exposure whereas occupational exposure to low doses of ionizing radiation failed to induce an AR. In addition, SNPs in genes of the DNA repair machinery could be a possible explanation of the individual heterogeneity observed.

P124
Distribution of glutathione S-transferases M1 and T1 genes in population living in the Semipalatinsk nuclear test site region

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It is known, that a key role in detoxification of xenobiotics glutation S-transferase (GST) superfamily plays important role, which converts toxins into watersoluble, unharful for cells products and deduce from an organism. The GST (M1 and T1) genes family controls synthesis of enzymes of the second phase detoxification and are characterized by significant population polymorphism, and the certain alleles can play a role of contributing factors during a carcinogenesis. In this context the purpose of the given research was to study a polymorphism of GSTT1 and GSTM1 genes in population living in territory of former Semipalatinsk test site and in control group, people who were not exposed to radiation. 326 men living in territory of Semipalatinsk region and 250 men - control group, people living in Ush-Tobe (Almaty) were surveyed. DNA samples were received by standard method. Evaluation of GSTM1 and GSTT1 genes polymorphism was carried out by polymerize chain reaction (PCR). The analysis of GSTM1 and GSTT1 polymorphism has shown, that the percentage of GSTM1 (+/-) genotype in study group was equal to 21%, GSTM1 (-/-) - 79%; GSTT1 (+/-) - 44%, GSTT1 (-/-) - 56%; where as in control group aforementioned parameters was - GSTM1 (+/-) - 74%, GSTM1 (-/-) - 26%; GSTT1 (+/-) - 67%, GSTT1 (-/-) - 33%. The obtained data demonstrated that in radiation exposed group presence of GSTM1 null - genotypes was higher than in the control group. Significant differences in frequency of GSTT1 gene between study and control groups were not revealed. Several recent studies have showed that absence of GSTM1 gene and heavy smoking can be associated with higher risk of development of oncological diseases, particularly with diseases of respiratory system. Our results strongly suggest that distribution frequency of GSTM1 (-/-) genotype among radiation exposed group was significantly higher than in control group independently from smoking habits. For final conclusions it is also necessary to study polymorphism of DNA-repairing genes (XRCC1, XRCC3, XPD, XPG etc.) at the same population.
P125
Folate metabolism and the risk of Down syndrome pregnancies in young Italian women

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Impairments in folate metabolism and elevated homocysteine levels are risk factors for having a child with Down syndrome (DS). The G80A polymorphism of the reduced folate carrier gene (RFC-1) has been demonstrated by others to affect plasma homocysteine levels in combination with the C677T polymorphism in the methylenetetrahydrofolate (MTHFR) gene, and to interact with the MTHFR A1298C polymorphism in the risk of spina bifida. We performed the present study on 80 Italian mothers of DS individuals, aged less than 35 at conception, and 111 Italian control mothers, to study the role of combinations of the RFC-1 G80A, MTHFR C677T, and MTHFR A1298C genotypes to the risk of a DS offspring. The combined MTHFR677TT/RFC-180GG genotype was associated with an increased risk (OR 6) and to be 677(CT or TT)/80GG was borderline associated with an increased risk (OR 3.47). Combined RFC-180 G>MTHFR1298A>C genotypes were inversely associated with the risk (OR 0.37), whereas borderline associations were observed for the combined MTHFR677CT/1298AA (OR 3.88) and the MTHFR(CT or TT)/1298AA (OR 3.66) genotypes. Moreover borderline significant increased risk was observed for the combined MTHFR677TT/1298AA/RFC-180GG genotype (OR 10.2).

P126
The investigation of XRCC1 and p53 gene polymorphism for colorectal cancer risk

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Purpose: Repair functions of the cell are important for recognising and correcting genetic errors. Parts of these pathways are the X-ray cross-complementing group 1 (XRCC1) gene and the p53 tumor suppressor gene. Polymorphism of these genes is well-known, but less is their role in colorectal carcinogenesis. In this study we investigated the effect of XRCC1 exon 10 Arg399Gln and p53 codon 72 Arg/Pro polymorphysm on human colorectal carcinogenesis.

Materials and methods: Ninety-seven colorectal cancer patients and matched controls were genotyped for XRCC1 and p53 alleles, by PCR-RFLP and allele-specific PCR. Results: The Gln allele of XRCC1 occurred more frequently among cancer patients than in the control group (OR: 1.54 95 % CI: 0.83-2.86) but the difference was not statistically significant. The investigated p53 Pro allele showed significantly elevated risk for colorectal carcinoma formation. (OR: 2.17 95 % CI: 1.14-4.16). Conclusion: Our results suggest an important role of the studied polymorphisms in the human colorectal carcinogenesis. Possibly an increased number of participants would also lead to a statistically significant result in case of the XRCC1 exon 10 polymorphism.

P127
Association between XRCC1, APE1 and OGG1 genotypes and lung cancer risk according to smoking status

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Purpose: The association of XRCC1 (194Arg/Trp, 280Arg/His, 399Arg/Gln), APE1 (148Asp/Glu) and OGG1 (326Ser/Cys) polymorphisms and lung cancer risk was studied versus the smoking status in a case control setting to examine possible gene-mutagen interactions.

Materials and Methods: The study population consisted of 60 lung cancer patients and 60 matched healthy controls. Smoking was quantified using tobacco consumption categorized as pack-year (PY) groups with PY<10, 10≤PY<30 and PY≥30. Odds ratios were calculated for single polymorphisms and for combinations. PCR-RFLP and PCR-SnapShot assays were performed to examine the polymorphic sites.

Results: Neither the XRCC1 194Trp and 280His alleles nor the OGG1 326Cys allele were associated with lung cancer risk. A positive association with lung cancer was found for the XRCC1 399 and the APE1 148 polymorphic alleles (OR= 2.2, p= 0.066 and OR= 2.1, p= 0.114 respectively). Grouping the individuals by smoking history, this association was amplified for light and moderate smokers (PY<30: OR= 5.1, p= 0.010 and OR= 3.2, p= 0.065 respectively), and lost for heavy smokers (PY≥30: OR= 1.1, p= 0.76 and OR= 1.1, p= 0.73 respectively). Combined risk allele analysis resulted in OR's of 6.8 (p= 0.016) and 0.21 (p= 0.321) for respectively light and moderate smokers (PY<30), and heavy smokers (PY≥30) possessing 3 or more risk alleles.

Conclusion: The results of this study show that XRCC1 399Arg/Gln and APE1 148Asp/Glu polymorphisms modulate lung cancer risk attributable to cigarette smoking exposure, especially for low and moderate tobacco consumption.

P128
A susceptibility-to-adenocarcinoma signature in prostate tissue using high-resolution synchrotron IR micro-spectroscopy

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The prostate gland is a composite organ of the genitourinary tract consisting of non-glandular (fibromuscular) and glandular components, and is the most common site of pathology in the human male. Conventionally divided into tightly-fused zones, this morphology is of clinical significance in the development of age-associated conditions such as benign prostatic hypertrophy (BPH) or prostate cancer (CaP). BPH appears to arise exclusively within the transition zone whereas the multi-focal-entity of CaP is mainly confined to the peripheral zone. We obtained tissue sets consisting of cancer-free transition zone and peripheral zone, and adjacent cancer from individual patients undergoing radical retropubic prostatectomy. Tissues archived as paraffin-embedded blocks were sectioned (10-µm thick) and mounted on BaF2 windows before being de-waxed and air-dried. Spectra were collected employing high-resolution synchrotron IR micro-spectroscopy in transmission mode. Derived IR spectra representing cell-biochemical fingerprints were subjected to principal component analysis to determine whether wavenumber-absorbance relationships expressed as single points in “hyperspace” might on the basis of multivariate distance reveal biophysical differences between scanned epithelial cells located in different tissue regions. Discrete clustering suggesting that peripheral-zone cells align more closely with cancer cells point to possible structural characteristics that may reveal a susceptibility-to-adenocarcinoma spectral signature.

P129
ERCC2 polymorphisms and micronuclei levels in thyroid cancer patients after treatment with 131I

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Radioactive iodine (131I), used to treat thyroid cancer, increases the level of DNA damage, which could be modulated by individual polymorphisms. We evaluated the micronuclei (MN) frequency in peripheral blood lymphocytes of 15 thyroid cancer patients, before and one and six months after exposure to a therapeutic dose of 70 mCi of 131I, and correlated the data obtained with individual genetic polymorphisms in the ERCC2 gene.
Genetic and acquired susceptibility

The results showed that $^{131}$I exposure increased MN frequency at first month from 5.3 to 8.8‰ ($P<0.02$) and to 8.9‰ six months later ($P=0.05$). Wild-type individuals for G23591A ERCC2 polymorphism had a lower level of MN before treatment (1.6 versus 7.1‰; $P=0.002$). One month after exposure the increase of MN was less evident in non wild-type individuals (1.4 versus 7.8 ‰; $P<0.05$). These data suggest a dual role of this polymorphism on DNA repair. Wild type individuals seem to be protected against DNA damage arising from background exposure to environmental genotoxicants, but after exposure to ionising radiation the increase in DNA damage seems to be lower in non wild-type individuals. These data might contribute to explain inconsistent results observed in case-control studies dealing with the role of ERCC2 polymorphism in cancer susceptibility.

P130
GPXPro198Leu and OGG1Ser326Cys polymorphisms and risk of colorectal adenomas and adenocarcinomas

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We assessed the association between polymorphisms in two genes involved in DNA repair of oxidative stress, GPX and OGG1, and risk of colorectal adenomas or adenocarcinomas in two cohorts. The Norwegian cohort NORCCAP consisted of 166 cases with adenocarcinoma, 974 with adenomas and 397 controls, and in the Danish cohort Diet, Cancer and Health we identified 387 cases with carcinoma and 782 controls.

No associations were found between the polymorphism GPX Pro198Leu and risk of colorectal adenomas or adenocarcinomas in the Norwegian cohort. In the Danish cohort there was a trend ($P=0.08$) towards a lowered risk of colorectal cancer, RR=0.84 (CI: 0.64-1.09) and RR=0.73 (CI: 0.47-1.12) for heterozygous and homozygous carriers of the variant allele, respectively.

In the Norwegian cohort carriers of the variant allele OGG1 Ser326Cys polymorphism had a lowered risk of colorectal cancer, OR=0.56 (CI: 0.33-0.95). No association was found with risk of adenomas. This indicates that a low repair capacity of oxidative DNA damage may not be a risk factor for colorectal adenomas or carcinomas.

P131
Individual sensitivity to genotoxic agents by the analysis of SCEs in G2-phase arrested cells and the potential role of endogenous mutagenesis

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Genetic traits that confer susceptibility to DNA damage from exposure to genotoxic agents play an important role in carcinogenesis. The cytogenetic methods used are based on the conventional analysis of metaphase chromosomes and thus G2-arrested cells cannot be analyzed. Here, we applied SCE analysis in Calyculin-A induced G2-prematurely condensed chromosomes (G2-PCCs) and the SCE frequencies determined using a modified FPG staining. The individual sensitivity to the genotoxic potential of various chemicals evaluated directly in the G2-phase of cultured peripheral blood lymphocytes from different donors. The objectives were (1) to verify whether SCE analysis in metaphase chromosomes underestimates the mutagenic potential of various possible carcinogens, (2) to investigate whether assessment of individual sensitivity to genotoxic agents can be carried out even at exposures that arrest lymphocytes in G2-phase, (3) to examine whether the individual sensitivity to genotoxic agents in terms of SCEs can be associated with polymorphism of GSTT1 gene. The presence or absence homozygous deletion of GSTT1 gene was determined using PCR. Higher SCE frequency was scored in G2-phase PCCs than in metaphase chromosomes but less variability among individuals was observed. SCE frequencies were obtained even in G2-phase-arrested cells. The preliminary results do not associate GSTT1 polymorphism with increased endogenous SCE-frequencies.
P132
Association between the β2 adrenoreceptor (ADRB2) gene polymorphisms and cardiovascular phenotypes during laryngoscopy and tracheal intubation in a Turkish population

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β2 adrenoreceptor (ADRB2) genetic variation contributes to blood pressure regulation and hemodynamics changes by mediating peripheral vasodilation. Laryngoscopy and tracheal intubation associated with hemodynamic changes. Of the ADRB2 gene four single nucleotide polymorphisms (SNPs), one of at codon 16 (Arg16Gly); the other at codon 27 (Gln27Glu) are both common and functionally important. The Glu27 ADRB2 genotype results in attenuated down-regulation compared with the wild type Gln27 receptor, whereas Gly16 exhibits enhanced down-regulation compared to Arg16. Therefore, we carried out a study in 103 unrelated adult patients (ASA grade I-II) to examine the effects of ADRB2 Arg16 and Glu27 SNPs and the cardiovascular phenotypes. We measured arterial systolic and diastolic pressure, heart rate (HR) and rate pressure product (RPP) before induction of anaesthesia and 1 min following laryngoscopy and tracheal intubation. Genomic DNA was genotyped for the SNPs of ADRB2 genes using ASPCR-RPLP assays. The allelic frequencies of mutated Gly16, Glu27 alleles were found as 66%, and 51%, respectively. Our findings showed that patients who possessed glutamine homozygote of ADRB2 at codon 27 exhibited significantly greater in the increases of HR and RPP than patients with glutamic acid after laryngoscopy and tracheal intubation.

P133
Effects of glutathione S-transferase (GST) polymorphisms on chromosomal aberrations and micronucleus frequencies in a Turkish population

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Genetic polymorphisms of drug metabolizing enzymes, influencing the metabolic activation and detoxification of carcinogens, appear to affect cytogenetics biomarkers. Chromosomal aberrations (CAs) and Micronuclei (MN) have been applied as biomarkers of genotoxic exposure and early effects of genotoxic carcinogens. Glutathione S-transferase (GST) polymorphisms showed inter-individual differences have been indicated to affect susceptibility to toxic activity of xenobiotics and spontaneous or induced level of cytogenetic biomarkers. Thus, knowledge of the GSTs polymorphisms that influence the frequencies of CAs and MN in a normal population may provide an important guide to the risk assessment of human exposure to mutagens and carcinogens. In this study, GSTM1, GSTT1, GSTP1 genotypes and CA and MN frequencies were determined for 100 Turkish healthy individuals aged 18-50 years. Individual information on DNA damage and its correlation with the genotypes of the individual will allow an assessment of individual susceptibility to cancer.

