65 **7H-BENZO{C}FLUORENE, A CANDIDATE CARCINOGENIC PAH WITH MAJOR HEALTH IMPLICATIONS.** Goldstein L[1], Goth-Goldstein R[2], Russell M[2], Singh R[3], Weyand EH[3]. [1]EPRI, Palo Alto,CA 94304. [2]Lawrence Berkeley National Laboratory, Berkeley. CA 94720. [3]Rutgers- The State University of New Jersey, College of Pharmacy, Piscataway, NJ 08855.

7H-Benzo[c]fluorene (B[c]F) has been known for a long time as a component of complex mixtures such as coal tar or cigarette smoke. B[c]F has been identified recently as a major DNA adduct-forming component of coal tar in lungs of mice fed a coal tar containing diet. Since ingested benzo[a]pyrene (B[a]P) does not induce lung tumors, B[c]F may play a larger role than B[a]P in lung tumors induced by coal tar or other ingested mixtures. We have investigated if human cells have the ability to form B[c]F:DNA adducts as detected in mouse lung. MCF7 (human breast cancer) and HepG2 (hepatoma) cells were treated with increasing concentrations (0.2 - 10 µg/ml) of B[c]F for 20 hours. Adduct formation was evaluated using ^{32}P-postlabeling. Two adducts were detected in each cell line. One of the adducts observed corresponded to that observed in the lungs of mice treated with pure B[c]F. Adduct level increased in a dose-dependent manner. Induction of *CYP1A1* and *CYP1B1*, two genes coding for cytochrome P450 enzymes involved in PAH metabolism, was measured in parallel. CYP1A1 and CYP1B transcript levels increased with increasing B[c]F dose similar to levels found after B[a]P treatment. These results clearly demonstrate that MCF7 and HepG2 have the capacity to metabolically activate B[c]F to derivatives that covalently modify DNA. The similarities in adduct patterns also suggest that the activation pathway is similar in human cells and in mouse lung. However, total B[c]F adduct levels were considerably lower than the levels observed with equimolar concentrations of B[a]P. This research was supported by funds from EPRI.

66 ***IN VITRO* MUTAGENIC EVALUATION OF *CANNABIDIOL* (CBD), A MARIHUANA COMPOUND, ON HUMAN LYMPHOCYTES BY THE MICRONUCLEUS AND COMET ASSAY TESTS.** Gomes L[1], Gattás GJF[1]. [1]MEDICAL SCHOOL - SÃO PAULO UNIVERSITY.

The plant *Cannabis sativa* is an abuse drug that has also therapeutic qualities as analgesic or antiemetic agent. The *cannabidiol* (CBD) is a nonpsycoative cannabinoid present in large amount in the plant that has been indicated in the control of epilepsy and dystonic movement disorders due to its depressive activity on neuromuscular transmission and response in man. Preliminary studies had shown that the CBD may increase the frequency of chromosomal aberrations in human lymphocytes treated *in vitro*. In the present investigation, the frequency of MN and comets was evaluated in human lymphocytes obtained from peripheral-blood samples taken from six donors mean age 27.7 ±2,1 years. The cultures were exposed to CBD dissolved in ethanol (0,01 ml/ml medium) in concentrations of 0,00,1; 0,01; 0,1; 1,0 and 10,0 µg of CBD per ml of medium. Cultures with ethanol and without treatment were also done. The CBD dissolved in ethanol did not increase the frequency of MN in 1000 binucleated lymphocytes ($p>0,25$) as revealed by non-parametric tests. However, the frequency of comets was higher in the cultures with CBD dissolved in ethanol, or ethanol alone, when compared to control ones. The cultures with 0,01 mg of CBD/ml medium and the cultures with ethanol contributed statistically for this difference ($p<0,05$). The comets were analyzed by morphological and image systems and the comparisons between them showed a statistically significant correlation ($p<0.01$). The results suggested mutagenic effect of CBD *in vitro* that could be detected only by comet assay. Taking into consideration the therapeutic potential of the CBD,and the marihuana use, other mutagenic tests should be undertaken, especially in the *in vivo* systems before this substance can be prescribed.

67 **SINGLE CELL GEL (COMET) ASSAY OF TRANSITIONAL CELLS FROM PATIENTS WITH HISTORY OF UROTHELIAL NEOPLASM.** Gontijo AMMC[1], Elias FN[1], de Oliveira MLCS[1], Salvadori DMF[1], de Camargo JLV[1]. [1]Departamento de Patologia, Faculdade de Medicina, UNESP, Botucatu, SP, Brazil.

After resection, primary human urinary bladder cancer (UBC) often recurs (50-80%). Thus, rigorous follow up of the patients is important for early detection of recurrences and intervention aiming at an alleviation of the morbidity and mortality by the disease. A crucial step in bladder carcinogenesis is genomic instability, which can be triggered by DNA lesions, such as double-strand breaks. The present study was performed to evaluate DNA damage by the single cell gel (SCG)/Comet assay in urothelial cells of patients with history of previous UBC, currently with normal urinary cytology and urinary bladder cystoscopy. Bladder washes were obtained from 60 patients by barbottage. As tobacco use may cause DNA damage in urothelial cells, patients were divided into 4 groups: control (12), smokers (18), non-smokers with history of UBC (15), and smokers with history of UBC (15). A modified alkaline SCG assay was performed, which permitted the specific analysis of urothelial cells, excluding leukocytes present in bladder washes due either to inflammation or trauma. Tail moment values of comets were obtained by image analysis and values are represented as means of groups. The non-smoker group with history of UBC presented increased DNA damage (7.10) ($p = 0.04$). Increased tail moment was also observed in smokers (6.38) when compared to controls (1.94) ($p = 0.03$). Therefore, augmented DNA damage occurs in urothelial cells of smokers and of patients with history of UBC but with normal urinary cytology. Hence, genomic instability might affect the urothelium of these patients, confirming their status as risk patients for bladder cancer.

68 **CYP1B1 EXPRESSION AS A RISK FACTOR FOR BREAST CANCER.** Goth-Goldstein R[1], Erdmann C[2], Russell M[1]. [1]Lawrence Berkeley National Laboratory,Env. Energy Technology Div., Berkeley, CA 94720. [2]Lawrence Berkeley National Laboratory, Computing Sciences Div., Berkeley, CA 94720.

The cytochrome P450 isozymes CYP1A1 and CYP1B1 activate polycyclic aromatic hydrocarbons (PAHs) to ultimate carcinogens. High levels of these enzymes can increase the formation of DNA adducts that can lead to initiation of cancer. Besides environmental carcinogens, these enzymes also metabolize steroid hormones, including estradiol. CYP1B1 in contrast to CYP1A1 metabolizes estradiol to the potentially carcinogenic 4-hydroxy estradiol. Because of these associations we investigated whether expression of CYP1B1 in breast tissue represents a risk factor for breast cancer. We determined CYP1A1 and CYP1B1 expression in non-tumor breast tissue specimens from 26 breast cancer patients and 32 cancer-free individuals. Using a semiquantitative RT-PCR assay, we measured CYP1B1 and CYP1A1 expression relative to the constantly expressed β-actin gene. We found large variation in expression between individuals, about 170-fold for CYP1B1 and 400-fold for CYP1A1. For most samples, CYP1B1 transcript levels were 2-7 times higher than CYP1A1. Comparing CYP1A1 and CYP1B1 transcript levels in non-tumor breast tissue of mastectomy patients and cancer-free individuals, there was a slight, though statistically not significant, increase in CYP1A1 in breast cancer patients versus controls, and a statistically significant increase in CYP1B1 transcript levels in breast cancer patients versus controls. The mean CYP1B1 level was twice as high in breast cancer patients than in controls. We conclude that CYP1B1 is the major PAH-activating enzyme in breast and that high levels present a risk factor for breast cancer. CYP1B1 could play a role in breast cancer etiology by activating either environmental chemicals or endogenous estrogen to carcinogens. Supported by USAMRMC Grant Number DAMD17-98-1-8062.

