1082 A GENOTOXICITY STUDY WITH P-ARAMID RFP (RESPIRABLE-SIZED, FIBER-SHAPED PARTICULATES).

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The objective of this *in vitro* assay was to assess the potential for p-Aramid RFP (respirable-sized, fiber-shaped particulates) to induce chromosomal aberrations in cultured human peripheral blood lymphocytes without metabolic activation. The highest concentration tested in this assay was limited by the physical characteristics of p-Aramid RFP. The test article was suspended in fully supplemented RPMI culture medium with 1% Pluronic F68. All dosing was achieved using a dosing volume of 90% (900 µL/mL) and the vehicle control cultures were treated with 900 µL/mL of fully supplemented RPMI culture medium with 1% Pluronic F68.In the chromosomal aberrations assay, the treatment period was for 3.0 hours and 19.0 hours without metabolic activation, and cultures were harvested 22.0 hours from the initiation of treatment. Replicated cultures of human whole blood lymphocytes were incubated with 6.30, 12.6, 25.2, 50.4, 101, 201, and 401 µg/mL. Cultures treated with concentrations of 6.30, 12.6, 25.2, and 50.4 µg/mL for 3.0 hours and 6.30, 12.6, 25.2, and 201 µg/mL for 19.0 hours were analyzed for chromosomal aberrations. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed. The results demonstrated that p-Aramid RFP was negative for inducing chromosomal aberrations in cultured human peripheral blood lymphocytes without metabolic activation.

1083 SPONTANEOUS HYDROLYSIS AND AN ACTIVE REPAIR PROCESS DETERMINE THE OVERALL RATE OF REMOVAL OF DNA-PROTEIN CROSSLINKS FROM FORMALDEHYDE-EXPOSED CELLS.

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Formaldehyde is an ubiquitous chemical with numerous sources of human exposure in both environmental and occupational settings. Formaldehyde is also a rodent carcinogen and it forms DNA-protein crosslinks (DPC) as the dominant form of DNA damage in exposed cells. Neither the mutagenic potential nor repair mechanisms of these important lesions are known at this time. In this study, we conducted detailed analysis of the stability of formaldehyde-induced DPC *in vitro* and in cultured cells. Both DNA-histone crosslinks formed *in vitro* and DPC induced *in vivo* exhibited a similarly high rate of spontaneous hydrolysis with the average half-life of 26.3 hr at 37°C and physiological pH/ionic conditions. The rate of the DPC removal from intact cells was even more rapid and was similar among three different human cell lines (HF/SV fibroblasts, Ad293 kidney and A549 lung cells). The average half-life was 12.5 hr; range 11.6-13 hr. After adjustment for the spontaneous loss, an active repair process was calculated to eliminate DPC from cells with the average t1/2=23.3 hr. Nucleotide-excision repair appears to play no significant role in the removal of DPC since repair-deficient XP fibroblasts had kinetics of the adduct elimination similar to other cells. Depletion of intracellular glutathione had no effect on the initial levels of DPC or the rate of their repair. A rapid loss of DPC from exposed cells determined in part by hydrolytic instability of these lesions suggests that application of DPC measurements as a biological dosimeter of formaldehyde exposure could be limited to situations with very recent exposures.

1084 MECHANISM OF METHYLENE DI-PHENYL DIISOCYANATE GLUTATHIONE CONJUGATE MICRONUCLEI INDUCTION DISTINGUISHABLE FROM THAT OF METHYLENEDINALINE.