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P134
Influence of MPO, CYP1A1 and CYP1A2 genotypes on urinary mutagenicity in smokers

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Aim of the study is to find potential susceptibility factors, on genetic basis, capable of modulating individual responses to genotoxic smoke exposure and the consequent risk of smoking-related cancers. The influence of genetic polymorphisms of three phase I (activating) enzymes myeloperoxidase (MPO-
Genetic and acquired susceptibility

463G→A), cytochrome P450 (CYP1A1 (3801T→C (allele*2A) and CYP1A2 (2467 T→delT (allele*1D), -163 C→A (allele *1F)), on smoke-induced urinary mutagenicity (YG1024 Salmonella typhimurium strain with S9 mix), according to an internal indicator of tobacco smoke exposure (urinary nicotine plus its metabolites) was studied in 95 current smokers. Only high exposed smokers (N=48) i.e. with urinary nicotine plus its metabolites ≥ 0.69 mg/mmmole creatinina (median level)), with at least one -2467 delT variant and fairly those with -163A significantly increased urinary mutagenicity (-2467 T/T versus T/delT and delT/delT, p= 0.051 and p=0.019; frequencies of subjects with urinary mutagenicity ≥ 66° percentile value -163A/A and -163 C/A versus -163C/C genotype, p= 0.0091 and 0.0324). In a multiple regression analysis the increase in urinary mutagenicity was mainly associated to the CYP1A2-2467 delT/delT (t=2.147, p=0.035) but not to other polymorphisms. In conclusion CYP1A2 -2467 T/delT polymorphism is a genetic factor capable of modulating the response to high genotoxic smoke exposure and the consequent risk of smoking-related cancers in heavy smokers.

P135
Interaction of low-activity EPHX1 and GSTP1 genotypes with smoking in the etiology of HNSCC

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Many polymorphic enzymes can be regarded as potential susceptibility factors for cancer in smokers. A case-control study was performed to evaluate the role of five polymorphic genes, namely GSTP1, EPHX1, NQO1, hOGG1 and XPD, in the etiology of head and neck squamous cell cancer (HNSCC), which is strongly associated with tobacco use. Data were first analyzed with a bioinformatic approach, i.e. with a machine learning algorithm named XCS that predicted that carriers of low-activity EPHX1 and GSTP1 genotypes, smoking more than 12 packyears, are at increased risk of developing HNSCC. Logistic regression and hierarchical model analyses were computed to validate XCS results and to estimate the specific weight of each risk factor and their interactions. In the main effect analysis, cancer risk resulted significantly higher only for low-activity EPHX1 (OR=2.57, 95%CI 1.3–5.1). However, the joint effect of low EPHX1-GSTP1 activities and smoking (≥12 packyears) was more than additive in enhancing HNSCC risk to OR=15.11 (95%CI 4.51–50.58), thus confirming the results of XCS. The present study suggests that bioinformatic and statistical approaches can be used as complementary tools to evaluate the role of genetic and environmental risk factors in cancer susceptibility.

P136
c-MYC Asn11Ser is associated with increased risk for familial breast cancer

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c-MYC is a multifaceted protein that regulates cell proliferation, differentiation and apoptosis. Its crucial role in diverse cancers has been demonstrated in several studies. Here, we analysed the influence of the rare c-MYC Asn11Ser polymorphism on familial breast cancer risk by performing a case-control study with a Polish (cases n = 349, controls n = 441) and a German (cases n = 356, controls n = 655) study population using the TaqMan allelic discrimination assay. All cases have been tested negative for mutations in the BRCA1 and BRCA2 genes. A joint analysis of the Polish and the German study population revealed a 54 % increased risk for breast cancer associated with the heterozygous Asn11Ser
variant (OR = 1.54, 95 % CI 1.05 - 2.26, p = 0.028). The breast cancer risk associated with this genotype increases above the age of 50 years (OR = 2.24, 95 % CI 1.20 - 4.21, p = 0.012). The wild type amino acid Asn of this polymorphism is located in the N-terminal MYC transactivation domain and is highly conserved not only among most diverse species but also in the N-MYC homologue. Due to the pivotal role of c-MYC in diverse tumours this variant might affect the genetic susceptibility of other cancers as well.

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P137
Investigation of DNA damage associated to XRCC3 Thr241Met polymorphism: a pilot study using an a priori subject selection using frozen lymphocyte samples.

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The influence of DNA repair polymorphisms on the individual susceptibility to genotoxic agents can be evaluated by using intermediate endpoints, such as genotoxic biomarkers, after treatment of peripheral lymphocytes with challenging agents. Therefore, in order to optimise the use of resources, an experimental design whereby study subjects are first genotyped and characterized for confounding factors, allowing the selection of most suitable subject for subsequent challenge assays, is recommended.

In this pilot study, designed to verify the individual susceptibility associated with the XRCC3 Thr241Met polymorphism, blood samples were obtained from 57 healthy subjects, peripheral lymphocytes isolated, aliquoted and stored frozen. After genotyping, samples of lymphocytes from 10 couples of wild type and variant carriers, matched by age, sex and smoking habits, were challenged with 0.015μg/ml mytomycin C (MMC) or 2Gy gamma radiation. Micronuclei and bridges in binucleated lymphocytes were analysed from 8 couples. Levels micronuclei induced by MMC and radiation were different in 4 and 3 pairs (p < 0.05 by chi square), respectively, with no relation to the XRCC3 genotype. Both challenging agents also increased bridges. After treatment with radiation, bridges were slightly more elevated in wild type individuals compared to variants (p=0.03 by paired t test), while no difference was observed in cells treated with MMC. The results of this pilot study indicate that the XRCC3 Thr241Met polymorphism may be associated to increase susceptibility to radiation. For a confirmation of these preliminary findings, larger study groups should be compared. To this aim, the a priori selection of suitable study group from existing lymphocytes banks may represent a convenient approach.
9. Dietary modulation of genotoxicity/carcinogenesis and nutrigenomics
P138
Folate status and chromosome damage

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Results from three studies, shows that there is a correlation between low folate status and a high frequency of micronucleated very young erythrocytes (fMN-Trf-Ret). This correlation was determined in blood from persons, both men and women, with a normal serum folate status. A correlation between folate status and chromosome stability has been observed by others but then restricted to certain subpopulations e.g. folate deficient persons.

The frequency of micronuclei in very young erythrocytes in human peripheral blood (fMN-Trf-Ret) can be determined. In a blood sample, a separation of these very young cells (Trf-Ret) from older erythrocytes makes this micronucleus determination possible (L.Abramsson-Zetterberg et al. (2000), Environ. Mol.Mutagen 36: 22-31). This separation is made with an immuno-magnetic method.

Micronuclei are formed mainly during the last cell division of the erythropoiesis, a process occurring continuously at a high rate. Thus, variation in genotoxic exposure can be studied over short periods, which is different from e.g. assays using peripheral lymphocytes. The use of flow cytometer allows a great number of cells (about 500 000 per sample) to be analysed, giving the high sensitivity, which is a prerequisite in monitoring studies.

P139
The chemopreventive effects of (-)-epigallocatechin gallate and ellagic acid in N-ethyl nitrosourea-exposed mice

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The natural plant polyphenols, (-)-epigallocatechin gallate (EGCG) and ellagic acid (EA), were examined to understand the underlined mechanisms of their anti-genotoxic effects. Mice were orally fed with EGCG or EA for consecutive seven days before challenging with N-ethyl nitrosourea (ENU). By using the highly sensitive and specific gas chromatography/mass spectrometry (GC/MS), pretreatment with 200, 100 mg/kg EGCG and 200mg/kg EA significantly reduced ENU-induced DNA damage (7-methylguanine) in mice (P<0.05). Moreover, EGCG and EA could markedly inhibit ENU-induced hprt mutations in mouse splenic T lymphocytes (P<0.05). Since 7-methylguanine is believed to be repaired by N-methylpurine-DNA glycosylase (MPG) in vivo, the expression of mRNA of MPG in mouse liver tissue was examined by RT-PCR. EGCG and EA considerably increase the expression of MPG mRNA. These data suggest that the protective effects of EA and EGCG against chemical-induced DNA damage and mutations might at least partly result from its modulating effect on DNA repair.

P140
Modulation of DMBA-induced expression of cytochrome P450 and phase II enzymes by plant phenols

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7,12-Dimethylbenz[a]anthracene (DMBA) is an effective mouse skin carcinogen. As the other PAHs, DMBA requires metabolic activation in order to exert its carcinogenic activities. Our earlier studies showed that protocatechuic, chlorogenic and tannic acids and resveratrol, besides scavenging reactive DMBA metabolites, modulate the activity of cytochrome P450 dependent enzymes and glutathione-S-transferase. The aim of the present study was to examine the effect of these compounds on the expression of CYP1A1/1A2, CYP1B1, GST pi and theta in female BALB/C mouse epidermis by Western blot assays.
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Phenolics were applied topically at the dose of 16 μmoles one hour before a single application of 10 nmoles of DMBA in acetone. The constitutive expression of all tested enzymes was observed, however their expression levels were different. Topical application of initiation dose of DMBA increased mainly the level of CYP1A1/1A2 and CYP1B1. Pretreatment of mice with tannic acid and resveratrol one hour before DMBA decreased the DMBA-induced level of both isozymes cytochrome P450. The all tested compounds did not affect on the total activity and the level of GST π and theta, but tannic acid slightly decreased of the level of both GST isoforms. The results of our study indicate that although moderate, the effect of phenolic acids and resveratrol on expression of CYP1A1/1A2, CYP1B1, GST theta and π in mouse epidermis may play certain role in their anticarcinogenic activity.

P141
Modulation of NFκB activity by plant phenols in mouse epidermis

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Plant phenols, including phenolic acids and stilbene derivatives have been shown to inhibit the tumor promotion stage in animal carcinogenesis models, including mouse skin. The transcription factor NF-κB plays an important role the activation of genes which are involved in the promotion of initiated cells. In this study we evaluated the NF-κB activation by potent tumor promoter, phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) and its modulation by three structurally different phenolic acids and resveratrol. Pretreatment of mice with 16 μmoles of tannic acid or resveratrol 15 minutes before TPA treatment, significantly reduced the degradation of IκBα (p35) as measured by immunoblotting analysis. Nuclear translocation of NF-κB /p65 was also the most affected by these two phenols. NF-κB DNA binding activity was measured by ELISA –based procedure and showed diminished level of TPA induced binding as result of tannic acid and resveratrol pretreatment. Simple phenolic acid, protocatechuic and hydroxycinnamic acid derivative, chlorogenic acid did not show significant effect on NF-κB activation. These results indicate that polyphenol tannic acid and stilbene derivative, resveratrol may affect the TPA promotion in mouse epidermis by reducing the NF-κB activation.

P142
Antioxidative activity of fresh white cabbage and sauerkraut provides essential protection of other food components (ex vivo) and cultured cells (simulated in vivo)

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White cabbage and its fermented counterpart – sauerkraut - are vegetable products traditionally served in Central Europe with meals rich in meat and fats. Our research concentrates on finding out whether this culinary behaviour is a mere tradition or provides health promoting effects, hence deserves to be included in current dietary recommendations. One of important activities exhibited by plant foods is their ability to prevent oxidative damage of other food products and also cellular components in vivo. We investigated protective properties of cabbage and sauerkraut juices with regard to both these abilities. The protection of lard and rapeseed oil against oxidation was assessed after 6 hr heating at 100°C by determining changes in peroxide number, extinction coefficient at 233 nm and fatty acid composition. The protective effects at the cellular level were studied by comet assay and involved estimation of DNA damage in HT-29 cells exposed to hydrogen peroxide together with the juices for 30 min (in situ protection) or preincubated with juices for 24 hr then exposed to hydrogen peroxide (induction of endogenous antioxidative barrier). In all cases both juices offered substantial protection against oxidative damage, though no induction of cellular antioxidants was observed. In particular, a very strong protective effect of fresh cabbage against oxidative damage of fats during heat processing suggests that the mentioned culinary tradition should be preserved.
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P143
Modulation of the H\textsubscript{2}O\textsubscript{2} induced DNA damage and cell death in PC12 cells by the natural antioxidant Ergothioneine

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Reactive oxygen species (ROS) both from endogenous and exogenous sources are involved in several pathological processes including neurodegenerative diseases; neurodegeneration that can be potentially modulated by treatment with free-radical scavengers and antioxidants. In the present study the antioxidant capacity of the natural antioxidant ergothioneine (EGT) was analyzed in PC12 cell model. The ability of EGT to prevent H\textsubscript{2}O\textsubscript{2}–dependent cell death was tested by MTT and Comet assay. H\textsubscript{2}O\textsubscript{2} insult was challenged with increasing concentration of antioxidant with two different incubation periods. At the concentrations of H\textsubscript{2}O\textsubscript{2} tested, 250 and 500 M, with the pre-treatment of 23h with EGT, 250 M and 1mM, followed by 1h of H\textsubscript{2}O\textsubscript{2} incubation resulted in increased cell viability, data in agreement with a decrease DNA damage visualized by Comet assay. Moreover protein analysis reveal that, against the H\textsubscript{2}O\textsubscript{2} insult, EGT acts as MAPKs specific inhibitor. These results clearly indicates that EGT may play a protective role in rescuing oxidative stress induced apoptosis, likely by activating antioxidant intracellular pathway involving MAPKs cascade.

P144
Modulatory effect of xanthohumol on genotoxicity of heterocyclic amine (IQ)

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Xanthohumol (3-[3,3-dimethyl allyl]-2,4,4-trihydroxy-6-methoxychalcone) is a prenylated chalcone that occurs only in the hops, Humulus lupulus L. (Cannabaceae). Hops are used to add bitterness and flavour to beer, therefore the main dietary source of xanthohumol is beer. Based on the in vitro studies xanthohumol has been characterized as a broad-spectrum cancer chemopreventive agent acting at the initiation, promotion and progression stages of carcinogenesis. At the initiation stage xanthohumol modulates carcinogen metabolism. The heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) is a potential human carcinogen found in cooked food that requires initial metabolic activation by cytochrome P450s. In Salmonella typhimurium TA98 where standard rat liver S9 fraction mediated exogenous activation was used, xanthohumol suppressed mutagenic activity of IQ in a dose dependent manner (EC\textsubscript{50} 14 µg/plate). In human hepatoma (HepG2) cells, which have retained the activities of certain phase I and II enzymes xanthohumol strongly inhibited their EROD activity. Exposure of HepG2 cells to IQ in the presence of 10 µg/ml xanthohumol for 24 hours completely prevented IQ induced DNA damage, which was measured with the comet assay. Xanthohumol alone did not induce DNA damage. These results provide additional evidence of high cancer preventive potential of xanthohumol. The potential health-maintaining effects of xanthohumol as a cancer chemopreventive agent have already led to the development of experimental beers with high levels of xanthohumol (ca. 5 mg/L), however further investigations in particular farmacokinetic and animal model data are need in order to be able to evaluate the effects and side effects of xanthohumol consumption on human health.