69 **GENOTOXICITY OF 1,3-DICHLOROPROPANE, 2,2-DICHLOROPROPANE, AND 1,1-DICHLOROPROPENE IN SALMONELLA AND *E. COLI* PROPHAGE-INDUCTION ASSAYS.** Granville CA[1], Warren SH[2], Huggins-Clark G[2], George SE[2], Claxton LD[2], DeMarini DM[2]. [1]Dept. of Environ. Sci. & Engin., UNC, Chapel Hill, NC 27599. [2]US EPA, NHEERL, RTP 27711.

1,3-Dichloropropane (1,3-DCP), 2,2-dichloropropane (2,2-DCP), and 1,1-dichloropropene (1,1-DCP) have been detected in approximately 1% of ground water sources. Their introduction to the environment probably occurs through soil fumigants. Little toxicological data are available on these compounds, but their structural similarity to 1,3-dichloropropene, a rodent carcinogen, has made them a high priority for research. These compounds are on the 1998 US EPA Contaminant Candidate List and were deemed "high priority for research" in 1999. Thus, the genotoxicity of these compounds is being studied in Salmonella and the E. coli prophage-induction assay. The role of glutathione-S-transferase (GST) in the activation of 1,3-DCP also has been investigated based on a 1985 study that reported positive results. Preliminary results show that 1,3-DCP and 2,2-DCP are weakly mutagenic without S9 in the prophage-induction assay, whereas 1,1-DCP is negative. Likewise, preliminary tests on 2,2-DCP and 1,1-DCP in Salmonella strains TA98, TA100, and TA1535 were negative; 1,3-DCP also was largely nonmutagenic in these strains. However, a weak positive response to 1,3-DCP was observed in TA1535 at 8.85 mM in the presence of S9. Preliminary tests for activation of 1,3-DCP by GST in RSJ100 (TA1535 expressing rat GST5-5) and TA1535 expressing human GSTT1-1 were negative. These results suggest that 1,1-DCP is not mutagenic, whereas 1,3-DCP and 2,2-DCP can cause DNA damage that induces prophage. Thus, 1,3-DCP and 2,2-DCP may cause DNA strand breaks, which would induce prophage, but would not revert the Salmonella strains used in this study.[Abstract does not necessarily represent policy of the US EPA.]

70 **RANDOM MUTAGENESIS OF HUMAN 3-METHYLADENINE DNA GLYCOSYLASE.** Guo H[1]. [1]Dept of Pathology, HSB K-058 Box 357705, UWSOM, Seattle, WA 98195-7705.

DNA glycosylases initiate Base Excision Repair by recognizing and removing damaged or inappropriate bases. The human 3-methyladenine DNA glycosylase (AAG, also known as MPG, ANPG, and APNG) recognizes a diverse range of alkylation damaged bases. A crystal structure of AAG has been reported (1). In order to further address AAG's mechanism for substrate recognition, we created large libraries harboring random substitutions in the region surrounding the AAG active site. We generated 2 independent libraries of mutant AAGs, each with greater than 10^6 diversity. One library is composed of mutants spanning 2 regions totaling 27 nucleotides, averaging 2-3 amino acid substitutions per mutant. The other library encompasses mutations over 3 regions totaling 30 nucleotides, also averaging 2-3 a.a. substitutions per mutant. A third library encompasses all 5 mutated regions and is the most random, with 5 average mutations. Similar to the wildtype AAG, mutants within these libraries complement alkylation repair deficient *E. coli* and rescue cells from the alkylating agent Methyl Methane-Sulfonate (MMS). Active AAG mutants were isolated from repeated rounds of MMS selection. On average, greater than 2% of the first two libraries are active. As expected, less than 0.5% of the more random, combined library are active. Among these active mutants, amino acid substitutions are distributed adjacent to and within the substrate recognition pocket, including at evolutionarily conserved residues. Active mutants are being characterized kinetically for the ability to excised altered bases. Mutants obtained from large-scale random substitutions will provide further insight into AAG function. 1. Lau, A. Y., Scharer, O. D., Samson, L., Verdine, G. L. and Ellenberger, T. (1998) Cell, 95, 249-258.

71 **THE COMET ASSAY: THE TEST OF CHOICE AS THE SECOND IN VIVO TEST FOR IN VITRO POSITIVE PHARMACEUTICALS?.** Hartmann A[1], Suter W[1]. [1]Novartis Pharma AG, Genetic and Experimental Toxicology, WSH2881.5.14, CH-4002 Basel, Switzerland.

Registration of pharmaceuticals requires a comprehensive (in vitro as well as in vivo) assessment of a possible genotoxic potential. In case of in vitro positives, guidelines request, besides the in vivo bone marrow MNT, a second in vivo test. The comet assay is becoming established as a sensitive and easy-to-perform test. We used the comet assay for further clarification of in vitro positives and for other specific questions. Fourteen new drug candidates were tested so far. Test were performed for various reasons: positive in vitro tests; CAT (5 compounds), Ames (1), MLA TK (1); findings in long term rodent studies (4); unclear mechanism of (genotoxic?) action(2). The advantage of the comet assay is that tests can be performed in parallel with the same animals used for the MNT in vivo to save animals. Furthermore, the target tissues can be investigation of, e.g., organs in which tumors were seen in the long term rodent tests. The results of the comet assay in vivo were used for decision making. We yet have filed 4 reports with regulatory authorities / ethical committees which accepted this test. The development of two compounds out of the 14 investigated was discontinued because of a positive outcome of the in vivo comet assay: one compound induced DNA-Protein crosslinks in stomach mucosa cells; the other compound was negative in the bone marrow MNT but induced DNA damage in liver cells of rats under certain conditions. An overview will be given on the use of the comet assay in vivo in our company.

72 **MEASUREMENT OF STABILISED TOPOISOMERASE CLEAVAGE COMPLEXES USING THE TARDIS ASSAY.** Harvey JS[1], Burman M, Lynch AM, Rees RW. [1]Genetic Toxicology, SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR, UK.

We have used the TARDIS (Trapped in Agarose DNA Immunostaining) assay to investigate the mechanism of clastogenicity of several topoisomerase I and II inhibitors. Topoisomerases are essential nuclear enzymes that regulate the topological state of DNA during replication, transcription and repair. Topoisomerase enzymes bind covalently to DNA and produce temporary strand breaks thus creating a transient gate (cleavage complex) through which another DNA strand can pass. After strand passage the break is ligated and the DNA structure is restored. Type I topoisomerases create a single strand break in DNA, while type II topoisomerases create a double strand break. Numerous cytotoxic drugs are known to disrupt the catalytic DNA breakage-reunion cycle of topoisomerases. These compounds can stabilise the normally transient cleavage complex, which can ultimately lead to clastogenicity and/or cell death. The TARDIS assay allows the direct detection of topoisomerase molecules covalently bound to DNA in situ. This is indicative of the formation of drug stabilised cleavage complexes in individual cells and may be predictive of clastogenicity. In addition, the semi-quantification of immunofluorescence using image analysis can provide a relative measurement of drug induced stabilised cleavage complex formation. Therefore the TARDIS assay may be used to predict topoisomerase mediated clastogenicity in mammalian cells.

73 **GENOTOXIC RESPONSE OF CALIFORNIA SEA LION (*ZALOPHUS CALIFORNIANUS*) LYMPHOCYTES TO BPDE AND H_2O_2 USING THE COMET ASSAY.** Hastings-Smith DA[1], El-Zein R[2], Ward Jr. JB[1]. [1]The University of Texas Medical Branch at Galveston, Galveston, TX 77555. [2]M.D. Anderson Cancer Center, Houston, TX 77030.