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Methlyene di-phenyl diisocyanate (MDI) is widely used in the production of polyurethane products, such as wood binders and foams. Diisocyanates are very reactive compounds that can undergo nonenzymatic hydrolysis to form methylenedianiline (MDA), or react under physiological conditions with primary amines to form ureas and/or with thiols to form labile thiol acid esters. MDA is a carcinogen in animals and a suspected carcinogen in humans. We have previously reported that exposure of Brown Norway rats (BNR) to MDI induces micronuclei (MN) in bone marrow polychromatic erythrocytes. In vitro studies suggested that both MDI glutathione (MDI-GSH) conjugates and MDA are potential genotoxic metabolites of MDI. Addition of MDI directly to cell cultures did not induce micronuclei. The mechanism of micronuclei induction of these metabolites was explored in the present study. Chinese hamster lung fibroblasts were incubated with MDA or MDI-GSH. Micronuclei kinetichore from cytokinesis-blocked cells were labeled by immunofluorescent staining. Cells were washed, methanol fixed, treated with Tween 20 buffer, then labeled with antikineticore and fluoresceinated goat anti-human IgG antibodies to discern the presence of centromere within the micronuclei. Vincristine, DMSO and glutathone were used as positive, solvent and negative controls, respectively. MDA induced MN were negative with respect to anti-kineticore antibody binding. This is consistent with induction of chromosomal fragments by MDA binding to DNA. MDI-GSH induced MN had a significant increase in the number of anti-kineticore antibody labeled MN. MDI-GSH also increased the number of cells in metaphase. These results suggest that MDI-GSH MN induction was mediated through disruption of microtubules, whereas MDA MN induction was by a DNA-binding mechanism.

1085 *IN VIVO* DETECTION OF CHEMICALLY-INDUCED MUTATIONS IN TRANSGENIC FISH CARRYING CII/CI MUTATIONAL TARGETS.

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We examined the responses of the cII/cI transgenes in fish (medaka, *Oryzias latipes*) following treatment with either, the model mutagen, ethylnitrosourea (ENU), or a drinking water disinfectant by-product, potassium bromate ($KBrO_3$). The assay consisted of recovering the bacteriophage lambda vector which harbors the cII/cI target genes using *in vitro* packaging, followed by plating phage-infected indicator bacteria to quantify the frequency of cII/cI mutants. The assay is based on the role that the cII protein plays in the ability of the bacteriophage lambda to commit to either the lytic or lysogenic cycle using specialized E. coli hosts. The lysogens produced by the lambda phage with the wild-type cII/cI are favored, and indistinguishable in the E. coli lawn, while lambda phage that carry a cII/cI mutation are selected by forming plaques on the bacterial lawn when incubated at 24°C. Despite the small size of the fish, the vector was recovered with high efficiency (>0.3-2 $X10^6$ PFUs/reaction) which facilitated practical analyses of cII/cI mutant frequencies (MF) in whole fish, as well as in liver, and testes. Exposure-dependent inductions in MF (minimum two-fold induction above 2.9 $X\ 10^{-5}$) were observed in whole fish, testes, and liver after 15 days following 1 h exposures to 80 mg/L and 120 mg/L ENU. A ~two-fold induction of cII/cI mutants was observed in fish following a 28 day chronic exposure to 400 mg/L $KBrO_3$. These results further support the practical viability of transgenic fish as models for mutation detection in genotoxicity, and with distinct relevance to aquatic environmental health hazard assessment.

1086 RADICAL GENERATION CAPACITIES OF AMBIENT PARTICULATE MATTER IN RELATION TO ITS GENOTOXICITY.

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The purpose of this study was to investigate the generation of radicals by ambient particulate matter and to relate these data to (oxidative) DNA damage in alveolar type II cells. Particle morphology and chemical composition was determined by TEM/EDX and AAS analysis of transition metals. Fenton-driven radical formation by particles was evaluated by electron spin resonance (ESR) and by unwinding of supercoiled f X 174 RF1 DNA. DNA damage was measured by single cell gel electrophoresis and immunohistochemical analysis of 8-hydroxydeoxyguanosine. In line with previous observations, PM as well as PM supernatant cause depletion of supercoiled plasmid DNA. Both particles and supernatant produced hydroxyl radical-DMPO adducts in the presence of hydrogen peroxide, and this effect was completely abrogated upon pre-incubation with desferrioxamine (5 mM). PM supernatants also caused DNA damage in type II cells, and this damge was reduced upon pretreatment with desferrioxamine. Our data provide evidence that airborne particulate matter generates hydroxyl radicals in acellular systems, and that this may result in enhanced DNA damage in lung cells.

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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number (2), begins on page 423.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 448.

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