P145
Protective effects of zinc in yeast Saccharomyces cerevisiae

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Antimutagen compounds play an important role in decreasing damage induced by oxidants. Zinc is a microelement essential for biological functions, during physiological growth and immune function. It’s vital for the functionality of more than 300 enzymes, for the DNA stabilization and the gene expression.
Considering that zinc is a component of superoxide dismutase, it’s probably that it plays an important role in the protection against oxidative damage. Epidemiological studies suggest that zinc deficiency may be associated with increased risk of cancer. Despite the importance of zinc in human health, its role in antimutagenesis and anticarcinogenesis has not been well elucidated.

The aim of this work was to investigate the effect of zinc sulphate in yeast cells of Saccharomyces cerevisiae D7 strain and to evaluate its ability to protect against genotoxic damage. The genotoxic and mutagenic potential of zinc sulphate have been previously investigated.

Antimutagenesis experiments were performed using hydrogen peroxide as mutagen, with different concentrations of ZnSO₄. The results demonstrated that zinc had antimutagen activity in growing cells in two experimental conditions. Hydrogen peroxide caused significant decreases of cellular survival; zinc recovered survival percentage until 100%. Zinc sulphate decreased the mitotic gene conversion rate induced by H₂O₂, at concentration of 10µM, 20µM, and 50µM. Zinc sulphate reduced significantly point mutation frequency induced by H₂O₂ at all concentrations assayed.

In conclusion, the data suggest that zinc sulphate exerted a protective effect on yeast cells in oxidative stress conditions. The effect is not specific for a single genetic damage.

P146
Antimutagenicity of vegetable extracts on the mutagenesis induced by plant metabolites of aromatic amines

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Studies have demonstrated that many edible plants, as whole vegetables or their main ingredient, contain antimutagenic substances as well as the enzymatic machinery to activate environmental mutagens/carcinogens. Given the important interaction of these antagonistic components in plants, the purpose of this study was to evaluate antimutagenic activity of vegetables in the Mexican diet as uncooked ingredients in salads: parsley (Petroselinum sativum), celery (Apium graveolens) and germinated alfalfa (Medicago sativa). Extracts of these plants were analyzed against mutagenic activity of 4-nitro-o-phenylenediamine (NOP), m-phenylenediamine (m-PDA) and 2-aminofluorene (2-AF), using the Ames reversion mutagenicity assay with Salmonella typhimurium TA98 as indicator organism and coriander (Coriandrum sativum) cell cultures as metabolized system. These aromatic amines (AA) are metabolically activated into mutagens by both animals and plants. NOP is a direct-acting mutagen whose potential can be enhanced by plant metabolism. m-PDA and 2-AF are activated into potent mutagens producing frameshift mutations. The plant cell/microbe coinubcation assay was used as activating system for AA transformation and plant extract interaction. Aqueous extracts of raw parsley and celery significantly reduced mutagenicity of the three metabolized AA. Germinated alfalfa did not decrease NOP mutagenicity, but 2-AF and m-PDA diminished it slightly. This suggests that the chlorophyll contained in the extracts could have reduced mutagenicity. Acknowledgments PAPIIT-UNAM-Proyect-IN222202.

P147
Effect of the red beet extract on leukocyte DNA measured by comet assay.

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Beetroot (Beta vulgaris) contain a variety of components, which were reported to possess chemopreventive potential. The aim of our current study was to assess the influence of ingested beetroot extract on the damage of blood leukocytes DNA, caused by the standard environmental carcinogens. Standard laboratory diet of male Wistar rats and Sprague-Dawley females was enriched for 28 consecutive days with 2.5 ml beetroot extract. On the 27th day the male rats were i.p. treated with KBrO₃, N-diethylnitrosamine (DEN), or CCl₄. Female rats were treated i.p. with 7,12-dimethylbenzanthracene (DMBA). The comet assay procedure was performed in the whole blood samples. DEN, CCl₄ and DMBA were powerful DNA-damaging agents, KBrO₃ was not active. Beetroot-enriched diet partly protected DNA from the strand breaks only in the case of DEN, no such effect was found for CCl₄ and DMBA. The rate of DNA damage in beetroot receiving
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animals after CCl₄ administration was even greater than in rats receiving CCl₄ only, suggesting that the beetroot DNA-protective action in vivo is carcinogen-specific.

P148
Influence of lignin on reduction of oxidative DNA damage in lymphocytes and testicular cells isolated from rats and mice.

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Lignin is one of the major components of plant cells walls. It is a highly branched aromatic biopolymer composed of phenylpropane units with hydroxyl and carbonyl substitutions. The phenolic hydroxyl groups of lignin can act as effective scavengers of free oxygen radicals produced by oxidants.

The aim of the present work was to investigate the protective effect of biopolymer lignin on the level of DNA damage induced by oxidative stress in vitro in peripheral blood lymphocytes and testicular cells freshly isolated from rats and mice. As model oxidative agents were used H₂O₂ and visible light plus photosensitizer Methylene Blue. For detection of DNA damage were used classical (for detection of DNA single strand breaks after H₂O₂-treatment) and modified (for detection of oxidative DNA lesions after treatment with visible light-excited Methylene Blue) comet assay.

We found out that pre-treatment of cells with lignin, compound that possess adsorptive and antioxidative capacity caused a significant decrease of H₂O₂-induced DNA damage and visible light-induced oxidative DNA lesions in tested primary cells isolated from rats and mice.

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P149
The anti-clastogenic effect of Zhu-Ling Mushroom (Polyporus umbellatus) in vitro and in vivo

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Zhu-Ling Mushroom (Polyporus umbellatus) is a commonly used Chinese Medicine for renal and liver disease. In the present study the chemoprotective effects of polysaccharides of Polyporus umbellatus (PUPs) on cyclophosphamide (CP)-induced chromosome damage were studied in human lymphoblastoid TK6 cells and in ICR mice. In TK6 cells, pretreatment of PUPs at the dose of 300 µg/ml 30 min before exposure to CP significantly reduced CP-induced micronuclei (MN) (P<0.05). Furthermore, pretreatments of PUPs at a dose of 50 mg/Kg by i.p injection 30 min before CP treatment resulted in statistically significant decrease in the frequencies of CP-induced MN in the peripheral blood reticulocytes of ICR mice (P<0.05). These results show that PUPs could protect against CP-induced chromosome damage both in vitro and in vivo.
P150
Cytogenetic effects of grape extracts from Greek varieties of Vitis vinifera and plant polyphenols on mitomycin C-induced genotoxicity in cultured human lymphocytes

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The grape (Vitis vinifera), one of the world’s largest crops, and wine have been part of human diet for more than 6,000 years. In recent years, grape extracts, wine and polyphenolics present in them have been the center of attention for the beneficial effects in human health. The present study was carried out in order to evaluate the modulatory influence of grape extracts from two Greek varieties of Vitis vinifera (Assyrtiko Santorini and Mandilaria Santorini) and polyphenols found in them on sister chromatid exchanges (SCEs) induced by the mutagenic anticancer drug mitomycin C (MMC) in human peripheral blood lymphocytes. Treatment with caffeic acid (3,4-dihydroxycinnamic acid), gallic acid (3,4,5-trihydroxybenzoic acid) and rutin hydrate significantly potentiated the frequency of MMC-induced SCEs. Our previous studies have also shown that caffeic acid, gallic acid and rutin hydrate enhanced reactive oxygen species (ROS) mediated breakage of plasmid DNA induced by MMC in the presence of iron (Fe). This pro-oxidant action was probably attributed to the Fe reducing properties of polyphenols. As well as, cell culture systems tend to be highly oxygenated, unlike the in vivo situation. Therefore, the pro-oxidant action of polyphenols may be related more to the oxygenated environment than to the natural action in the body. In general, a positive result in a genotoxic test has been considered undesirable to human. However, other polyphenols as resveratrol have been reported to increase the frequency of SCEs, while it has also shown many beneficial effects in pharmacological aspects.

On the contrary to caffeic acid (3,4-dihydroxycinnamic acid), another plant phenolic, ferulic acid (4-hydroxy-3-methoxy cinnamic acid) had no effect on MMC action indicating that the 3,4-dihydroxy group may be essential to the activity of caffeic acid. Furthermore, protocatechuic acid (3,4-dihydroxybenzoic acid) as opposed to gallic acid (3,4,5-trihydroxybenzoic acid) did not affect MMC clastogenicity suggesting that the 5'-hydroxy group of gallic acid may be required for its genotoxicity. The only polyphenol showing a protective effect was quercetin, which in contrast to its glycosylated form rutin hydrate decreased MMC-clastogenicity. That the 5'-hydroxy group of gallic acid may be required for its genotoxicity. The only polyphenol showing a protective effect was quercetin, which in contrast to its glycosylated form rutin hydrate decreased MMC-clastogenicity. It has been estimated that around one-third of all human cancers may be related to the diet. The use of dietary antimutagens and anticarcinogens has been seen as the most promising approach to protection of human health. Basil (Ocimum basilicum L.) is well known medicinal and aromatic plant. Antimutagenic effects of essential oil of basil (EO) and its pure constituents, linalool, myrcene and 1,8-cineole were evaluated in the Ames test using Salmonella typhimurium TA100. UV-C irradiation and two chemical mutagens, 4-nitroquinoline-N-oxide (4NQO) and 2-nitropropane (2-NP) were used for induction of mutagenesis. UV-induced mutagenesis was significantly reduced by EO (76%), linalool (64%) and myrcene (74%), while 1,8-cineole showed moderate inhibitory effect (32%). All tested substances inhibited mutagenesis induced by direct acting model mutagen/carcinogen 4NQO (52% for both EO and 1,8-cineol and 67% for myrcene); linalool is under study. In the presence of 2-NP (both with and without S9), EO and 1,8-cineole showed moderate inhibition (30 and 35%, respectively), while remaining two substances did not show inhibitory effects. Although there are limitations in extrapolation bacterial antimutagenicity data...
P152
Differential effects of soy isoflavones and their combination with tamoxifen in modulating tumor parameters and ERα expression

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The controversy of incorporating soy and/or soy-derived compounds into the diet of women prescribed tamoxifen continues to be an impetus for research. Recently, we have shown that a diet combination of daidzein, a soy isoflavone, and tamoxifen is more effective than tamoxifen alone in reducing tumor parameters such as tumor burden, tumor incidence, and tumors per rat in the Sprague-Dawley/DMBA rat model of mammary carcinogenesis. Conversely, genistein, the purported “active component” of soy, abrogated the efficacy of tamoxifen. Tumor data alone is of great importance, but the next step is to elucidate the mechanisms underlying the effects of the diets. Due to the antiestrogenic properties of tamoxifen and the variable agonist/antagonist effects of daidzein and genistein, a logical first step is to investigate their influence on estrogen receptors, ERα and ERβ. Preliminary results, determined by immunohistochemical analysis, concur with the generally accepted role of ERα in mammary cancers. ERα is upregulated in the ductal epithelium of normal mammary glands of animals fed ineffective diets and downregulated in those animals fed effective diets. Further research is necessary for defining the exact mechanism of action of soy components and their combination with tamoxifen in this animal model of carcinogenesis and how this knowledge can be applied to humans.
10. Reactive oxygen species and oxidative stress
P153
Evaluation of a bioreductive agent by the comet assay: DNA strand breaks, oxidative damage and repair

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The purpose of the present work is to elucidate the mechanism of action of the bioreductive agent Q-85. Bio-reductive agents are designed to take advantage of the particular metabolic characteristics of the resistant hypoxic regions of the solid tumours.

Caco-2 cells were treated with 0.01, 0.05 and 0.1 µM of Q-85 in hypoxic conditions and with 0.5, 1 and 5 µM in well oxygenated conditions during 2 hours. The number of viable cells was determined by the Trypan blue exclusion method, at different times after the treatment (0, 24, 48 and 72 hours). Just after the treatment and 24 hours later the alkaline comet assay was carried out. The enzymes formamidopyrimidine glycosilase (FPG) and endonuclease III (Endo-III) were used to detect the oxidative damage. Comets were classified by the visual score method.

Viability percentages after the treatment were 100% in all the cases. The proliferation rate of the treated cells was similar to the proliferation rate of the control cells. There was a significant increase in the number of strand breaks in both conditions; the incubation with FPG and ENDO III revealed an oxidative damage. After 24 hours there was an important repair of the DNA strand breaks and the oxidative damage.

P154
No evidence of correlation between oxidative stress and cytogenetic damage in Chronic Obstructive Pulmonary Disease (COPD)

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COPD is a major cause of chronic morbidity and mortality in industrialised countries. It is characterized by a progressive airflow limitation resulting from an abnormal inflammatory response of the lungs. Increased oxidative stress has been associated with several diseases that are also characterised by chromosomal instability.

Due to the well known presence of oxidative stress in COPD, we investigated whether chromosome instability was also present in this disorder.

Whole blood lymphocytes from 49 COPD patients and 66 controls were cultivated in vitro and cytogenetic damage was evaluated by the micronucleus (MN) and sister-chromatid-exchange (SCE) frequency.

No significant increase in MN and SCE frequencies was observed in COPD patients compared to controls. Both PI (Proliferation Index), calculated from SCE test, as well as NDI (Nuclear Division Index), calculated from MN test, were unchanged, strongly suggesting that the status of the cell cultures and the capacity of cells to divide were the same in the two groups. As COPD is strictly related to smoking habit, the role of smoke is discussed.

P155
Differential response to oxidative stress induced DNA damage, chromosomal aberrations and apoptosis in ataxia telangiectasia

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Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by neuronal degeneration accompanied by ataxia, telangiectasias, acute cancer predisposition, and sensitivity to ionising radiation (IR). The AT phenotype has been suggested to be a consequence, at least in part, of an inability to respond appropriately to oxidative damage. The aim of this work was to correlate primary DNA damage,
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chromosomal aberrations and apoptosis induction in response to oxidative stress in AT cell lines. The effect of L-carnitine on the capability to respond to oxidative damage was analysed in comparison to mannitol, a known scavenger of reactive oxygen species. L-carnitine, a trans-mitochondrial carrier of acetyl and long chain groups, seems to act as an agent possessing protective effects against oxidative stress. AT cell lines were pre-treated with L-carnitine or mannitol and treated with t-butyl hydroperoxide to induce oxidative damage and comet assay, apoptosis and chromosomal aberrations analysis have been performed. The results obtained indicate a clear enhanced protective effect of L-carnitine in AT cell lines in terms of reduction of primary damage, chromosomal aberrations and apoptosis, in comparison to control cells. Our data suggest a possible role of L-carnitine in the cell cycle checkpoint control or in enhancing DNA repair capability.

P156
The effects of vitamin C on oxidative mutagenesis in microbial tests

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It has been established that DNA damage induced by reactive oxygen species is involved in mutagenesis and carcinogenesis. The study of antigenotoxic potential of antioxidants is of great importance for prevention of human health.

Vitamin C is well known as potent antioxidant, but its prooxidative effects have also been reported. Antigenotoxic properties of vitamin C were examined in E.coli K12, E.coli WP2 and S.cerevisiae D7 reversion assays as well as in S.cerevisiae comet assay. We used t-Butyl hydroperoxide (t-BOOH) to induce oxidative mutagenesis and H2O2 to induce strand breaks in comet assay.