Pollution of marine environments near human population centers can expose marine mammals to carcinogenic and mutagenic chemicals. Humans residing in these areas may share some of the same food sources, exposing them to similar risks. The California sea lion (*Zalophus californianus*) is a marine mammal that develops cancers. Here we report preliminary results of a novel use of the Single Cell Gel Electrophoresis Assay (Comet Assay) with sea lions euthanized due to trauma or carcinomas. Sea lion lymphocytes isolated from whole blood using Vacutainer CPT$^{(r)}$'s were cryopreserved, and later thawed into three cultures for a 72 hour incubation. At 67 hours, one culture of the sample was treated with benzo(a)pyrene diol epoxide, and at 71.5 hours the second culture of the sample was treated with hydrogen peroxide, the third culture of the sample was used as the internal control. The samples were harvested at 72 hours for the comet assay using the Trevigen CometAssay^{TM} kit and protocol. Fifty cells were randomly scored per exposure per sample using the Kinetic Imaging Komet 4.0 system (Integrated Laboratory Systems). The mean tail length for the 2 groups of animals, trauma (n=5) and cancer (n=6), was calculated and a paired t-test was performed to determine a difference between the treated and untreated samples within each group. The mean tail length for treated cells in both groups was 3-fold higher ($p<0.01$) than that of the untreated controls (21.53 ± 5.75). Our results indicate that the comet assay may be a sensitive biomarker for environmental exposure in sea lions. California sea lion blood samples provided by The Marine Mammal Center in Sausalito, Ca. Supported by NIGMS GM 18908-02, T32 ES07254, UTMB Centennial Center for Environmental Toxicology and MDACC.

74 **CHROMOSOMAL ABERRATIONS, SOMATIC MUTATIONS, AND CANCER RISK ASSESSMENT: A PUBLIC HEALTH PERSPECTIVE.** Hattis D[1]. [1]Marsh Institute, Clark University, Worcester, MA.

The essence of a "public health perspective" is an orientation to prevention of adverse health outcomes. To this end public health seeks to (1) identify the causes of illnesses and injuries, and (2) quantify the population aggregate potential prevention benefits of reducing or eliminating specific causal agents so that different prevention options can be appropriately prioritized. Biomarkers such as chromosomal aberrations and measures of somatic mutation have the potential to make the connections between exposures and adverse health outcomes clearer, more specific to particular individuals and groups, and more quantitative. The potential to clarify causal relationships is well illustrated with recent experience with trichloroethylene and kidney cancer, where specific mutations in a particular tumor suppressor gene have made a much more definitive causal connection possible. More generically, there is the capability to make wide-ranging measurements of the prevalence of different DNA lesions in humans and relate them both backward in the causal sequence to responsible environmental agents, and forward to the incidence of somatic mutations along known molecular pathological pathways to cancers. Combined, this opens up excellent possibilities to identify and reduce exposures to the causal agents. Finally, the potential for innovative approaches to quantification is illustrated by recent findings relating differences in easily measured chromosomal aberration frequencies to long term excesses in population aggregate cancer risks.

75 **MUTATION INDUCTION IN NEONATAL MICE TREATED WITH ANTIRETROVIRAL NUCLEOSIDE ANALOG DRUGS.** Heflich RH[1], Von Tungeln LS[1], Hamilton LP[1], Dobrovolsky VN[1], Bishop ME[1], Gamboa da Costa G[2], Beland FA[1]. [1]National Center for Toxicological Research, Jefferson, AR 72079. [2]Centro de Química Estrutural, Instituto Superior Técnico, Lisboa, Portugal.

Zidovudine (AZT) is an anti-retroviral nucleoside analog used in the treatment of HIV infection and acquired immunodeficiency syndrome. AZT is also used pre- and post-natally to prevent the transmission of HIV to the offspring of HIV-positive women. The long-term effects of these treatments to children are not known. AZT is carcinogenic when administered transplacentally or neonatally to mice, and it has been suggested that this carcinogenicity is due to a genotoxic mechanism. To investigate the genotoxic potential of AZT, we assessed the induction of mutations and micronuclei in mice treated neonatally. In addition, since single-drug treatment with AZT is being superseded by multidrug combinations, assays were also conducted with lamivudine (3TC) and a combination of AZT and 3TC. Male and female B6C3F$_1$/Tk$^{+/-}$ mice were treated daily on days 1-8 of age with total doses of 4.0 mg AZT, 4.0 mg 3TC, or a mixture of 4.0 mg AZT and 4.0 mg 3TC. One and two days after the last dose, bone marrow polychromatic erthyrocytes were obtained to assess the induction of micronuclei. Three weeks following treatment, the frequency of mutants was determined in the *Hprt* and *Tk* genes of spleen lymphocytes. AZT and AZT/3TC, but not 3TC, caused a significant increase in micronuclei. Neither AZT nor AZT/3TC increased mutant frequencies in the *Hprt* gene, while both treatments caused significant increases in the *Tk* gene of male mice, with AZT/3TC giving the greater value. The increase in *Tk* mutant frequency by AZT and AZT/3TC was associated with loss of the wild-type *Tk* allele (loss of heterozygosity). The data suggest that AZT is genotoxic in neonatal mice, and that 3TC potentiates the mutagenic response.

76 **GENOTOXIC AND ANTIGENOTOXIC EFFECT OF CHAMOMILE ESSENTIAL OIL IN SOMATIC AND GERM CELLS.** Hernández-Ceruelos CA[1,2], De la Cruz-Maya C[1], Madrigal-Bujaidar E[2]. [1]Laboratorio de Citogenética, Depto. Ciencias Biológicas, Facultad de Estudios Superiores Cuautitlán, U.N.A.M., México. [2]Laboratorio de Genética, Depto. Morfología, Escuela Nacional de Ciencias Biológicas, I.P.N., México.

Matricaria chamomilla (chamomile) has been used since ancient times like remedy against different diseases. The plant possesses several active compounds that may be involved in its therapeutic effects, for example flavonoids, farnesene, alpha-bisabolol, and chamazuleno; the last two chemicals are also strong antioxidant agents. The aims of this study were: a) To determine if the chamomile essential oil (CEO) was genotoxic in bone marrow cells of mouse, b) To determine if the CEO was able to inhibit the genotoxicity of daunorrubicin (DAU) an inductor of free radicals in somatic cells and c) To evaluate if the antigenotoxic potential of CEO may be expressed in germ cells. The study was made by scoring the SCEs in the bone marrow and spermatogonial cells of mice treated as follow: a) for the first purpose we tested 4 doses of the CEO (0, 10, 100, 1000 mg/kg), we did not found increase in the rate of SCEs, or modification in the mitotic index and cellular proliferation kinetic. b) In this phase animals were treated orally with the CEO (0, 5, 50, 500 mg/kg) and at the same time with an i.p. injection of DAU (10mg/kg), the results showed that CEO had a dose-dependant inhibitory effect over DAU (76.96% as the maximum SCE decrease with the highest dose) but no protective effect was observed concerning to DAU´s cytotoxicity. c) With respect to spermatogonial cells we used the same doses but DAU administered 12 h after CEO; we found that the oil produced a dose-dependant inhibitory effect, with 94.73 % as the maximum SCEs decrease. In conclusion this study showed that CEO has a strong capacity to prevent DNA damage produced by a free radical inductor in somatic and germ cells.

77 **INCREASED TRANSLOCATION FREQUENCY OF CHROMOSOMES 7, 11 AND 14 IN LYMPHOCYTES FROM PATIENTS WITH NEUROCYSTICERCOSIS.** Herrera LA[1,2], Rodriguez U[3], Gebhart E[4], Ostrosky-Wegman P[1]. [1]Instituto de Investigaciones Biomédicas, UNAM. Mexico City 04510, Mexico. [2]Instituto Nacional de Cancerologia, SSa. Mexico City, Mexico. [3]Instituto Nacional de Neurologia y Neurocirugia, SSa. Mexico City, Mexico. [4]Intitut für Humangenetik, Univ. Erlangen-Nürnberg, Germany.

Neurocysticercosis (NCC) has been associated with a high frequency of DNA damage in human lymphocytes and more recently with the development of haematological malignancies. Chronic inflammation, a common feature of helminthic infections, has been proposed to play a key role in carcinogenesis induced by parasites. However, this mechanism is more likely to occur during local tumorigenesis rather than in a systemic neoplasia such as the reported for patients with NCC. In this paper we determined the frequency of aberrations in two different sets of chromosomes in lymphocytes obtained either from NCC patients or from uninfected individuals. Our results indicate that translocations involving chromosomes 7, 11 and 14 were more frequent than those occurring in chromosomes 1, 2 and 4. The constant rearrangement of some regions of the first set of chromosomes that take place during T- and B-lymphocyte maturation prone these chromosomes for translocations, a situation that could be increased by a persistent antigen stimulation. This chromosome instability should be considered as a cancer risk factor in NCC patients in addition to other concurrent sources of DNA damage, such as the exposure to environmental mutagens or even to soluble factors secreted by *Taenia solium* cysticerci.