Vitamin C reduced t-BOOH-induced and spontaneous mutagenesis in repair proficient and MMR deficient strain of E.coli K12 respectively, as well as t-BOOH-induced mutagenesis in S. cerevisiae D7 strain. However, in E.coli K12 strains carrying plasmid with microsatellite sequences, treatment with vitamin C slightly stimulated microsatellite instability. Vitamin C also showed mutagenic effects in WP2 oxyR strain. The observed effects were probably due to its prooxidative potential. In comet assay, antagonistic results were obtained: while low concentrations inhibited oxidative damage, higher concentrations stimulated it.

Obtained results indicate that vitamin C can exhibit antimutagenic or co-mutagenic effects depending on the dose and other experimental conditions.

P157
Glutathione S-transferase genetic polymorphism and oxidative stress parameters in lung cancer patients

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Glutathione S-transferase (GST) genotype and parameters of oxidative stress were analyzed among lung cancer patients and controls, matched by gender, age, including histological type of cancer, smoking habit and occupational exposure. The study included 404 patients with diagnosed lung cancer and 410 controls. According to the investigation, following conclusions were proposed: 1) The absence of relationship between GSTM1, GSTM3, GSTP1 and lung cancer susceptibility was found, 2) Among lung cancer patients, GSTT1 null and GSTP1 Ile/Val+Val/Val/GSTT1 null genotype were less frequent when compared to controls, suggesting protective role of these genotypes in lung cancer development, 3) Among lung cancer individuals, glutathione peroxidase (GPx), GST and superoxide dismutase (SOD) activity in red blood cells, ceruloplasmin (Cp) activity and selenium (Se), zinc (Zn), copper (Cu) level in plasma differed significant as compared to controls, 4) Microelements level and antioxidant enzymes activities might modify the estimated lung cancer risk, 5) Relationship between GPx, GST, Cp activity, Zn level and GST genotype was mainly observed among controls, 6) The changes in antioxidative enzymes activity, microelements level and frequency of GST genotypes among lung cancer patients and the relationships between investigated parameters, did not show synonymously their role in lung cancer aetiology. However, they might be useful for complex study of antioxidative defence among cancer individuals, including genetic and biochemical mechanisms.
P158
Down-regulation of iron regulatory protein 1 activities and expression in SOD1 knockout mice

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Recent evidence from studies on bacteria and yeast deficient in superoxide dismutase shows that iron metabolism in these organisms is altered. We have used mice lacking Cu,Zn-SOD as an experimental model to study the regulation of iron metabolism in SOD-deficient mammals. We have demonstrated a decrease of aconitase activity of IRP1 in SOD1−/− mice as compared to WT mice. In addition, we have also found that the expression of IRP1 in Cu,Zn-SOD deficient mice is (80-90%) decreased. This result has been obtained indirectly by measuring total IRP1 RNA-binding activity of liver cytosolic extracts in the presence of 2% 2-mercaptoethanol (total IRE-binding activity) and by western blotting using a specific anti-IRP1 antibody. This down-regulation of IRP1 expression was partially due to the decrease in IRP1 mRNA level (RT-PCR). The expression of ferritin subunits was unaffected in SOD1−/− mice. We have not found any differences between SOD1−/− and wt mice as regards neither hepatic non-heme iron content nor labile iron pool level measured in peritoneal exudate cells. In summary, our results suggest that down-regulation of IRP1 in SOD1-deficient mice has no implications on several parameters of hepatic iron homeostasis.

P159
Development of an in vitro assay for the investigation of oxidative DNA damage induced by cigarette smoke condensate

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Cigarette smoke contains a number of chemicals, some of which may be involved in oxidative damage of biomolecules, including DNA (Pourcelot et al. 1999). As a method of investigating these potential effects on DNA, the use of the modified in vitro Comet assay with the lesion specific enzymes Formamidopyrimidine glycosylase (Fpg) and Endonuclease III (ENDO III) were investigated. As a positive control for the validation of this assay KBrO₃ was found to induce a consistent dose-response for oxidative DNA damage in both purine and pyrimidines in H292 cells. These results show that KBrO₃ is an ideal positive control for the induction of oxidative DNA damage without being cytotoxic at the doses used. This facilitated the use of this assay for the analysis of the effects of tobacco smoke condensate on H292 cells. These analyses showed a dose response for FPG sensitive damage but not for ENDO III sensitive sites. This study indicates that the assay may be useful in identifying components within the tobacco smoke that can cause oxidative stress. These results may give an indication of the genotoxic potential of tobacco smoke condensate, which constitutes the particulate phase of tobacco smoke. As the particulate phase of tobacco smoke constitutes 5-10%, future work will investigate the effects of whole cigarette smoke.

P160
ALDH3A1 prevents apoptosis of corneal cells induced by DNA damaging agents and oxidative stress

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We have previously shown that aldehyde dehydrogenase (ALDH) 3A1 protects human corneal epithelial cells against ultraviolet- and 4-hydroxynonenal-induced oxidative damage. The aim of this study was to determine whether ALDH 3A1 can protect against cellular oxidative damage induced by DNA damaging agents (mitomycin C and VP-16) and/or oxidative stress (H2O2). The cytotoxic effects of mitomycin C, VP-16, and H2O2 were evaluated by MTT assays, DNA fragmentation, cell morphology, and Western blot analysis in both vector- and ALDH3A1-transfected human corneal epithelial and rabbit corneal keratocyte cell lines. ALDH3A1-trasfected cells were found to be more resistant (3-fold) to the cytotoxic effects of all three agents compared to the vector-transfected cells tested, as determined by MTT assays. DNA fragmentation assays and cell morphology revealed that treatment with either mitomycin C, VP-16, or H2O2 induced apoptosis in vector-transfected cells, but not in ALDH3A1-expressing cells. Corneal cells expressing ALDH3A1 had an extended cell cycle and were prevented against formation of 4-hydroxynonenal-protein adducts and GSH depletion that was induced in vector-transfected cells after treatment with mitomycin C, VP-16, or H2O2. In conclusion, these data support our long standing hypothesis about the protective role of ALDH3A1 against oxidative damage.

P161
The role of oxidative stress and reduced glutathione in microcystin-LR induced genotoxicity

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Microcystins are liver specific toxins produced by different species of freshwater cyanobacteria. They are potent inhibitors of protein phosphatases 1 and 2A, which is directly related to their cytotoxicity and tumor promoting activity. They have been shown to induce DNA damage in vitro and in vivo, however, the mechanisms of their genotoxic activity remain unclear.

In our study we investigated the role of oxidative stress and intracellular reduced glutathione (GSH), which is the principal nonprotein thiol involved in the antioxidant cellular defence in microcystin-LR (MCLR) induced genotoxic effects. We showed that MCLR at non-cytotoxic concentrations induced a time and dose dependent increase of intracellular reactive oxygen species (ROS) formation in HepG2 cells. The role of intracellular reduced glutathione (GSH) in MCLR induced DNA damage was studied using two approaches: prior to the exposure to MCLR the cells were pre-treated either with N-acetylcysteine (NAC), a precursor of GSH synthesis or with D,L-buthionine-[S,R]-sulfoximine (BSO), a specific inhibitor of GSH synthesis. Pre-treatment with NAC nearly completely protected the cells against DNA damage induced by MCLR while pre-treatment with BSO markedly increased formation of MCLR induced DNA strand breaks. We also observed that exposure of HepG2 cells to MCLR induced changes in the levels of intracellular GSH and non-protein sulfhydrils. In MCLR exposed cells the activity of glutathione reductase, which catalyses the reduction of glutathione disulfide to reduced glutathione (GSH), showed a modest increase, while expression of γ-glutamyl-cysteine-synthetase, the key enzyme in GSH synthesis, showed a significant, time dependent increase, indicating de novo synthesis of GSH. We conclude that the increased GSH concentration measured during exposure to MCLR resulted from an increase in de novo GSH synthesis, an that formation of MCLR-GSH complexes is the predominant mechanism of MCLR detoxification.
11. Ecogenotoxicology
P162
Genotoxicity assessment of freshwater and seawater samples using the yeast RAD54-GFP GreenScreen assay

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A collection of UK estuarine and freshwater effluent samples were provided by the Environment Agency (England and Wales). In this study, genotoxicity and toxicity were assessed using the RAD54-GFP assay (GreenScreen EM). In parallel studies, other toxicity and genotoxicity endpoints were recorded. Results and reproducibility of data will be presented. The data demonstrate high reproducibility and that Saccharomyces cerevisiae grows well in seawater and can be used to produce meaningful data from such samples.

P163
Biological monitoring of cyanobacterial blooms in Polish reservoir of drinking and recreational water and the mutagenic activity of their extracts

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Cyanobacterial blooms are one of the common consequences of the increasing eutrophication of surface waters. The production of cyanobacterial toxins in drinking and recreational waters represents a growing danger to human and animal health. In this study the cyanobacterial blooms in the Sulejów Reservoir (central Poland) were monitored during the last summer. The biomonitoring was included the assessment: the cyanobacterial species in the blooms; the concentrations of microcystins in the cells and water using HPLC, ELISA and PPIA methods; the mutagenicity of cyanobacterial extracts for prokaryotic cells using SOS Chromotest and UMA test. The high cyanobacterial biomass, mainly Microcystis aeruginosa was observed at the end of summer. The concentrations of microcystins were 0.076 to 0.218 µg/l in the water and 2.80 to 5.83 µg/l in the cyanobacterial extracts. Cyanobacterial extracts (CEs) were toxic (growth factor decreased from 1 to 0.4) but not mutagenic in the UMA test. In the SOS Chromotest, CEs were toxic and mutagenic. The induction factor was up to 2.2 and SOS-inducing potency to 2.7. The toxicity of CEs was related to the microcystins content.

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P164
Mussels (Mytilus galloprovincialis) transplanted to the estuary of an impacted river. A biomarker approach

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Although its modest extension, the basin of Cecina has been indicated as pilot basin within the Water Frame Directive (2000/60 EC). It flows across Tuscany and is impacted by several human activities including mining and tanning industry, geothermal power plants and agriculture. Chemical analyses showed that sediments and biota are contaminated by heavy metals and pesticides. Cecina river enters the Central Tyrrhenian Sea (South Tuscany), an area with an high touristic importance.

Cellular responses were investigated in order to evaluate possible early warning signals. Mussels from a commercial farm were caged for one month in a reference site and into the estuary of the river. Lysosomal membrane instability was detected in haemocytes and lipofuscin accumulation was observed in cryopreserved digestive glands. Lysosomal enlargements were also quantified by electron microscopy. Genotoxic effects were evaluated by Comet assay and Micronucleus assay in gill cells. Organisms deployed at the impacted site showed a lower (p<0.01) lysosomal membrane stability and a larger (p<0.05) diameter in comparison with controls. They also displayed lower (p<0.01) levels of DNA
integrity, accompanied by higher (p<0.05) frequencies of micronucleated cells, indicating the presence of environmental stress in the area under investigation.

Part of this work was supported by Fondazione Cassa di Risparmio di Volterra.

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**P165**

**Cyto- and genotoxic effects in human lymphocytes induced by cyanobacterial extracts from the Polish water reservoir**

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Cyanobacterial blooms in drinking and recreational water reservoirs are the worldwide problem and in Poland. Microcystins are hepatotoxic cyclic heptapeptides produced mainly by Microcystis aeruginosa. In this study, cyto- and genotoxicity of microcystic cyanobacteria extracts (MCEs) from Sulejow reservoir were assessed for human lymphocytes using XTT test and the comet assay, respectively. Cytotoxicity of MCEs at the concentrations from 10 to 80 µM after 24 hrs exposure of human lymphocytes was tested using XTT method. Toxicity of cyanobacterial extracts differed from each other for lymphocytes and not related to the concentration of microcystin in the extracts. In the comet assay, DNA damage as a single strand breaks and an oxidative damage of DNA (after treatment of FPG enzyme) were assessed. Lymphocytes were exposed to cyanobacteria extracts for 3, 6 and 12 hours. The concentrations of microcystin in the cell cultures were from 1 to 10 µM. All MCEs induced DNA damage in lymphocytes. After 3 hrs exposure of cells to MCEs the highest level of DNA damage was observed. Supported by Research Grant PB 0546/P05/2003/25

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**P166**

**In vitro evaluation of potential genotoxic effects of Bisphenol A in fish cells.**

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Bisphenol A (BPA) is a bicyclic aromatic compound occurring mainly as an intermediate in the production of polycarbonate products and epoxy resins. Its estrogenicity is well documented using both in vivo and in vitro systems. Due to its possible presence in sewage and waste water effluents, there is interest in investigating the potential genotoxicity. Therefore, in this study, the genotoxicity of BPA (0.000 - 18.000 µg ml⁻¹) was assessed in metabolically competent cultured rainbow trout gonad cells (RTG-2) using the comet and cytokinesis-block micronucleus (CBMN) assays. Prior to the comet assay, cytotoxicity of BPA was assessed using a fluorescence dual stain technique (i.e. Calcein AM/Eth D III). Validation of the comet assay was carried out using a range of concentrations of ethyl methanesulphonate (EMS). EMS and colchicine (COL) were used to validate the CBMN assay using Giemsa and anti-kinetochore antibody staining. Cell viability was not significantly affected following exposure to BPA over the concentration range used. A significant increase in DNA strand breaks (tail DNA-%) was obtained using 1.000 and 18.000 µg ml⁻¹ of BPA (comet assay). In the CBMN assay, all concentrations tested showed increased presence of kinetochore–positive micronuclei, demonstrating the aneugenic activity of BPA. This study showed genotoxic effects of BPA on fish cells, indicating a potential hazard to aquatic organisms.
P167
Do fish cells exhibit differential sensitivity for genotoxic but not for cytotoxic effects of chemicals?

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It is becoming increasingly clear that origin and properties of the target cell influence toxic responses to environmental contaminants. In this context, use of fish cells has increased in recent years in particular for identification of environmental hazards and in reducing the use of animals, adopting the ‘tiered approach’. Several studies, employing different endpoints have indicated that with the exception of some cell specific cytotoxicities and taking into account some confounding factors; there is no general difference in basal cytotoxic sensitivity of mammalian and fish cells under in vitro conditions. However, a paucity of information pertaining to relative genotoxic response exists in the literature. Using a range of concentrations of reference chemicals with different modes of action (i.e. ethyl methane sulphonate, benzo(a)pyrene), we have therefore tested the following hypothesis: compared to mammalian cells (CHO-K1), fish cells (EPCA1 and RTG-2: latter being metabolically competent) exhibit differential sensitivity at the DNA and cytogenetic levels (comet and cytokinesis-block micronucleus assays, using Giemsa and anti-kinetochore staining) but not for cytotoxic effects (trypan blue and fluorescent Calcein AM/EthD III vital staining). The general assumption that mammalian and fish cells exhibit similar sensitivity for cytotoxicity assays was supported by the present study but the measured genotoxic response differed. Care should therefore be taken while comparing and evaluating genotoxic potential of chemicals under in vitro conditions on cells of phylogenetically different origin.

P168
Detection of PAH adducts in the European eel by competitive enzyme linked immunosorbent assay (ELISA), with chemiluminescent detection.

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A common mechanism of genotoxicity by marine pollutants is through covalent interaction with DNA forming stably modified nucleotides. Adducts might trigger cancer development and their monitoring in aquatic organisms is of interest for the possible impact of environmental genotoxins on human health. The use of antisera against selected bulky adducts, including those of PAHs, has been extensively described for immunohistochemical analysis in humans and recently also on eels. The cross reactivity of the polyclonal antiserum developed for BPDE-I-modified DNA has been highlighted to emphasize the potential of immunoadsorbs to detect different, but structurally related, PAH-adducts. In this work, we investigated genotoxic outcomes from the enzymatic metabolism of 7,12-dimethyl-benz[a]anthracene (DMBA), a potent carcinogenic PAH, in the European eels (Anguilla anguilla). ELISA assay and immunofluorescent stainings for BPDE-I-DNA evidenced the occurrence of diol-epoxide DNA adducts, together with increased biotransformation rates of CYP1A rates shown by EROD activity. The possibility to assess PAH adducts in a few micrograms of extracted DNA indicated the utility of the antibody approach for the study of DNA damage induced by different environmental PAHs.

P169
Use of the land snail Helix aspersa for biomonitoring the impact of atmospheric pollution and electromagnetic fields in urban areas

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The land snail Helix aspersa was used to assess the environmental impact of atmospheric pollution in the urban area of Ancona (Italy), with an integrated ecotoxicological approach. Snails were translocated in several zones differently impacted by vehicular traffic. Analyses of chemical contaminants were integrated with those of biotransformation enzymes, metallothioneins and peroxisomal proliferation. The efficiency of antioxidant defenses and the total oxyradical scavenging capacity (TOSC) were evaluated as biomarkers.
of oxidative stress conditions. Cytotoxic effects on the integrity of lysosomal membranes were assessed as neutral red retention time. The genotoxic impact was evaluated as loss of DNA structural integrity (comet assay and micronuclei) in haemocytes, and as occurrence of 8-oxo-dG by immunoperoxidase staining in the digestive gland. The overall results indicated a significant accumulation of metals and PAHs with a contemporary appearance of biological alterations in organisms from more trafficated sites. Prooxidant effects and oxidative damages to lysosomes and DNA were also measured in snails exposed to electromagnetic fields, supporting the potential of H. aspersa as key species for terrestrial biomonitoring.

P170
Influence of oxidative and enzymatic-mediated effects in detection of DNA strand breaks in the Mediterranean mussel, Mytilus galloprovincialis.

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The single cell gel electrophoresis (SCGE) in marine invertebrates often revealed a higher background of DNA strand breaks (SB) as compared to vertebrates, explained as different packaging features of chromatin. SCGE experiments were performed on blood cells of the Mediterranean mussel (Mytilus galloprovincialis) using different conditions of EDTA, proteinase K (PK), Trolox and aurintricarboxylic acid (ATA) to investigate if protocol constraints may influence the SB detection. SCGE results were compared with those from the land snail Helix aspersa and the seabass Dicentrarchus labrax. The effects of various conditions were also evaluated on genomic DNA extracted from the digestive gland. Mussels showed native high SB, which were reduced combining increased EDTA concentrations with the use of PK, and supplementing comet solutions with Trolox (soluble antioxidant) and ATA (inhibitor of Ca\(^{2+}/\text{Mg}^{2+}\)-dependent nucleases). Conversely snails and seabass showed lower basal DNA SB and comet results were not significantly affected by protocol modifications. A decreased DNA fragmentation was also observed using EDTA, Trolox and ATA for isolating the whole genomic DNA. These results suggest that chemical features of internal fluids, through oxidative and enzymatic-mediated effects may influence the protocol for detecting basal SB in mussels. This occurrence should be adequately considered when studying genotoxicity of aquatic pollutants.

P171
Mutagenic potential of (presumed) control soil samples

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The purpose was to assess the mutagenic potency of presumably control soil samples (i.e. not contaminated by a known industrial activity). The studied soil were 11 samples collected in cities (> 100,000 inhabitants), 16 in villages, 7 in agriculture fields, and 17 in forests or natural areas. As described in Courty et al. (2004), soil samples (collected 0-5 cm deep) were dried, sieved (2 mm), homogenised before dichloromethane/acetone extraction, solvent exchange to DMSO and sterilizing filtration. The PAH content of each sample was also measured. The micromethod adaptation of the Ames test on Salmonella typhimurium was performed with and without metabolic activation system (rat liver homogenate). The results showed a wide range of effect levels: a clear mutagenic activity was induced by the extracts of all 11 city soils studied, of 9 of the village soils, and of only one of the agriculture soils, while none of the natural or forest soil samples showed mutagenic extracts. This work demonstrated that soil mutagenic potency was strongly correlated to human activity, but not to PAH content.
P172
Niacin modifies UV-induced genetic damage in fish skin cells

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Fish living in clear, shallow waters may be susceptible to the acute and chronic effects of ultraviolet (UV) radiation, including genomic instability and photocarcinogenesis. Mammalian research has demonstrated that the dietary status of niacin (vitamin B3) has the potential to influence genomic stability, DNA repair, and the immune system. However, very little information is available for fish, despite the rapid expansion of aquaculture and increased concern for fish health. Therefore, this study investigated the efficacy of niacin (NAM) supplementation in reducing UV-B induced genotoxicity in a fish skin cell line (EPC-A1) using the Comet assay. The Comet assay was validated using a reference genotoxin (EMS) and a range of UV-B doses (0 – 600 Jm⁻²). Cytotoxicity was initially assessed using two different cell viability assays (trypan blue and Calcein AM/EthD II) with the dual fluorescence assay considered more reliable. Following validation, the potential modifying effects of niacin supplementation (0.18-1.0 mM, 24 h) on UV-B induced DNA damage was evaluated. Validation studies showed clear dose-response relationships for EMS and UV-B induced DNA damage (% tail DNA), confirming the sensitivity of the assay for detecting genotoxicity in fish cells. Furthermore, initial results simply that niacin supplementation at the 1.0 mM level significantly reduced UV-B induced DNA damage. This suggests that niacin may be an important dietary factor in protecting fish from environment-induced genomic instability.

P173
Genotoxicity evaluation of algal extracts in Oncorhynchus mykiss

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Harmful Algal Bloom and marine algal toxin incidents have been recently spreading, causing damages to the marine environment and threatening human life through contaminated seafood. The number of phycotoxins which are being characterized is increasing, stimulating the development of tools in the monitoring of contamination.

DNA is an important target of environmental toxicants and the loss of DNA integrity may determine the induction of mutations, birth defects and cancer in vertebrates. Consequently the DNA integrity was proposed as an effective biomarker in biomonitoring.

During summer 2003 an algal bloom of Polysiphonia fucoides affected birds and fish on the Swedish East coast.

Extracts of the red algae have been used for in vivo experiments on rainbow trouts (Oncorhynchus mykiss). DNA damage was assessed by Micronucleus test and Comet assay. Diffusion Assay allowed the detection of apoptotic cells. In addition, EROD activity and oxidative stress parameters were measured. Benzo(a)pyrene (20mg/kg) was used as positive control. Results showed an increase (p<0.01) of single strand breaks in trouts erythrocytes treated with algal extracts, accompanied by a slight increase of micronuclei, underlying a weak genotoxic effect under the experimental conditions used.

P174
A strategy of ecological gene assessment

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Ecological genes are considered as ones controlling the interaction between a cell or an organism and external environment and providing for their stability. At present the strategies to reveal cDNA and such gene functions are realized for studying the mechanisms of aluminium plant tolerance. The overall aim of this investigation is to examine the various barley cultivates on their sensitivity to aluminium (0.5 mg/l) and iron (0.3 mg/l) as well as to their combined effect. Fourteen cultivates of barley were selected for the experiment. The effect of Fe and Al chlorides on barley seeds was estimated from morphometric (seed
germination energy) and cytogenetic (mitotic index and frequency of chromosome aberrations) parameters. It is found that various barley cultivars are aluminium tolerant differently. The increased concentration of Fe in a solution for germinating various cultivars of barley has practically no effect on variations in the parameters of germination energy and mitotic index of seedlings as compared to the control values; at the same time the presence of Fe with Al reduces the toxic aluminium effects that is especially pronounced in Al-sensitive cultivates. Tolerant cultivates are assumed to induce a transduction signal, as a result of which activated are the genes similar to transferrin or lactoferrin genes of animals. These gene products are capable to inactivate aluminium toxicity. A strategy to identify a family of ecological genes is discussed.
12. Methods development
P175
Validation of PAXgene system for RNA isolation from children and adults whole blood


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Objectives: To validate the PAXgene system for collection of blood from children and subsequent RNA isolation, with special emphasis on field conditions. Materials and methods: Whole blood has been collected into PAXgene and blood samples (2.5 ml) were stored at RT for 7.5 to 8.0 h. In next step, samples were frozen and kept at -80°C until processed for RNA isolation with PAXgene Blood RNA Isolation Kit. The DNase treatment was omitted as test RNA isolations were free from DNA contamination. Results: The RNA was successfully isolated from children and adults, with RNA quantity of 3.0 µg up to 21 µg per blood sample (for both groups). The quality of RNA was in general good. However, there was, however, substantial genomic DNA contamination (about 20 % of RNA samples). Conclusion: In order to assure the best quality of RNA, we highly recommend DNase treatment in a course of RNA isolation.

P176
Quantitative determination of O6-methyl-2’-deoxyguanosine, 8-oxo-7,8-dihydro-2’-deoxyguanosine and 1,N6-etheno-2’-deoxyadenosine in DNA by LC-MS/MS

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Health risks associated with exposure to environmental DNA-damaging agents could be viewed as increments over background DNA damage. A quantitative assessment depends on the development of appropriate analytical techniques. A liquid chromatography-triple quadrupole mass spectrometry method has been developed and validated for dynamic range, accuracy, and precision for the analysis of the pre-mutagenic endogenous DNA adducts O6-methyl-2’-deoxyguanosine (O6-mdGuo), 8-oxo-2’-deoxyguanosine (8-oxodGuo), and 1,N6-etheno-2’-deoxyadenosine (dAdo). O6-mdGuo standard was synthesized, including deuterated O6-[2H3]mdGuo to be used as internal standard. Standards of 8-oxodGuo and dAdo were commercially available. Using an on-line sample preparation with column switching we were able to determine the three adducts simultaneously and with minimum workup with high specificity and selectivity. Limits of detection (LOD; signal to noise ratio >3) determined in a matrix of the 4 normal 2’-deoxynucleosides were 12, 49, and 24 fmol on column for O6-mdGuo, 8-oxo-dGuo, and dAdo, respectively. Expressed as adduct concentrations, and based on a sample of 31 µg DNA hydrolysate (100 nmol nucleotides), this is equivalent to detection limits of 120, 490, and 240 adducts per 10⁹ nucleotides. These LODs suffice to investigate background adduct levels in biological samples, as verified by preliminary analysis of crude enzymatic hydrolysates of DNA from untreated L5178Y mouse lymphoma cells.

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P177
Improving the comet assay

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The comet assay is a simple, sensitive method for measuring DNA damage. But it has limitations: 1) Sample throughput: it is not suitable for use in large-scale epidemiological studies, because few gels can be processed at one time. 2) Scoring: even with computerised image analysis, this is very time-
Methods development

consuming. 3) Only certain lesions are detected – strand breaks, plus sites which can be attacked by lesion-specific endonucleases. 4) Calibration and standardisation are problematic.

We are working on improvements to the assay, including the following: Gels set on plastic film with a multi-well format; Analysing very small numbers of cells in a restricted area of the gel; Scoring based on measurement of differential staining of head and tail DNA, so that a simple ‘snapshot’ of comets will give a measure of damage; Incorporating the enzyme uvrABC in the assay to permit detection of bulky adducts; Inclusion of cells with a known level of damage as an internal standard.

A progress report will be presented. We are looking into the best ways of making these technical improvements available to the comet community. We also hope to improve quality control and develop a system of laboratory accreditation. This will need to be done on a commercial basis, but aiming at covering costs rather than making a profit. We will be collecting views on what ‘customers’ really want.

P178
Detection and separation of nucleoside-5’-monophosphates of DNA by conjugation with the fluorescent dye BODIPY and capillary electrophoresis with laser-induced fluorescence detection

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We investigated the separation and detection of the 5’-monophosphates of 2’-deoxynucleosides selectively conjugated with BODIPY FL EDA (4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diazas-indacene-3-propionyl ethylene diamine hydrochloride) at the 5’-phosphate group using capillary electrophoresis with laser-induced fluorescence detection (CE-LIF). BODIPY conjugates of the four common deoxynucleoside-5’-monophosphates (2’-deoxyguanosine-5’-monophosphate, 2’-deoxyadenosine-5’-monophosphate, 2’-deoxycytidine-5’-monophosphate and thymidine-5’-monophosphate) were prepared and subjected to CE-LIF to serve as standard compounds for peak assignment and to develop separation conditions for the analysis of DNA. BODIPY conjugates were detected and resolved by CE-LIF after digestion of DNA or an oligonucleotide to 5’-monophosphates by nuclease P1 and fluorescence labelling without further purification step. Comparative analyses of calf thymus DNA digested either with micrococcal nuclease/spleen phosphodiesterase to 3’-monophosphates or with nuclease P1 to 5’-monophosphates showed that both versions of the fluorescence postlabelling assay were equally efficient and sensitive. Moreover, using the same assay 2’-deoxyuridine and 2’-deoxy-5methylcytidine were identified in bisulfite treated DNA after nuclease P1 digestion indicating that fluorescence postlabelling of 2’-deoxyribonucleoside-5’-monophosphates with BODIPY FL EDA and detection by CE-LIF has the potential to determine DNA damage and genomic DNA methylation.

P179
Development of the cytokinesis-block micronucleus assay on Caco-2 cells.