78 **THE IN VITRO MICRONUCLEUS ASSAY IN HAMSTER AND HUMAN CELLS: (I) CYTOTOXICITY-ASSOCIATED POSITIVE RESULTS AND (II) ASSESSMENT OF OPTIMAL SAMPLING TIME.** Hill RB[1], Greenwood SK[1], Galloway SM[1]. [1]Merck Research Labs, West Point, PA 19486.

The in vitro micronucleus (MN) assay has been proposed as an alternative to the assay for chromosome aberrations (Abs). We have described chemicals that induce Abs in vitro but are non-mutagenic and not rodent carcinogens, and are thought to induce Abs indirectly and/or through cytotoxicity associated mechanisms. Here we show that MN are also induced by these compounds at cytotoxic doses (cell counts <50% of controls). Sampling times were 21, 24 and 27 h for hamster (CHO) cells and 3 samples between 20 and 32 h for human (TK6) cells. In CHO cells small increases in MN were induced by bisphenol A, menthol, dithiocarb and the metabolic poisons 2,4-dichlorophenol and sodium iodoacetate. In TK6 cells results were negative or equivocal, just as Ab responses to these chemicals were lower in TK6 than in CHO cells. Suitable sampling times were assessed with mitomycin C (MMC), cyclophosphamide (CP), cytosine arabinoside (AraC) and dimethylnitrosamine (DMN). These true clastogens induced MN with little to moderate reductions in cell counts. In CHO cells there was a fairly wide time range for the MN response with MMC and CP (21 - 30 h) and AraC (17 - 24 h). DMN had a weak increase at 24 h, much stronger at 48 h, consistent with its expected maximum response after two cycles. In TK6 cells, MMC and CP were negative at 21 h, with the greatest MN increases at 27 - 30 h. DMN gave a weak increase at 30h, and was clearly positive by 48h. We found little or no increase in sensitivity in the MN assay in CHO cells from scoring Cytocholasin B-induced binucleate cells compared with mononucleate cells, even for the cytotoxic compounds. Thus, the in vitro MN test is not immune to cytotoxicity-associated "false-positive" results despite the fact that cells must progress through mitosis for MN analysis.

79 **MUTATION LOAD OVER THE LIFE OF THE MOUSE IS AGE AND TISSUE SPECIFIC BUT NOT OBVIOUSLY RELATED TO RATES OF CELL PROLIFERATION.** Hill KA[1], Halangoda A[1], Heinmoller PW[1], Chitaphan CC[1], Sommer SS[1]. [1]Molecular Genetics, City of Hope National Medical Center/Beckman Research Institute Duarte CA.

It is of interest to examine mutation load (mutation frequency and alterations in mutation pattern and spectrum) with tissue type and age. The Big Blue assay was used to measure mutation load in whole fetus (13.5 dpc) and eight tissues (forebrain, cerebellum, thymus, heart, liver, adipose tissue, kidney and sperm) in post-natal mice at 10 days and 3, 10, 14, 16, 17, 22, 25 and 30 months of age. The previously observed constancy of mutation frequency at early and mid-adulthood and two tissue-specific time courses of mutation frequency in old age were confirmed. Tissues that divide rapidly, moderately or not at all have quantitatively similar time courses of mutation from 10 d to 30 mo. Mutation load is unchanged from 3 to 30 mo in neurons and sperm, non-dividing and rapidly dividing tissues, respectively. The nondividing tissues, heart and adipose have increases in mutation load in old age, similar to the moderately dividing tissues, liver and kidney. Additional interesting findings include the following. 1) There is a single core mutation pattern in all tissues that is unaltered even when mutation frequencies change more than five fold. 2) Tandem GG to TT mutations occur in liver, and adipose tissue and show increases with age and certain hotspots in liver. 3) Doublet mutations are enhanced over that expected for two independent events. 4) The distance between doublet mutations fits an exponential distribution consistent with their occurrence in the same cell cycle. The constancy of mutation load in sperm suggests that no advanced paternal age effect on mutation load will be found in mice; compatible with the absence of an advanced paternal age effect observed in the human F9 gene (Hum Gen 105:629). These data may be a helpful reference for future study of endogenous and exogenous mutagenesis.

80 **VALIDATION OF A MULTICOLOR FISH ASSAY TO DETECT CHROMOSOMAL ABERRATIONS IN MOUSE SPERM USING RECIPROCAL TRANSLOCATION CARRIERS.** Hill F[1], Marchetti F[1], Liechty M[2], Bishop J[3], Hozier J[2], Stubbs L[1], Wyrobek AJ[1]. [1]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA. [2]Applied Genetics Laboratories, Melbourne, FL. [3]National Institute of Environmental Health Sciences, Research Triangle Park, NC.

De novo aberrations in chromosome structure represent important categories of paternally transmitted genetic damage. We conducted validation studies of a sperm FISH assay (CT8) that was recently developed to detect chromosome structural aberrations in sperm of mice. This assay used a set of centromeric (digoxigenin) and telomeric (biotin) probes, specific for chromosome 2, to detect partial duplications and deficiencies that may originate during or before male meiosis. Another probe set, specific for chromosome 8 (biotin/digoxigenin dual labeled), was used to distinguish aneuploidy from diploidy. Analyses of over 50,000 sperm indicate that the baseline frequency of B6C3F1 mouse sperm carrying partial duplications and deficiencies of chromosome 2 is 6.0 per 10,000. To validate the assay, sperm from two mice heterozygous for the reciprocal translocation T(2;14), which were expected to have a high frequency of unbalanced sperm, were evaluated. Analysis of ~2000 sperm showed that ~15% carried unbalanced products of chromosome 2. Of these, ~13% were duplications or deficiencies of the telomeric region, while ~2% were duplications or deficiencies of the centromeric region. These findings indicate that the CT8 FISH assay is a promising method for detecting structural chromosome aberrations in mouse sperm and that it has potential widespread application in genetics, physiology and genetic toxicology. [Work was performed under the auspices of U.S. DOE by LLNL under contract W-7405-ENG-48 with funding from NIEHS IAG Y01-ES-10203-00 and by AGL under NIEHS SBIR N44-ES-72003.]

81 **MUTAGENIC AND RECOMBINAGENIC EFFECTS OF TWO METHYLATING AGENTS IN BACTERIA.** Hoffmann GR[1], Crowley DJ[2], Theophiles PJ[1]. [1]Holy Cross College, Worcester, MA 01610. [2]Mercer University, Macon, GA 31207.

Methylazoxymethanol (MAM) and dimethyl sulfate (DMS) both alkylate DNA but they differ in the distribution of methyl groups among sites in the DNA bases. DMS principally attacks highly nucleophilic nitrogen sites, especially the N7 position of guanine. Unlike DMS, MAM also methylates the O6 position of guanine. We studied MAM and DMS in bacterial assays that detect the induction point mutations and the formation of chromosomal duplications by genetic recombination. Duplications were detected by selecting for Trp+ prototrophs in a strain of Salmonella typhimurium carrying the aroC321 allele. Base-pair substitutions were measured in the same strain by selecting for reversion of the hisG46 allele. Both methylating agents were recombinagenic and mutagenic in the aroC321 assay, with MAM being the more potent mutagen and DMS the stronger recombinagen. Mutagenesis was also measured in lacZ strains of Escherichia coli that revert by specific base-pair substitutions and frameshift mutations. In this assay DMS induced several classes of base-pair substitutions (GC to AT, GC to TA, and AT to TA), as well as lower frequencies of +1, -1, and -2 frameshifts. In contrast, MAM was more specific as a base-pair substitution mutagen, inducing only GC to AT transitions. It also induced +G, -G, -A, and -CG frameshifts, although more weakly than it induced base-pair substitutions. The lacZ results show that both DMS and MAM are effective inducers of base-pair substitutions and modest inducers of frameshift mutations and that DMS exhibits a broader spectrum of mutagenic activity than does MAM. The genetic effects of MAM and DMS in the aroC321 and lacZ assays can be related to patterns of alkylation and mutagenic effects of alkylating agents in other assays.

82 **FACTORS AFFECTING MICRONUCLEUS FREQUENCY IN EXFOLIATED BUCCAL AND UROTHELIAL CELLS OF ADULTS AND CHILDREN.** Holland NT[1], Hubbard A[1], Schumacher L[2], Gunn L[1], Golden D[1], Duramad P[1], Smith MT[1]. [1]School of Public Health, University of California, Berkeley, CA. [2]Children's Hospital Oakland Research Institute, Oakland, CA.

Effects of environmental pollution may differ for children and adults. However, few studies have examined biomarkers of genetic damage in children. Development of non-invasive methods is particularly important for community-based biomarker studies involving children. Exfoliated epithelial cells from the mouth and bladder of adults can be easily collected and subjected to cytogenetic analysis as well as analysis of DNA-polymorphisms. Our first step was to determine if cytogenetic analysis could be performed in children using micronucleus assay. Micronucleus frequency is a sensitive measure of genetic damage that reflects both chromosome breakage and aneuploidy. In collaboration with the Children's Hospital Oakland, CA, buccal and urothelial cells have been collected from 81 healthy African-American children, ages 5-12, and 49 parents/guardians from the inner city East Oakland, CA. Here, data are presented on cytogenetic biomarkers, including micronucleus and degenerated cell frequencies in two types of exfoliated cells. Prevalence of degenerated cells was similar in children and adults, in both groups it was significantly higher in urothelial cells (24 and 33%, respectively). The lowest level of micronuclei was observed in children's buccal cells (0.6/1000 cells). The frequency of micronucleated cells was slightly higher in urothelial cells of children than buccal cells ($P<0.001$); but this difference between cell types was not apparent for adults. An age-dependent increase in micronuclei in urothelial cells of children was observed ($P<0.01$). Several factors may contribute to the background level of micronuclei including time of the year, sex and age.

83 **A HUMAN GENE MAPPING TO 11P15 COMPLEMENTS THE DNA POST REPLICATIVE REPAIR DEFECTIVE CHO MUTANT UV-1.** Holmes AA[1], Vannais D[2], Waldren CA[2], Stamato T[3]. [1]Cell and Molecular Biology Grad Program, Colorado State University, Fort Collins, CO 80525. [2]Radiological Health Science, Colorado State University, Fort Collins, CO 80525. [3]Lankenau Medical Research Center, Wynnewood, PA 19151.

Damaged DNA is operated on by a bank of repair systems, some error free, others error prone. The latter can lead to mutation and cancer. CHO-UV1 is a mutant of CHO which is defective in post replication recovery (PRR), defined by its aberrant kinetics of DNA elongation and replication following UV irradiation. Excision repair in UV1 is biochemically normal and it complements the defect in known CHO and human excision repair mutants. UV1 is extremely hypersensitive to killing by DNA alkylating and cross-linking agents typified by MNNG and MMC, respectively, slightly sensitive to UV, but has normal resistance to ionizing radiation. It is hypomutable for EMS and UV induced $HPRT^-$ mutants, indicating a deficiency in error prone repair. PRR in mammalian cells is poorly understood, and few other PRR^- mutants are known. The extreme hypersensitivity of UV-1 to killing by MMC provides a phenotype for identifying human genes involved in PRR. We have shown that introduction by microcell fusion of human chromosome 11 into UV1 fully corrects the hypersensitive phenotype. Deletion mapping with fluorescent *in situ* hybridization puts the complementing gene in 11p15. Chromosome 11 STS marker PCR analysis has further localized the human complementing gene to a 700 kbp region of chromosome 11p15 between positions 12.1-12.8 Mbp. Transfection into UV1 of BACs from this region promises to allow identification of the complementing gene so that its functions can be elucidated. Supported by NCI/CA36447, TG09236.

84 **EFFECT OF C-MYC OVER-EXPRESSION AND LOSS OF MSH-2 EXPRESSION ON SOMATIC INTRACHROMOSOMAL RECOMBINATION.** Hooker AM[1], Morley AA[1], Sykes PJ[1]. [1]Department of Haematology and Genetic Pathology, Flinders University and Medical Centre,Bedford Park, 5042, South Australia, Australia.

The rate at which mutations accumulate in tumour cells is greater than the spontaneous mutation rate in normal cells. Cancers therefore exhibit genomic instability. Somatic intrachromosomal recombination (SICR) is a mechanism which can result in inversions and deletions in the DNA, events which are commonly observed in tumours. Oncogenes and suppressor cancer genes are known to be involved in differentiation, proliferation and apoptosis. Recently, there has been evidence that deregulated cancer genes may themselves play a role in genomic instability. We investigated the role of over-expression of the c-myc proto-oncogene and loss of msh-2 expression on genomic instability using SICR as an endpoint. Tumour model mice with these deregulated cancer genes were bred with pKZ1 mice. pKZ1 mice possess an *E.coli* lacZ transgene in inverse orientation with respect to a promoter complex. When an inversion occurs in the transgene the lacZ gene can be expressed and the lacZ gene product (β-galactosidase) can be detected histochemically in frozen tissues. We analysed SICR inversion events in spleen and marrow of pKZ1/Eμ-myc, pKZ1/heterozygous msh-2 knockouts and pKZ1/homozygous msh-2 knockout double transgenics. Compared with their pKZ1 litter-mates a significant 2X increase in SICR frequency was observed in pKZ1/Eμ-myc spleen and marrow and a significant 10X decrease in SICR was observed in the pKZ1/homozygous msh2 knockout mice. pKZ1/msh2 heterozygous knockout mice exhibited the same SICR inversion frequency as their pKZ1 littermates. This is the first time that c-myc over-expression has been shown to increase SICR in any system. The reduction observed in pKZ1/msh-2 homozygous knock-out mice suggests that the msh-2 gene may be important for SICR as well as mismatch repair in vivo.

85 **UTILIZATION OF AFFYMETRIX MU11K GENECHIPS TO EXAMINE GENE EXPRESSION CHANGES CAUSED BY MITOMYCIN C (MMC) IN L5178Y TK+/- MOUSE LYMPHOMA CELLS.** Hu T[1], Gibson DP[1], Torontali SM[1], Carr GJ[1], Tiesman JP[1], Aardema MJ[1]. [1]Procter & Gamble, Miami Valley Laboratories, P.O. Box 538707, Cincinnati, OH 45252.

Positive responses in in vitro genotoxicity assays due to indirect mechanisms at doses not expected to occur in humans create the need for time consuming and costly follow-up testing. Because of this, many potentially useful chemicals/drugs are dropped from further consideration. Development of mechanistic "screening" assays based on gene expression changes have the potential to quickly identify chemicals acting via an indirect mechanism of genotoxicity. To this end, we are participating in the International Life Sciences Institute (ILSI) project: Application of genomics to mechanism-based risk assessment. The ILSI genotoxicity subgroup is conducting interlaboratory studies of gene expression changes caused by model genotoxins in order to define "fingerprints" for use in discriminating indirect-acting genotoxins from direct-acting genotoxins. Our first test chemical is MMC which is a DNA crosslinking agent. We treated L5178Y mouse lymphoma cells with 0.01, 0.06 and 1 ug/ml MMC for 4 hours. The cells were harvested after a 20 hour recovery period for the assessment of micronuclei (MN) and cytotoxicity. Samples were collected for analysis of gene expression at 2h, 4h, 8h, 16h, 20h and 24h from the beginning of MMC treatment. A significant increase in micronuclei was found at 0.06 ug/ml (6.5%; 20% reduction in live cell count) and 1 ug/ml (13.7%; 55% reduction in live cell count) MMC treatment. MMC at 0.01 ug/ml did not induce a significant increase in MN and was not cytotoxic. This concentration will be useful for examining gene expression changes at doses that precede genotoxicity. We have finished the gene expression data collection phase of this study by using Affymetrix Mu11k GeneChips. Data mining is in progress and significant gene expression changes will be reported.