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Carrying genotoxicity experiments on intestinal cells is attractive for the study of food contaminants. However, only few in vitro assays have been developed on enterocytic cells. Previous experiments were conducted on human carcinoma colonic Caco-2 cells by the micronucleus assay without cytochalasin (Jacquin & Fessard, 2003). However, problems were encountered for the determination of the cytotoxicity. In order to improve the method, we added to this assay a cytokinesis–block step and a fluorescent detection of the cells. The protocol which was finally chosen is described here after. After one day of subculture, cells were exposed with various concentrations of MMS (5 to 20 µg/ml) during 24 hours followed by a 30 hours incubation with 4.5 µg/ml cytochalasin B and 1.5 hours of recovery. After trypsinization, a quick osmotic shock and a fixation step, the cells were spread on cold slides. Staining of cyttoplasm and nuclei was performed with phalloidin-FITC and with DAPI respectively. More than 70% of binucleated cells were obtained in the control cultures. A significant increase of micronuclei in binucleated cells was obtained with 10 µg/ml MMS.
Methods development

P180
An investigation to determine the feasibility of mixing S. typhimurium strains for Ames screening

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The ideal Ames screen would be one that required less test article than the standard assay but had equivalent specificity and sensitivity. We are investigating a potential way whereby this could be achieved using mixtures of some of the standard S. typhimurium strains.

Preliminary work was performed using strain mixtures TA1537+TA100, TA1535+TA98, TA98+TA100, TA1535+TA1537, TA98+TA1537 and TA100+TA1535 with volume ratios ranging from 100:0 to 0:100. Standard plate-incorporation methodology was used for treatments with water, dimethylsulphoxide and the direct acting mutagens 2-Nitrofluorene, Sodium Azide and 9-Aminoacridine at concentrations known to elicit a significant increase in revertants (5, 2 and 50 µg/plate respectively). Good correlations between actual revertant counts and theoretical counts were obtained for treatments with mixtures TA1537+TA100, TA1535+TA1537 and TA100+TA1535, with no compromise on specificity. For all treatments with strain TA98 poor correlations were observed, and thickening of the background lawn was seen following treatments with mixtures containing this strain.

Using mixtures of TA1537+TA100, TA1535+TA1537 and TA100+TA1535 at volume ratios of 50:50 the sensitivity of the assay system was investigated by examining dose-responses with 2-Nitrofluorene, Sodium Azide and 9-Aminoacridine.

Ongoing work is being performed with other direct acting mutagens, and toxicity measures are being investigated. Mixing certain Ames strains appears a promising approach to allow a qualitative assessment of potential mutagenicity, whilst reducing test article usage.

P181
Photo Comet assay and photo micronucleus test in vitro: Contributions to the validation in a collaborative study

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A collaborative study with seven participating laboratories was conducted to evaluate the performance of protocols for the photo micronucleus test (PMNT) and the photo Comet assay (PCA) with Chinese hamster V79 cells. Thirteen coded test chemicals were selected based on their ability to absorb UV light of which eight were classified as photo-genotoxic and five as non-photogenotoxic (three phototoxic, two non-phototoxic) according to published data.

Results obtained showed a good reproducibility, both within and between laboratories. Sensitivity in detecting the photo-genotoxic compounds was higher in the PMNT than in the PCA. Specificity of both models appears to be low as the three phototoxic compounds assumed to be non-photogenotoxic (based on literature data) showed positive findings. However, these results could also suggest that the available published data were inadequate for a correct pre-study classification.

In summary, the data establish a good basis for future evaluations of both assays and for their eventual validation as models for the prediction of photogenotoxicity and potential photocarcinogenicity. (This work was supported by the German Federal Ministry of Education and Research, BMBF-project No. 0312916A/B/C/D)
P182
General synthesis of etheno derivatives

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DNA damages, mediated by bifunctional chemical agents are known to form covalent cyclic adducts with DNA bases. While cyclic adducts as such may be classified as mutagenic DNA lesion, those bearing additional functional groups that are able to interfere with the base-pairing are of particular interest. Thus, evolution has developed complex enzymatic pathways that recognize and repair DNA damage. The rate of cleavage was found to differ with the lesion and was also affected by neighbour sequences geometry and the resulting local conformational changes, which can be sequence-dependent. Etheno adenine nucleotides are commonly applied as fluorescent probe for various biochemical studies such as: structure and function of nucleic acids, protein visualization, enzymatic studies, investigation of nucleotide binding-site, conformational analysis of nucleotides, and pharmacology of nucleosides. Extended conjugation of the N1,N6-etheno-bridge by an auxochrome is desirable to increase the fluorescent properties. Therefore, extension with conjugated double bonds or aromatic rings will be a good approach. Therefore, we have developed a method based on the halo substitution of basic etheno adduct.


P183
Development of an LC-MS/MS method for quantifying N7-(2-hydroxyethyl)-guanine adducts.

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Ethylene oxide (EO) is widely used in the chemical industry and is also generated endogenously. Although classed as a human carcinogen, epidemiological evidence relating to its ability to induce human cancers is inconsistent. EO causes DNA damage, primarily reacting with the N7 position of guanine, forming N7-(2-hydroxyethyl)-2-deoxyguanosine (7HEG) adducts. The ultimate aim of the project is to directly identify sources of endogenous 2-hydroxyethyl DNA adducts and accurately establish background levels in animal models, thereby allowing the measurement of increased damage specifically due to exogenous EO exposure. To quantify 7HEG isolated by neutral thermal hydrolysis, an LC-MS/MS assay has been developed and optimised for this particular adduct. When operated in the MRM mode, monitoring the transitions m/z 196>152 and 201>157 (equivalent to loss of the hydroxyethyl group) and using synthesised 15N5-labeled internal standard, adducts can be detected as low as 0.5fmol. Analysis of rat liver DNA revealed background levels of 1.5±0.9 adducts/10^6 bases in control animals (n=3), whilst treatment with single i.p. doses of EO (0.05-1.0mg/kg) induced adducts in a dose related manner, reaching a maximum of 48±0.9/10^6 bases. In future studies, this assay will be used in conjunction with AMS to measure background adducts and those formed as a result of treatment with isotope labeled precursors of ethylene and EO itself. Funded by the American Chemistry Council (MTH0311-02).
P184
Evaluation of the Litron XCell MN Kit for the flow cytometric enumeration of micronuclei in L5178Y mouse lymphoma cells treated with MMS.


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Litron Laboratories’ prototype XCell MN Kit uses a differential staining technique to identify micronuclei via flow cytometry, allowing discrimination between DNA fragments derived from apoptotic / necrotic cells and true micronuclei. We have compared the XCell MN Kit with conventional microscopy using L5178Y cells treated with methylmethane sulphonate (MMS).

L5178Y cells were treated with MMS (5 to 70 µg/mL) for 4 hours and harvested 20hr later, in three separate studies. Toxicity was assessed by relative survival and cultures at 40% of control and above were analysed. Culture samples were processed through the XCell MN Kit staining procedure for flow cytometric analysis or stained with acridine orange for manual micronucleus analysis.

Pooled data showed dose-related increases in % micronuclei (%MN) for both methods. Although %MN values were lower for flow analysis, comparable fold increases in %MN relative to the vehicle controls were observed up to 50µg/mL MMS (up to 44.9 and 33.1-fold for flow and manual analysis respectively).

Significant (P < 0.05) increases in %MN were observed at and above 10µg/mL and 20µg/mL MMS for flow and manual analysis respectively. This is consistent with previous data presented by Litron Laboratories and suggests their method is readily transferred between laboratories.

P185
Use of archived DNA for genotyping

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The isolation of DNA from archived samples poses several problems: The DNA was kept soluble in phosphate buffer, and separation of the DNA from phosphate buffer can be difficult. Furthermore each time the DNA is purified, DNA is lost in the purifying process. These factors can lead to low concentration of isolated DNA and DNA, which sometimes is of poor quality. The isolated DNA was used for genotyping and analysing for members of the glutathione-S-transferase (GST) family of enzymes, GSTM1, GSTP1 and GSTT1, N-Acetyltransferase (NAT2*14A, NAT2*5A, NAT2*6A and NAT2*7A/B), the excision repair cross-complementing 1 (ERCC1) gene and the xeroderma pigmentosum complementation group D (XPD) gene.

A total of 198 samples of DNA were used in the study and so far genotyping has been performed on GSTM1, GSTP1, GSTT1, ERCC1 and N-Acetyltransferase.

One of the problems regarding the use of archived DNA for genotyping is the interpretation of no signals as a real deletion of the investigated gene, or the result of the DNA being of poor quality and/or of too low a concentration?

P186

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Ethylene oxide (EO) is a widely used chemical intermediate and is also formed endogenously from the metabolism of ethylene. In contrast to ethylene, EO reacts with cellular macromolecules forming protein and DNA adducts. A method has been developed for the simultaneous detection and quantitation of five different 2-hydroxyethyl (HE) DNA adducts, using LC-ESI-MS/MS with MRM. This sensitive and highly specific assay can detect HE-DNA adducts down to levels of 10 femtomoles of adduct standard. HE-purine adducts are excised by neutral thermal hydrolysis at 100°C for 30 min and the remaining DNA is then
enzymatically digested to nucleosides, and the products separated by HPLC. Fractions are collected, resulting in a mixture of nucleoside HE-adducts, which are then combined with the base adducts and quantitated by LC-MS. Samples of a control (untreated) pSP189 plasmid have been shown to contain both N7-HEG and N1-HEdA, at levels of 220 and 6 adducts per $10^8$ nucleotides, respectively. This assay is currently being used to quantitate levels of HE-DNA adducts formed in DNA treated with a range of doses of EO and could also be applied to the measurement of 2-hydroxyethyl adducts in control and exposed animals or human populations. This work was funded by CEFIC-LOS G, CEFIC EO&DerSG, and the ACC Olefins Panel.

P187
Optimisation of Comet assay methodology for isolating cell nuclei from the liver.

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The Single Cell Gel Electrophoresis Assay (Comet assay) is used in Genetic Toxicology as a second in-vivo test and investigative tool. The liver is often a key target organ for many test compounds and therefore hepatocytes are commonly assessed for DNA damage using the Comet assay. In order to compare whole cell versus cell nuclei methodologies a method for isolating cell nuclei from the liver has been validated in our laboratory. This has been achieved by performing Comet analysis and histological staining and adapting current methodology to ensure that cell nuclei, not whole cells, are being tested in the Comet assay.

The liver was homogenised with several combinations of loose and tight pestles. Centrifugation speed and time, and analysis of cell pellets versus supernatant following centrifugation were also assessed. All parameters were optimised using the Comet assay and histological staining.

The optimum method for isolating cell nuclei combining results from Comet analysis and histological staining of the cell nuclei suspensions, was determined to be: homogenisation with 3 strokes of a loose pestle, followed by centrifugation of the supernatant at 40g for 5 minutes, and resuspension of the cell pellet for Comet analysis.

Centrifugation of the homogenised liver was determined to be essential in obtaining isolated cell nuclei, and centrifugation speed and time were shown to influence Comet assay results.
13. Genotoxicity testing
P188
The novel thiadiazolidindione (TDZD) family of GSK-3β inhibitors for the treatment of Alzheimer’s disease: no genotoxicity and selection of NP031112.

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Alzheimer’s disease (AD) is a progressive, degenerative dementia characterized by decreased cognitive functions with associated decline in cholinergic transmission, brain senile plaques and neurofibrillary tangles.

NEUROPHARMA, involved in the research of new drug in the AD treatment, has developed a family of compounds called thiadiazolidindiones (TDZD) which have shown to be selective ATP-non competitive GSK-3β inhibitors. To investigate the mutagenic potential and its putative safety, three TDZD compounds were selected: NP00111, NP01139, and NP031112.

A screening approach based in a reduced Ames assay and in an in vitro micronucleus test was performed. The three TDZD showed negative results in these assays. Since other drugability properties were superior in NP031112, this TDZD was selected for regulatory genetic toxicology assays. Our candidate, NP031112, showed a dramatic improvement on behaviour in the Object Recognition Test in transgenic mice over-expressing human APP. The three ICH regulatory genetic toxicology studies (Ames assay, mouse lymphoma and in vivo micronucleus) were performed, demonstrating a lack of genotoxic potential.

All these results taken together showed that the TDZD chemical members studied had a safe genotoxicity profile, and that NP031112 possesses good properties for further non-clinical and clinical development for the treatment of AD.

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P189
Genotoxic effects of Ochratoxin A on the human kidney HK-2 cell line by the alkaline comet assay.

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The aim of the present work was to investigate the DNA strand breaks and oxidative damage of Ochratoxin A (OTA) using the alkaline comet assay in a human derived kidney cell line (HK-2). OTA is a potent renal carcinogen in male rats although its mechanism of action is not known. One hypothesis for OTA-mediated carcinogenicity is based on the formation of DNA adducts and oxidative DNA damage but the results are controversial.

For this purpose HK-2 cells (human renal origin) were treated with 50, 100, 200 and 400 µM of OTA for three hours. These concentrations were selected in a previous MTT assay because they gave percentages of viability above 80%. Parallel assays were carried out with and without metabolic activation using male rat S9 mix (10%). The comet procedure was carried out without and with formamidopyrimidine glycosylase (FPG) and endonuclease III (Endo III) enzymes to detect oxidative damage. Three independent assays were performed. Comets were classified by the visual score method.

No significant increase in the total comet score was detected. Therefore, under these conditions, OTA does not induce DNA strand breaks or oxidative damage. At 600 µM, there was an increase but this concentration was cytotoxic (75%) and for this reason the DNA damage can not be attributed to the toxin.

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P190
Comparative evaluation of peripheral blood flow cytometry micronucleus test in rats and mice

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The in vivo micronucleus test (MNT) is a standard assay for genotoxicity assessment at the chromosomal level. Peripheral blood erythrocytes have been accepted as an appropriate target for MN assessment. A
comparative evaluation of the peripheral blood flow cytometry MNT in rat and mouse treated with aneugens and clastogens was performed. Five aneugens (Mebendazole, Carbendazim, Nocodazole, Vinblastine, Vincristine) and two clastogens (Mitomycin C and Phenacetin) were selected for the rat micronucleus test. In the peripheral blood no significant MN induction was found with the aneugens, however, both clastogens induced a significant increase. In the rat bone marrow Vinblasticine increased the micronucleus frequency significantly. Mice treated with Vincristine, Vinblastine, Colchicine or Carbendazim showed a significant increase in peripheral blood MN frequencies. This data demonstrates that the selective analysis of young reticulocytes by using anti-CD71 antibodies-FITC conjugate (transferrin receptor), technique in the rat peripheral blood did not allow detection of large micronuclei, most likely because of their highly efficient removal in the the spleen. Based on our data it is clear that the flow cytometry rat peripheral blood micronucleus test is an appropriate assay for the evaluation of the genotoxic potential of clastogens but not of aneugens. In contrast, the mouse peripheral blood micronucleus test is a suitable assay for testing both classes of mutagens.

P191
Evaluation of the Genotoxic Potential of 3-Mono-ChloroPropane-1,2-Diol (3-MCPD) in vitro and in vivo using the Single Cell Gel /Comet Assay

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The objective was to examine the ability of 3-MCPD, a food contaminant regarded as a potential carcinogen, to induce DNA damages in vitro in CHO-K1 cells and in vivo in rat tissues using the comet assay. In vitro, cells were exposed to 0.5, 1, 2.5 or 5 mg 3-MCPD/ml for 3h. Two metabolites of 3-MCPD were also studied, β-chlorolactic acid (0.1, 0.25, 0.5 or 1 mg/ml) and glycidol (5, 10, 20, or 30 µg/ml). In vivo, SD male rats were administered oral doses of 3-MCPD at 25 or 60 mg/kg b.w. once daily for two days. Three hours after the second dosing, tissues (blood leucocytes, bone marrow, liver, kidneys, testes and mammary glands) were sampled. The comet assay was performed according to recommended methods. Our results obtained in vitro showed that β-chlorolactic acid did not induce DNA brakes, while slight damages were observed at high concentrations of 3-MCPD. Glycidol induced DNA damage at much lower concentrations (≥ 20µg/ml). In vivo, no DNA damaging effect was observed in the different rat tissues after 3-MCPD treatment. Our results are in accordance with the non-genotoxic status of 3-MCPD in vivo. Positive responses showed in some in vitro reverse mutation assays, in the presence of metabolic activation, may be due to glycidol, a genotoxic epoxide metabolite of 3-MCPD.

P192
How to setup and validate in vivo Comet assay in a contract research organization

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The in vivo Comet Assay (at pH > 13) is gaining popularity as an alternative or supplemental test to the standard battery for genotoxicity testing. It provides a means to investigate the ability of a compound to induce DNA damage in target organs. This abstract summarizes the approach taken by our laboratory to design protocols, and evaluate assay conditions to ensure the generation of reproducible results. Using Chinese hamster ovary (CHO) cells exposed to dimethyl sulfoxide (DMSO) or methyl methanesulfonate (MMS), the optimum assay conditions were determined to be 20 min for unwinding, and 30 min for electrophoresis at 0.7 V/cm at 2-10°C. For setting up in vivo studies, mice were exposed for 24 hr to a single oral dose of solvent, MMS (20, 40, 80 mg/kg), or cyclophosphamide (CP, 50,100 mg/kg). With the solvent control (DMSO), there was no increase in DNA damage (measured by the % tail intensity, %TI) in the liver (3.27±0.41), bladder (7.12±2.39), stomach (8.13±4.81), and lung (9.23±3.22). MMS at 40 mg/kg induced significant increases in %TI in the liver (21.13±3.94), bladder (30.92±12.29), stomach (21.19±6.65), and lung (30.21±16.01). MMS (10% solution) given topically elicited 60.68±10.4% tail intensity in mouse skin. In the validation experiments, a single oral dose of MMS (40 mg/kg) reproducibly induced DNA damage (%TI) following 3-hr exposure (liver, 41.51±6.19 vs saline, 1.25±0.78; bladder, 26.25±17.39 vs saline, 1.76±0.19) and 24-hr exposure (liver, 18.01±5.36 vs saline, 1.78±1.70; bladder, 7.51±3.09 vs saline, 1.69±1.21).
P193
Mutagenicity in Ames Salmonella assay of benzyl chloride depending on exposure conditions

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The mutagenic response of benzyl chloride (BCl), an intermediate product of organic synthesis (benzyl alcohol, dyes and perfumes), which is a volatile liquid compound, was investigated in the Ames/salmonella assay. A comparative exposure between the Ames test, using standard methods (direct plate incorporation and preincubation method), and two different simple exposure systems using i) a method where the plates were enclosed in closed container ii) an exposure to BCl under its volatile form using a vaporisation-diffusion method, were studied. Additionally, different times of exposure to BCl were evaluated. The results of this work showed that the use of jars increased the sensitivity of the test. The most immediate explanation for these finding is that a high concentration of BCl is present in the jar. In addition, the testing of BCl under its volatile form using the vaporisation-diffusion method proved to be more sensitive than the Ames conventional method of exposure. The mutagenic effect of BCl was clearly obvious after 5 or 48 hours. The necessity of this minimal exposure time should be taken into consideration, for a better evaluation, when direct continuous flow exhausts are used for the exposure of bacteria.

P194
Genotoxicity of hair dyes: Specific aspects and strategic considerations

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Permanent hair dyes are formed by oxidative reactions of small colourless precursors inside the hair fiber. The precursors mainly belong to the group of aromatic amines, known for its structural alerts for mutagenicity. The use of permanent hair dyes has been linked to bladder cancer in consumers and hairdressers (Gago-Dominquez et al., Int. J. Cancer, 2001), which triggered EU regulatory activity with special emphasis on genotoxicity. However, basic in vitro genotoxicity tests like Ames test, mouse lymphoma test, chromosomal aberration or micronucleus test, exhibit major limitations such as the lack of human-like metabolic capacity, toxicokinetics, use of cell lines not relevant to the target organs and oversensitivity compared to in vivo situations. A recent analysis of over 700 chemicals tested in in vitro genotoxicity tests demonstrated that, whilst efficient at detecting rodent carcinogens, 75%-95% of rodent non-carcinogens induced false positive results in at least one of these assays (Kirkland et al, Mutat. Res, in press). Instead of following current standards of in vivo follow up testing, we aim at clarifying positive in vitro results on the basis of in vitro experiments that adequately cover skin metabolism, skin penetration and genotoxicity. A test strategy will be presented which is not only aiming at the replacement of animal experiments but also at the generation of results with higher significance for the dermal route of exposure.

P195
In vivo genotoxicity of the cyanotoxin, microcystin LR, investigated by the comet assay

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Microcystins produced by freshwater cyanobacteria are potent hepatotoxins involved in human and cattle poisonings. Microcystin-LR (MC-LR), the most toxic variant, is a liver tumor promoter. However few data are available on the genotoxicity of this toxin in vivo. The objective of this study was to investigate if MC-LR, administered by gavage to mice, induces DNA damage using the alkaline comet assay. Swiss female mice were treated orally with a saline solution of MC-LR at 1 and 2 mg/kg. After 3 and 24 hours, the induction of DNA strand breaks was evaluated in whole blood, bone marrow, liver, kidney, ileum and colon. DMH (1,2-Dimethylhydrazine dihydrochloride) 20 mg/kg was used as a positive control. Whatever the organ considered, no increase in DNA strand breaks was observed in MC-LR treated mice for both
dose and time exposure. Previous studies showed liver DNA fragmentation up to 3h after ip administration of 21.5 µg/kg MC-LR to mice. This discrepancy between studies can be explained by the administration route. Moreover these effects, due to oxidative DNA damage, are repaired very quickly. Further experiments will be conducted by the in vivo comet assay with MC-LR ip administration for comparison.

**P196**

Characterization of DNA damage induced with anti-HIV drug zidovudine (AZT) in human cells; protective effects of antioxidants

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The genotoxic effects of AZT on human hepatoma HepG2 and colon carcinoma CaCo-2 cells and their reduction by antioxidants were examined by three modifications of the comet assay (SCGE). The total amount of induced DNA damage was measured by standard SCGE. The level of double strand (ds) DNA breaks was evaluated by neutral (pH=9.0) version of SCGE. The level of alkali-labile (AL) DNA lesions was measured by SCGE processed in parallel at pH≥13.0 and pH=12.1. Tested antioxidants were applied for 1 h before AZT-treatment. Our results showed that AZT induced in both cell lines significant increase of DNA lesions, but majority of DNA lesions were in Caco-2 cells converted to DNA strand breaks particularly under strong alkaline conditions, what characterizes AL sites in DNA. Ds DNA breaks were found in both cell lines. The kinetics of DNA rejoining revealed that AZT-induced DNA damage was repaired very slowly in both cell types. We hypothesize that the observed differences in the nature of AZT-induced DNA damage in hepatoma and colon cells may be associated with a different level of AZT phosphorylation in these two histopathologically distinct cell types and with a different distribution and interaction of metabolized and/or non-metabolized AZT molecules throughout the genome. All tested antioxidants manifested protective effects against AZT-induced DNA lesions.

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**P197**

Utilising the forward mutation assay to investigate thresholds of mutagenic activity in cultured human cells

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Current guidelines suggest a linear non-threshold (LNT) model should be applied to DNA reactive genotoxins. However, it appears obvious to many people that on theoretical grounds, thresholds or NOEL’s may exist for at least some genotoxins. Our current view is that thresholds of genotoxic activity have to be proven on a case-by-case basis. Furthermore, living organisms can be predicted to tolerate low levels of DNA damage, due to the activity of protective mechanisms such as DNA repair. We have been investigating the low dose region of compound exposure using the model genotoxin methyl methane sulphonate (MMS) and a larger dose range of the hormone 17-[β-oestradiol (E2) using the cell line AHH-1. In these studies, gene mutation activity has been monitored by the measurement of TK and HPRT mutations. Current data suggests a possible threshold for genotoxic activity at 1µg/ml for MMS in AHH-1 cells, with E2 showing mutagenic activity at concentrations exceeding 0.27 µg/ml. Additionally, data obtained from the cytokinesis blocked micronucleus assay indicate E2 to have a possible threshold of cytotoxicity at 1.89µg/ml. Molecular analysis of exon 3 (HPRT DNA) shows G:C > A:T transitions to be induced (11/40) by MMS, however only 2/39 spontaneous mutants were point mutations and both were deletions, which suggests a possible DNA repair related threshold for MMS.
P198
Genotoxicity of halogenated anesthetics studied in vitro by comet assay

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Inhalation anesthetics exert some side effects. Health effects for short-term (patients) and long-term occupational exposure differ. An increased hepatotoxicity, nephrotoxicity, migraine and reproduction disorders in operating room personnel were reported. Papers concerning genotoxic and cytotoxic effects of halogenated anesthetics are discordant.

The studies aimed for testing genotoxicity of volatile anesthetics (halothane, desflurane, isoflurane and sevoflurane) in human lymphocytes in vitro using comet assay. Because of a high volatility of drugs and to minimize DNA repair, all processes were carried out at 4°C. The negative control was water and 1 % DMSO (used as a solvent), when halothane (already proven as genotoxic) served as positive control. Mann-Whitney U-test was employed to estimate statistical significance.

An induction of DNA fragmentation by desflurane was as effective at that of halothane. Genotoxicity of isoflurane / sevoflurane did not differ significantly from controls. Genotoxic activity of desflurane was dose-dependent (0.1mM - 10mM). The most effective was a short treatment. Exposure prolongation was followed by shortening the comet tails but still longer than in control. It can be related to DNA repair and protective DMSO activity. However, the anesthetics toxicity is influenced also by pharmacokinetic parameters (blood-gas and fat-blood partition coefficients, minimum alveolar concentration and other). Comparison of these parameters indicated that desflurane is less harmful for humans than halothane.

P199
AMES Test : a powerful tool for rapid determination of mutagenic effects. Application to chemicals, pharmaceuticals and medical devices.

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The bacterial reverse mutation assay (AMES Test) using tester strains of Salmonella typhimurium and Escherichia coli is a useful tool as an initial in vitro assay for genotoxic activity of chemicals, pharmaceuticals and medical devices. Positive results in the AMES Test provide a valuable indication for the mutagenic activity in mammalian cells and in vivo systems as well. Here, the mutagenicity of test materials (n = 150), which were examined during the last 2 years at BSL BIOSERVICE, Scientific Laboratories GmbH, Planegg/Munich, Germany are presented. The results are discussed with respect to the nature and the mutagenicity of the different test items. Differentiation of test items with regard to categories such as chemicals and medical devices gives an indication to potential problems with the development of materials in the respective sector of the industry. With the comparison of results it is shown which tester strain shows the highest sensitivity with regard to the mechanism of the induction of mutations. To clarify why an evaluated result is described to be positive, the historical data of the spontaneous rates of revertants are pointed out for each tester strain. Similarly, the historical control data for the positive controls used for different tester strains is discussed. Additionally, the influence of metabolic activation with regard to toxification and detoxification is evaluated. Statistical analysis of the data is presented.
P200
Comparative evaluation of DNA damage by genotoxicants in primary rat cells applying the comet assay

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In this study, various compounds known to cause DNA damage (hydrogen peroxide, visible light-excited Methylene Blue, N-nitrosomorpholine and benzo[a]pyrene) were tested with different primary rat cells (lymphocytes, testicular cells, pneumocytes and hepatocytes) to determine the range of induced DNA damage. The level of DNA strand breaks was assessed by the alkaline version of comet assay, and the level of oxidative DNA damage was measured by the modified comet assay which includes digestion with specific repair enzymes (Fpg, EndoIII).

A dose-dependent increase of DNA breaks was observed after treatment with hydrogen peroxide in all cell types studied, the highest levels were detected in lymphocytes. Visible light-excited Methylene Blue caused significant oxidative DNA damage, which was comparable among all cell types used. Indirect-acting carcinogens (N-nitrosomorpholine and benzo[a]pyrene) were effectively metabolized by pneumocytes and hepatocytes and induced increased levels of DNA damage as revealed by comet assay. Based on these results we can conclude that the comet assay with primary rat cells represents a suitable and sensitive in vitro technique for genotoxicity testing.

P201
The genotoxicity of Tiamulin S in mouse germ cells

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In this work genotoxic effect of antibiotic preparation Tiamulin S (Hemovet, Vrsac, Pharmaceutical Company Yugoslavia) was investigated. The experiments were done using in vivo cytogenetic analysis (occurrence of chromosomal alterations) on BALB/c mouse germ cells. Clastogenic effect of Tiamulin S was monitored at three doses (0.01 ml/kg, 0.2 ml/kg and 0.4 ml/kg) through repeated experimental cycles. The results obtained have shown that Tiamulin S induces karyotype changes inducing both numerical (aneuploidies and polyploidies) and structural chromosomal aberrations (lesions, breaks and Robertsonian translocations). In light of our experimental data concerning the overall cytogenetic changes, we can conclude that there is highly significant (p<0.001) increase between untreated and treated groups of mice, as well as dose dependence. In addition, our experimental data demonstrate that doses of 0.2 ml/kg and 0.4ml/kg exert both cytotoxic and genotoxic potential.

P202
Mutagenicity of methyl methane sulphonate (MMS) in transgenic pUR288 plasmid mice

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MMS is a rodent carcinogen and mutagenic in most test systems. However, in lambda-based transgenic mouse mutation systems such as the MutaMouse it has generally failed to generate a mutagenic response. Here, the transgenic pUR288 plasmid mouse system was used to evaluate the mutagenicity of MMS, using 5 daily intraperitoneal administrations of 100 mg/kg and sampling at 3 or 35 days after the last treatment. The lacZ-containing plasmid DNA was rescued from the mammalian genomic DNA by an immunomagnetic bead assay and electrotransformed into bacterial cells for mutation analysis. After three days a significant increase in mutation frequency of about two- to threefold was seen in liver, lung, spleen and testes. At 35 days post-dose the mutation frequency in liver and lung remained high while in spleen and testis, which are highly proliferating tissues, it dropped to almost control levels. Restriction analysis showed that 30-50% of all induced mutations were deletions of ≥ 50 base pairs. The induction of DNA damage was demonstrated with the comet assay in the 3-day group in all investigated organs. These data...
Genotoxicity Testing

indicate that clastogens like MMS are detected in this model, and that MMS is mutagenic in multiple organs.