86 **PROTECTIVE EFFECT OF BAICALIN ON HEPATOCYTE APOPTOSIS INDUCED BY TNF-A AND ACTD IN RAT HEPATOCYTE IN VITRO.** Hu CH[1]. [1]CONG HU, No.54 Friendship North Street, Shijiazhuang, Hebei, 050081 P.R.China.

Baicilin is used as a traditional protective and tonic medical herb for treatment and prevention of liver and other internal organs diseases in China for over thousands years. To investigate the effect of baicalin on rat hepatocyte apoptosis induced by Tumor necrosis factor-a (TNF-a) and Actinomycin D (ActD), baicalin was administrated to cultured Wistar rat primary hepatocyte at 0.2 mg/ml, 2.0 mg/ml and 20.0 mg/ml respectively. TNF-a and ActD were applied into the hepatocyte culture medium 30 min after the administration of baicalin to induce the hepotocyte apoptosis. Twenty-four hours after the cellular apoptosis inducement, the apoptotic hepatocyte was assessed by agarose gel electrophoresis of DNA and the flowcytometric analysis (FCM). The hepatocyte's activity and albumin secretion function were detected and measured by MTT assay and Bromocresolum Viride method. The agarose electrophoresis of DNA fragment result showed that hepatocyte's apoptosis was successfully induced by TNF-a and ActD. The flow cytometry measurement showed that the apoptotic rate of the three different concentration groups of baicalin were lower than that of the apoptotic model group ($p<0.01$). However, the hepatocyte cellular activity and function in all three baicalin administrated groups, even the 0.2 mg /ml group were significantly higher than the control (non-treatment apoptotic model) group ($p<0.01$). The hepatocyte activity and function at baicalin concentration of 20.0 mg/ml group and 2.0 mg/ml group were remarkably higher than the 0.2 mg/ml group ($p<0.05$). The study demonstrated that baicalin might have the function not only to protect hepatocyte from apoptosis, but also to restore or improve the liver's normal function.

87 **ULTRAVIOLET LIGHT-INDUCED GENE EXPRESSION BY CDNA MICROARRAY ANALYSIS.** Huang JC[1], Guo YL[2], Li C[3], Huang W[1]. [1]Department of Medical Technology, School of Medicine, National Cheng-Kung University, Tainan, Taiwan. [2]Department of Environmental and Occupational Health, School of Medicine, National Cheng-Kung University, Tainan, Taiwan. [3]Department of Biochemistry and Molecular Biology, School of Life Sciences, National Chia-Yi University, Chia-Yi, Taiwan.

Ultraviolet irradiation causes CPDs and pyrimidine-pyrimidine (6-4) photoproducts on DNA. Cellular responses to such DNA damages are involved in multiple pathways, including DNA repair, transcription and cell cycle progression. In order to characterize the cellular pathways affected by UV irradiation, we have applied the cDNA microarray techniques to examine the UV-induced gene expression. We have recently constructed a human cDNA microarray chip, on which around 1200 cDNA genes are presented, including the genes for the environmental stresses and DNA damage responses, such as cytochrome P450 subunits, DNA repair, cell cycle regulation, apoptosis, signal transduction, tumor suppressors and oncogenes. HeLa cells were irradiated with 254 nm UVC light then allowed to recover for 30 minutes. The cellular mRNAs in the UV-irradiated and the control cells were reversely transcribed and hybridized to the genes on the chips. The hybridization signals were quantified using the ScanAlyze software and analyzed by the JMP statistical methods. Groups of genes involved in cell proliferation and protein degradation were identified to change in their expression levels after the UV treatment. Among them, the *jun-B* proto-oncogene was induced to 3-4 fold, implying that the transcription factor AP-1 may be affected by the UV irradiation. *In vivo* studies of the functions of the Jun-B proto-oncoprotein as well as the AP-1 complex in these cells are in progress.

88 **COMPARATIVE ANALYSIS OF GENE EXPRESSION CHANGES IN MOUSE TISSUES, MOUSE LYMPHOMA CELLS, AND MOUSE FIBROBLAST CELLS FOLLOWING MITOMYCIN C TREATMENT.** Islaih M[1], Flick LM[1], Deahl JT[1], Reid-Hubbard JL[1], Watson DE[1], Newton RK[1]. [1]Lilly Research Laboratories, Greenfield, IN 46140.

One way that cells respond to genotoxic stress involves an alteration of RNA levels of presumably affected genes. The focus of this study is to compare gene expression profiles from various pathways (e.g. DNA damage/repair genes) in mouse tissues to mouse *in vitro* cell systems (L5178Y or 3T3 cells) following treatment with a genotoxic agent (mitomycin C [MMC]). Male mice (ICR) were treated either with vehicle (PBS) or 5 mg/kg of MMC in PBS for 24 hr followed by collection of liver, spleen, and thymus. L5178Y and 3T3 cells were treated either with vehicle (PBS) or with 1 µg/mL of MMC in PBS for 4 hr followed by a cell harvest after 20 hours. Total RNA was extracted from liver, spleen, and thymus, as well as from L5178Y and 3T3 cell pellets. The RNA was reverse transcribed, labeled with fluorescent dye and hybridized onto Mu11K arrays following an Affymetrix protocol. In the case of DNA repair, oligonucleotide arrays representing genes involved in nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR) and DNA double-strand breaks repair (DSBs repair) were analyzed. Overall, among those genes represented on the Mu11K array, 30 genes were changed in the liver, 390 genes in the spleen, and 596 genes in the thymus. Genes involved in DNA damage/repair are differentially expressed among the various tissues. One DNA damage/repair gene (GADD45) was upregulated in the liver, three genes of a DNA repair pathway were downregulated in the spleen (Ligase I, PCNA, and MHR23A), and DNA topoisomerase II (TOPO II) and the growth arrest specific protein (GAS5) were upregulated in the thymus. The L5178Y cells treated with MMC displayed more DNA damage/repair genes (XPG, RAD51, RFC, TOPO IIb, BRCA1 and BRCA2) that are upregulated compared to the mouse tissues.

89 **DNA ADDUCTS DETECTED BY THE ^{32}P-POSTLABELING ASSAY IN JP-8 EXPOSED RAT HEPATOCYTES.** Jackman S[1], Grant GM[2], Stenger DA[3], Kolanko CJ[4], Nath J[1]. [1]West Virginia University, Genetics and Developmental Biology, 1120 Agricultural Sciences Building, P.O. Box 6108, Morgantown, WV, 26505. [2]George Mason University, 4400 University Dr., Fairfax, VA 22030-4444. [3]Center for Bio/Molecular Engineering, Code 6910, Naval Research Laboratory, Washington D.C., 20375. [4]Loats Associates Inc., Westminster, MD 21157-5146.

The deleterious effects of human exposure to jet propulsion fuel JP-8 is of increasing interest due to the extensive use of this fuel throughout the military and the civilian aviation industry. Chronic exposure to jet fuel has previously been shown to be associated with hepatic, renal, pulmonary, immunological, and neurological toxicity in animal models and through human biomonitoring. Primary military fuel JP-8 consists of a complex mixture of > 80% aliphatic hydrocarbons C9-C16, 18% polycyclic aromatic hydrocarbons (PAHs) and substituted naphthalene hydrocarbons. DNA adduct formation technology takes into account the overall effects of the individual components of complex mixtures, such as jet fuel. DNA adduct formation is detected and characterized by the ^{32}P-postlabeling assay incorporating n-butanol and nuclease P1 enhancement. Cultured rat hepatocytes (H411Es) subjected to varying concentrations of JP-8 in an ethanol carrier based upon previous fuel toxicity studies are analyzed. Preliminary results of exposure to JP-8 suggest DNA adducts formation due the PAH content, free radical production, lipid peroxidation and intermediary metabolic products. Results from these studies will facilitate the assessment of genotoxic risk for individuals exposed to jet fuel.

90 **HUMAN MINISATELLITES AND THE MONITORING OF GERMLINE MUTATION AND RECOMBINATION.** Jeffreys AJ[1], Barber R[1], Dubrova YE[1], Kauppi L[1], May CA[1], Neumann R[1], Slingsby T[1]. [1]Department of Genetics, University of Leicester, Leicester LE1 7RH, UK.