P203
In vivo Comet Assay on isolated kidney cells as a tool to discriminate genotoxic from epigenetic carcinogens or cytotoxic compounds

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The current study has aimed (1) to assess the capacity of the in vivo alkaline single cell gel electrophoresis assay (in vivo Comet Assay) to distinguish kidney carcinogenic compounds acting through true genotoxic mechanisms (streptozocin, aristolochic acids and 2-Nitroanisole) from the one acting through an epigenetic mechanism (D-limonene); (2) to determine the impact of the possible interference resulting from cytotoxic phenomena (streptomycin and indomethacin) on the kidney as target organ.

Single kidney cell suspensions from 4 animals per dose per type of treatment (3-6 hours and 22-26 hours) were obtained by a 2-step enzymatic digestion, i.e. in situ perfusion using collagenase followed by in vitro incubation with trypsin. The use of a specific method to obtain isolated cells was compatible with an easy cytotoxicity measurement and allowed to study the biological response of the parenchyme (no interference of other cell types like leucocytes).

All genotoxic carcinogens were found clearly positive with a statistically significant increase in the mediane Olive Tail Moment (OTM) when compared with control, in at least one treatment schedule. In return, both epigenetic and exclusively cytotoxic compounds failed to induce any significant increase in the mediane OTM. Furthermore, this study confirmed the need for 2 sampling times.

As a conclusion, the in vivo Comet Assay could be a useful tool to distinguish genotoxic from epigenetic mechanisms in case of an increase in the kidney tumor frequency in the rat. Cytotoxic effects did not interfere with the in vivo Comet Assay response.

P204
Study design evaluation of mouse lymphoma assay.

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The mouse lymphoma assay detects forward gene mutations at the autosomal thymidine kinase locus of heterozygous L5178Y/TK+/- cells. There is evidence that small TK-/- colonies may result from chromosomal damage to the TK locus and adjacent genes.

In the present study different parameters were examined to optimize the test protocol. Mouse lymphoma cells were treated with methyl methane sulfonate (MMS) for 4 hours and 24 hours. After an expression period of 72 hours cells were seeded for selection with trifluorothymidine at different cell densities (2000, 3000, 4000 cells/well). Mutant colonies were scored daily after an incubation time of 11 to 15 days using the criteria ¼ and ⅓ size of the well to distinguish between small and large mutant colonies. The mutation frequency was calculated from the number of mutant colonies corrected for cell viability.

The results obtained for different seeding parameters did not show any differences in induction of mutant colonies. A slight decrease in the proportion small versus large colonies was found for scoring colonies using the criterion ¼ of well. With prolonged incubation times the number of TK-/- colonies increased. However, the induction factors increased only moderately after 15 days.

Our data show a clear positive response in the formation of mutant colonies after treatment with MMS in all experiments performed. A shift in the number of small TK-/- colonies compared to the control was found indicating chromosome breakage.
P205
Utilising the CBMN assay to investigate thresholds of chromosome aberrations in cultured human cells

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This study investigates if chromosome aberrations induced by MMS occur in a thresholded or linear dose-response manner using the in vitro cytokinesis-blocked micronucleus (CBMN) assay in the MCL-5 cell line. A potential pragmatic threshold for chromosome aberration by MMS was identified at a dose of 0.80µg/ml. To further investigate the mechanism leading to the potential threshold of MMS it is useful to study pairs of alkylators that have diverse mechanisms of genotoxicity, and induce damage which is repaired in different ways. A potential threshold of N-methyl-N-nitrosourea (MNU) is therefore also being studied. Preliminary CBMN assay results from a dose response experiment suggest that either MNU does not act in a threshold manner, or that a threshold dose of MNU is lower than 0.25µg/ml.

In order to substantiate the existence of a potential threshold, identification of a mechanism is required. Firstly kinetochore labelling in conjunction with the CBMN assay demonstrated that MMS is a clastogenic agent, inducing mostly chromosome fragmentation. Secondly a potential threshold could be the result of the influence of DNA repair. Therefore a comparative study is being undertaken in the MT1 cell line, shown to be deficient in Mismatch repair (MMR). And a siRNA technique is being employed to silence the Base excision repair (BER) gene methylpurine glycosylase (MPG), and produce cells which are effectively MPG deficient.

P206
Genotoxic effect of sanitary effluents from a Danish hospital

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Very little is known about the genotoxic effects of sanitary effluents from hospitals. However, radionuclides, cytostatics and antibiotics excreted from patients with the urine and faeces are supposed to contribute to the genotoxic potential. In the present study we have examined sanitary effluent from four different bed sections in a Danish hospital using the Allium cepa anaphase-telophase chromosome aberration assay. Samples were taken from four separate wells representing the four sections. Prior to the genotoxicity testing the general toxicity was detected in the Allium growth inhibition test. The toxicity did not differ much between the four centres and all samples showed a dose response relationship. The EC50, EC30 and EC10 concentrations were used for the genotoxicity assay. For all four centres the genotoxicity was highest and statistically significant for the EC30 concentrations, which was in the range 74 to 119 ml/l. At the EC50 concentrations the chromosome aberrations for all four samples were lower than for the EC30 concentrations indicating an adverse effect on the cell cycle. The most frequent aberration observed was vagrant chromosomes. This could indicate that the hospital effluents have an adverse effect on the spindle mechanism of the cells. In conclusion the study showed significant genotoxic effect on Allium cepa root cells, but no significant difference between the four bed sections.

P207
Assessment of the genetic toxicology of the aqueous root extract of Cryptolepis sanguinolenta and its major alkaloid Cryptolepine.

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The aqueous root extract of Cryptolepis sanguinolenta (CSE) and its major alkaloid Cryptolepine (CLP) are used as traditional anti-malarial treatments in West Africa. Recent work has suggested that both may be prospective cancer chemotherapy agents also. CSE and CLP are cytotoxic in vitro, however, to date there has been limited genotoxicity evaluation. Previous work has shown that both compounds induce micronuclei at low levels of cytotoxicity in V79 cells. CSE was also mutagenic in a HPRT gene mutation assay in V79-MZ cells, while CLP was negative. To further elucidate the mechanisms of CSE and CLP induced DNA damage, studies were conducted using L5178Y mouse lymphoma cells. Three different end-
points were measured: micronucleus induction (including kinetochore labelling); mutation at the TK locus; and formation of topoisomerase II/DNA stabilised cleavage complexes (TARDIS assay). CSE (≥ 5 µg/mL) and CLP (≥ 0.2 µg/mL) induced statistically significant (p<0.05) increases in micronucleated cells at 24 hr (1.7-fold and 1.9-fold, respectively). Significant increases in TK(-/-) mutant colonies were seen after 4 and 24 hr treatments (4 hr: CSE 112.5 µg/mL 3.2-fold and CLP 2.37 µg/mL 3.7-fold; 24 hr: CSE 32.7 µg/mL 7.2-fold and CLP 0.69 µg/mL 2.4-fold). Kinetochore analysis indicated that micronuclei predominantly contained chromosome fragments (% Kn –ve Mn: CSE at 20 µg/mL 93%, CLP at 0.8 µg/mL 91%), and the proportion of small TK(-/-) colony mutants was elevated to 77% and 62% following treatment with CSE (150 µg/mL) and CLP (4.22 µg/mL), respectively; cf 23% small colonies in vehicle controls. These data suggest that CSE and CLP induce DNA damage primarily via clastogenic mechanisms. In contrast, there were no increases in the formation of topoisomerase II/DNA stabilised cleavage complexes above the vehicle controls, which suggests that CSE and CLP are unlikely to be topoisomerase II poisons.

P208
Comparison of three methods in the sensitivity assessment of the in vitro micronucleus test

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Many chemicals interact with genetic material, leading to chromosome aberrations. Such substances are classified as aneugenic or clastogenic. Different tests allow the characterisation and the classification of these chemicals. Among them, the chromosome aberration test and the micronucleus test permit detection of chromosome aberrations. Due to metaphase analysis, the first test presents some disadvantages. The second test is easier, the preparations can be scored more quickly and be assessed more objectively. Despite the fact that this type of assay is well-accepted and validated as an in vivo test (OECD Guideline 474), the in vitro method is not reflected in officially accepted test guidelines at OECD, just a draft is published to date (OECD draft proposal for a new guideline 487) and many disparities in the conductance of this test have become obvious. To contribute to standardisation, three different methodological approaches of the micronucleus test in vitro with V79 cells and cytokinesis block were compared at BSL Bioservice GmbH: GIEMSA staining, Acridine Orange staining (fluorescence) and flow cytometry. V79 cells were treated with three known clastogens (Ethyl methanesulphonate, Mytomycin C and Cyclophosphamide) and one aneugen (Colcemid®). All three assays yielded positive response for all test substances. However, sensibility and specificity differences between the three methods were noted. Thus, all three methods are appropriate for micronucleus detection, but variations between each other exist, in terms of result interpretation.

P209
Genotoxicity and endoreduplication inducing activity of the food flavouring eugenol

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Eugenol is a compound extracted from clove oil and marjoram, is widely used as a food flavouring and is present in spices, such as basil, cinnamon and nutmeg, and also used in dentistry as an antiseptic and analgesic. Structural similarities with the class IIB IARC carcinogen safrole raises questions on it’s carcinogenicity. We evaluated the genotoxicity of eugenol in V79 cells using chromosomal aberrations (CAS) and sister chromatid exchanges (SCEs), with and without rat liver biotransformation (S9). Eugenol induced a dose dependent induction of CAS up to 3 mM, with significant increases from 0 % (control) to 12 % aberrant cells at 3.0 mM, with cytotoxicity at higher doses. S9 increased CAS, from 0.5 % (control) to 18 % (3 mM) with particular emphasis on complex rearrangements. SCEs increased non significantly. We observed an increase of endoreduplicated cells from 0% at control levels to 2.3 % at 2.0 mM, which increased in the presence of S9 (5% at 2.5 mM). Our results confirm the genotoxicity of eugenol in vitro.
P210
Inter-laboratory comparison of MicroFlow micronucleus data obtained in rat peripheral blood and bone marrow: Results from Cyclophosphamide and Etoposide

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Inter-laboratory experiments were performed to evaluate the compatibility of rat bone marrow specimens with the MicroFlow® flow cytometric (FCM) scoring method that was originally developed to score micronuclei (MN) in rodent peripheral blood erythrocytes. The utility of the peripheral blood compartment relative to bone marrow was also investigated. In these experiments, performed at Pfizer, groups of 4 male rats were treated once a day for 4 consecutive days with vehicle control and etoposide at 14.25, 28.5 and 57 mg/kg bw/day, and cyclophosphamide at 5, 7.5 and 10 mg/kg bw/day. All doses were administered by oral gavage at a dose volume of 10ml/kg bw. Blood and bone marrow were collected approximately 24 hours after the final dose. Blood was processed as described in the MicroFlow manual. Bone marrow was processed using a cellulose column method, and with slight modifications to the standard labelling protocol for FCM evaluation. Bone marrow smears were also prepared and stained with acridine orange. Fractions of each peripheral blood and bone marrow sample were analyzed by flow cytometry both at Litron and Pfizer. Coded slides were analyzed at Pfizer. Between laboratories, very high correlation coefficients for FCM-based analyses were observed: $r = 0.986$ for peripheral blood specimens, and $0.973$ for bone marrow samples. Concordance between peripheral blood and bone marrow specimens was also high, with modest differences in response observed for both chemicals at intermediate dose levels. Cumulatively, these data suggest that with minor modifications, the peripheral blood FCM method can be reliably applied to both rat peripheral blood and bone marrow specimens, and that rat peripheral blood is an appropriate tissue for evaluating the genotoxic potential of chemicals.
14. Miscellaneous
P211
Role of the Mitogen Activated Protein Kinase (MAPK) pathways in the induction of IL-1β expression in murine J774A.1 macrophages exposed to platinum compounds

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Platinum compounds are potent sensitizers after topical exposure or inhalation. The aim of the study was to investigate the involvement of MAPK pathways in the induction of IL-1β expression in murine J774A.1 macrophages exposed in vitro to potassium tetrachloroplatinate (TCPP), ammonium tetrachloroplatinate (TCAP) and ammonium hexachloroplatinate (HCAP). Cytotoxic concentrations of the platinum compounds showed a very similar range (IC50: 238±30 µM; 269±39 µM and 245±31 µM, for TCPP, TCAP and HCAP respectively). In the macrophages stimulated with all test compounds increased expression of IL-1β mRNA (RT-PCR) and enhanced production of IL-1β protein (ELISA) was observed. Treatment of the cells with each derivative led to phosphorylation of both p38 MAPK and ERK 1/2 (Western blotting). Furthermore, blocking of the activation of p38 MAPK as well as ERK 1/2 by specific inhibitors (SB203580 and U0126, respectively) down-regulated the IL-1β expression. In contrast, the skin irritant sodium dodecyl sulphate did not trigger either p38 MAPK or ERK 1/2 phosphorylation nor induced IL-1β production.

To conclude, p38 MAPK and ERK 1/2 are essential for both the induction of IL-1β mRNA expression and the production of IL-1β protein by J774A.1 macrophages exposed to the tested platinum compounds.

P212
Transport of phthalate monoesters across the human placenta; perfusion method and pilot-tests

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Dual perfusion of a single cotyledon in the human placenta can contribute to a better understanding of the human placental barrier, transport-rate and -mechanisms of different substances and placental metabolism.

Immediately after birth the foetal circulation in a single cotyledon is re-established by cannulation of fetal artery and vein. The cotyledon is placed in a perfusion chamber where temperature, pH, pressure and pO2 are controlled. The maternal arteries are connected in the intervillous space. Test-substances are added to the maternal side and samples are drawn after different time intervals in both maternal and fetal reservoirs. Antipyrin rapidly diffuses across the placenta and therefore, this compound is used as a marker when assessing the transfer rate of other substances.

Pregnant women are daily exposed to phthalates. The main purpose of the project is to investigate if phthalates crosses the human placenta and thereby have potential to affect the health of the foetus. Preliminary studies of background levels show a rise in monoethyl phthalate, mono-n-butyl phthalate, monobenzyl phthalate and mono-(2-ethylhexyl) phthalate in both fetal and maternal reservoirs with highest concentration in maternal chamber suggesting a placental transport. The perfusion liquids did not contain monooctyl phthalate and monoisononyl phthalate and only small amounts of monomethyl phthalate and mono-(2-ethyl-5-hydroxyhexyl) phthalate.
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