Unstable GC-rich minisatellites in the human genome provide highly informative systems for exploring processes of tandem repeat DNA instability. Analysis of mutants in pedigrees and sperm has revealed a complex gene conversion-based mutational process apparently driven by an intense and highly localised meiotic crossover hotspot next to the repeat array. Sperm crossover typing in the MHC shows again that crossovers are heavily clustered into 1-2 kb long hotspots that all share a morphology very similar to that seen at minisatellites. Current evidence therefore suggests that meiotic recombination in the human genome is highly punctate, but at a resolution undetectable by conventional crossover analysis in pedigrees, and can actively drive at least some classes of repeat DNA instability. Minisatellites currently provide the only efficient system for monitoring germline mutation and have been used to explore whether environmental agents can induce repeat DNA instability. Exposure of male mice to ionising radiation results in a remarkable and apparently indirect induction of germline mutants via an exposure signal that can destabilise repeat DNA. This signal is transmissible through the germline, as shown by radiation-induced instability persisting into the unirradiated offspring of irradiated mice, raising concerns over long-term genetic effects. Similar studies of minisatellite instability in populations exposed following the Chernobyl disaster and from nuclear weapons testing at Semipalatinsk provide evidence for radiation-induced minisatellite mutation in humans. Studies on radiotherapy patients and Japanese A-bomb survivors show no evidence for induced germline minisatellite mutation, raising questions about the relative genetic effects of chronic/acute and external/internal exposure.

91 **DNA HYPERMETHYLATION AND LOSS OF EXPRESSION OF THE P16 TUMOR SUPPRESSOR GENE IN CADMIUM TRANSFORMED BALB/C-3T3 CELLS.** Joseph P[1], Lei Y[1], Muchnok T[1], Ong T[1]. [1]Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, CDC/NIOSH, 1095 Willowdale Road, Morgantown, WV 26505.

In spite of its weak mutagenic potential, cadmium has been shown to be carcinogenic in laboratory animals. The underlying molecular mechanisms responsible for cadmium (Cd)-induced cell transformation and carcinogenesis, however, are not clearly understood. We have undertaken studies investigating aberrant DNA methylation resulting in changes in the expression of cancer-related genes as a possible epigenetic mechanism for Cd carcinogenesis. Genomic DNA isolated from BALB/c-3T3 cells morphologically transformed with Cd was restriction digested with Mse1 (methylation non-sensitive) alone or with Mse1 and BstU1 (methylation sensitive). The resulting DNA was analyzed for differential methylation using a PCR-based technique - Methylation Sensitive Restriction Fingerprinting (MSRF). DNA fragments differentially methylated in the transformed cells compared with the non-transformed cells were identified by MSRF and sub-cloned into the TA-cloning vector. The results of MSRF were confirmed by southern hybridization analysis using the aberrantly methylated DNA fragments as the probes. DNA sequencing and sequence similarity analysis identified one of the aberrantly methylated DNA fragments as the p16 tumor suppressor gene. Further studies have shown that the expression of p16 tumor suppressor gene was significantly lower in the transformed cells compared with the non-transformed cells. Since DNA hypermethylation is known to result in gene silencing, it appears that the decreased expression p16 gene in the Cd transformed BALB/c-3T3 cells may be due to its hypermethylation. Further, decreased expression of the p16 gene may in part be responsible for the Cd-induced cell transformation and tumorigenesis.

92 **QUANTITATIVE CYTOTOXIC AND GENOTOXIC ANALYSIS OF DRINKING WATER DISINFECTION BY-PRODUCTS.** Kargalioglu Y[1], Minear RA[1], Chadaga AR[1], Wagner ED[1], Plewa MJ[1]. [1]University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

The addition of chemical disinfectants to drinking water results in the formation of toxic by-products. We examined and compared the cytotoxic and genotoxic properties of chlorinated versus brominated drinking water disinfection by-product (DBPs) standards and concentrated water mixtures. The cytotoxicity was determined in vitro by using rapid and quantitative microplate-based mammalian and bacterial assays. The rank order of cytotoxicity of DBPs in both cell systems were not identical however the brominated DBPs were more toxic than their chlorinated analogues. To assess the genotoxic properties of the DBPs and concentrated water mixtures in bacteria, we employed the *Salmonella typhimurium* histidine reversion assay. In mammalian cells we developed and calibrated a microplate based single cell gel electrophoresis method (SCGE) which allowed us to compare the genomic DNA damage induced by DBPs. The rank order of mutagenic potency of DBPs in bacteria did not correlate with the rank order of genotoxicity in mammalian cells. In the case of MX, it was highly mutagenic in *S. typhimurium* but was relatively less genotoxic in cultured chinese hamster ovary (CHO) cells. Therefore the widely used bacterial assays may not be good quantitative predictors for assessing the genotoxic potency of DBPs in humans. Supported by AWWARF grant 554 and U.S.EPA grant R825956.

93 **USE OF GENOTOXICITY ASSAYS TO PREDICT THE CARCINOGENICITY OF NICKEL-CONTAINING MATERIALS.** Kaspin LC[1], Verma A[1], Thakore K[1], Metwally ME[1], Landolph JR[1]. [1]Department of Molecular Microbiology and Immunology, USC/Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90033.

Nickel refinery workers exposed by inhalation to high concentrations of mixtures of certain nickel compounds showed higher incidences of respiratory cancers. However, mixed exposures prevent assessment of the carcinogenicity of specific nickel compounds. To determine the genotoxicity of nickel-containing samples, we treated mouse embryo fibroblasts with water soluble or insoluble nickel compounds, elemental nickel particles (filamentous and spherical) and powders of stainless steel containing 12% nickel (3.5 and 8.5 µm mean particle size), and quantitated chromosomal aberrations and morphological cell transformation. Abilities to induce chromosomal aberrations (sample dose = 1.0 µg/ml) were: Ni3S2 NTP (29.0%) > Ni0 f0.5-1 µm (17.0%) > Ni3S2 INCO (14.0%) > Ni0 s3-7 µm (9.0%) > Ni0 s1 µm (7.0%) > Ni0 f 2.2-3.3 µm = NiSO4 NTP = black NiO (6.0%) > NiSO4 anhydrous (5.0%) > NiCl2.6H20 (4.0%) > green NiO (3.0%) > SS 8.5 (1.5%) > SS 3.5 (1.0%). Transforming potencies (slopes of dose response curves for induction of morphological transformation) were: black NiO (9.6) > green NiO (4.9) > Ni3S2 INCO (2.8) > Ni3S2 NTP (1.1) (NiSO4 6H20 NTP (0.84) > Ni0 s1 µm (0.4) > NiSO4 6H20 Aldrich (0.3) (Ni0 s3-7 µm (0.3) > Ni0 f 0.5-1 µm (0.2) > Ni0 f2.2-3.3 µm (0.1). NiCl2.6H20, anhydrous NiSO4, and the 2 samples of SS powders did not induce significant transformation. The genotoxicity profiles for Ni3S2 and Ni oxides correlate positively with animal carcinogenicity data. Based on our results, negative carcinogenicity is predicted for soluble nickel compounds, anhydrous nickel sulfate, SS powders and elemental nickel powders of 14 µm particle diameter.

94 **GENOTOXIC EFFECTS OF CR^{6+}, CR^{4+} AND CR^{3+} IN THE DROSOPHILA WING SPOT ASSAY.** Katz AJ[1], Shi XL[2], Chiu A[3], Chiu N[3], Beaubier J[3]. [1]Biological Sciences, Illinois State University, Normal, IL 61701. [2]NIOSH, Morgantown, WV 26505. [3]U.S. EPA, Washington, DC 20210.

Chromium is a human carcinogen, although it's precise mode of action is unknown. Chromium can exist in various valence states including Cr^{6+}, Cr(5+), Cr^{4+} and Cr^{3+}. $Cr^{6+}$is readily taken up by cells where it is believed to be reduced to a lower valence state which then serves as the penultimate mutagen (Cr is not believed to directly interact with DNA). The Drosophila wing spot assay was used to evaluate the genotoxic effects associated with compounds containing Cr^{6+}, Cr^{4+} and Cr^{3+}. As expected, Cr^{3+} was not genotoxic in the assay (Cr^{3+} cannot easily pass through the plasma membrane). However, both Cr^{6+} and Cr^{4+} significantly ($p<0.05$) induced both small and large single spots and twin spots in the assay. Comparison of spot frequencies on wings of trans-heterozygous and inversion-heterozygous flies reveals that most of the spots induced by Cr^{6+} and Cr^{4+} arise from mitotic recombination. The genotoxicity associated with Cr^{4+} suggests that Cr^{4+} may be the penultimate mutagen. Furthermore, mitotic recombination can result in loss of heterozygosity and may play an important role in Cr-induced carcinogenesis.

95 **STRATEGIES FOR PROTECTION AGAINST AFLATOXIN-INDUCED HEPATOCARCINOGENESIS: INSIGHTS FROM CLINICAL TRIALS IN CHINA.** Kensler TW[1]. [1]Johns Hopkins School of Public Health, Baltimore, MD 21205.

One of the major mechanisms of protection against carcinogenesis, mutagenesis, and other forms of toxicity mediated by carcinogens is the induction of enzymes involved in their metabolism, particularly phase 2 enzymes such as glutathione *S*-transferases. Animal studies indicate that induction of phase 2 enzymes is a sufficient condition for obtaining chemoprevention. Indeed, monitoring of enzyme induction has led to the recognition or isolation of novel, potent chemopreventive agents such as 1,2-dithiole-3-thiones, terpenoids and the isothiocyanate sulforaphane. For example, oltipraz, a substituted 1,2-dithiole-3-thione, exhibits chemopreventive activity against different classes of carcinogens targeting multiple organs. Mechanistic studies in rodent models for aflatoxin B_1-induced hepatocarcinogenesis indicate that increased expression of phase 2 genes is of central importance to protection by oltipraz. To test the hypothesis that enzyme induction is a useful strategy for chemoprevention in humans, three key elements are necessary: a candidate agent, an at-risk population and modulatable intermediate endpoints. Towards this end, a placebo-controlled, double blind clinical trial of oltipraz was conducted in residents of Qidong, P.R. China who are exposed to dietary aflatoxins and who are at high risk for the development of liver cancer. Oltipraz significantly enhanced excretion of a phase 2 product, aflatoxin-mercapturic acid, in the urine of study participants administered oltipraz by mouth daily for one month. While this study highlighted the general feasibility of inducing phase 2 enzymes in humans, a longer term intervention is addressing whether protective alterations in aflatoxin metabolism can be sustained for extended periods of time in this high-risk population.

96 **CELL TRANSFORMATION AND GENE EXPRESSION INDUCED BY METAL WORKING FLUID IN BALB/C-3T3 CELLS.** Keshava N[1], Lin F[1], Huffman D[1], Chen ZY[1], Ong T[1]. [1]Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, WV 26505.

Metal working fluids (MWF) are commonly used in the lubricant production and compounding industries and in the manufacturing industries that perform machining, grinding, forming or treating operations. It is estimated that 1.2 million workers are potentially exposed to MWF through inhalation or skin exposures. To study the transforming potential of MWF, BALB/c-3T3 cells were exposed to MWF at varying concentrations for 24 hours. The media was washed and the cells were further allowed to grow for four weeks. The cells were stained with 10% Giemsa and Type 3 foci were analyzed. Ten focal colonies were isolated from Type 3 foci and transformed cell lines were derived. DNA and RNA were isolated from each transformed cell line. Gene amplification and gene expression of several proto-oncogenes (K-*ras*, c-*jun*, c-*fos*, c-*myc*, c-*sis* and *erb-B2*) and tumor suppressor genes (*p53* and *p16*) were studied using differential PCR. Random Amplified Polymorphic DNA (RAPD) analysis was carried out to study genomic changes using 10 different random primers. Results show that MWF caused a significant increase in transformation frequency in a dose-dependent manner. Also, the cytotoxicity data indicated a dose-dependent decrease in the cell number after MWF treatment. None of the genes studied were amplified in these transformed cells. However, increased expression of c-*jun* and c-*fos* was observed. No significant genomic changes were observed by RAPD analysis. These results indicate that MWF is capable of inducing morphological transformation in BALB/c-3T3 cells and that alteration in c-*jun* and c-*fos* expression is associated with MWF-induced transformation.

97 **TUMORIGENIC POTENTIAL OF METAL WORKING FLUID INDUCED IN BALB/C-3T3 TRANSFORMED CELLS.** Keshava N[1], Lin F[1], Chen ZY[1], Huffman D[1], Ong T[1]. [1]Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Morgantown, WV 26505.

Occupational exposures to Metal Working Fluids (MWF) occur in a variety of settings. Workers are exposed to MWF through skin contact and by handling parts, tools and equipment covered with MWF. Our earlier studies indicate that MWF is capable of transforming BALB/c-3T3 cells. Further, studies were performed to identify the tumorigenic potential, if any, of MWF-induced transformed cells in mice. Athymic nude mice, approximately 3-4 weeks, were injected either with MWF-induced transformed cell lines or non-transformed BALB/c-3T3 cells. The mice were observed for tumor formation and sacrificed when the tumor size was between 1-1.5 cm. The tumors were asceptically removed and tumor cell lines were derived. DNA and RNA were isolated from tumor cells and analyzed for genetic alterations. Differential PCR was performed to study gene amplification and expression of several proto-oncogenes (K-*ras*, c-*jun*, c-*fos*, c-*myc*, c-*sis* and *erb-B2*) and tumor suppressor genes (*p53* and *p16*). Random Amplified Polymorphic DNA (RAPD) analysis was carried out to study the genomic changes using 10 different random primers. All mice injected with transformed cells developed tumors within 34 days of injection. Mice injected with non-transformed BALB/c-3T3 cells did not develop any tumors. None of the genes studied were amplified in tumor cells. However, these cells showed increased expression of c-*fos* and *erb-B2*. No significant genomic changes were observed by RAPD analysis. These results indicate that MWF is capable of inducing cellular changes and changes in gene expression of certain oncogenes in BALB/c-3T3 cells and that these cells possess neoplastic potential. Studies are in progress to investigate the molecular mechanism of MWF-induced tumorigenesis.

98 **APPLICATION OF THE SHORT-EXTENSION NESTED PCR MUTATION ASSAY FOR GENOTOXICITY TESTING IN MITOCHONDRIAL DNA: EFFECTS OF BLEOMYCIN.** Khaidakov M[1], Manjanatha MG[1], Aidoo A[1]. [1]FDA Jefferson Laboratories, National Center for Toxicological Research, Jefferson, AR 72079.

In this study we have analyzed effects of bleomycin in mitochondrial DNA using short extension PCR method for detection of low-copy deletions. It is based on two PCR reactions with nested distantly spaced primers. An extension period used in these reactions does not allow amplification of the full-length fragment, and specifically selects for fragments containing deletions. Four-month-old F344 rats were subjected to a single injection of bleomycin (10 mg/kg). Animals were sacrificed 4 weeks after exposure and DNA from splenocytes was processed in SE-PCR assay. Samples from bleomycin-treated animals showed more than 30% increase in deletion incidence (p=0.053). Sequencing of 32 deletion-containing fragments revealed that deletions recovered in control group were typically flanked by direct repeats (72.7%) varying in size from 2 to 6 nucleotides. In bleomycin-treated group direct repeats were found in only 46.7% of breakpoints. Further analysis showed that deletion breakpoints in the treated group contained twice as many cleavage sites (GTX and GCX) for bleomycin (40% vs. 18.8%). In addition, the localization of breakpoints was not entirely random. We have found two clusters containing deletions from both groups which may be indicative of deletion hot-spots. Our additional finding include the presence of numerous polymorphisms in Fisher 344 mitochondrial DNA as compared to a wild-type sequence for SD rat mtDNA listed in the Genbank database. Also, in the deletion-flanking regions, several base substitutions were found, including three negative frameshifts, two 2 bp deletions and 17 base substitutions. These findings raise a possibility that the SE-PCR assay can potentially be used for analysis of point mutations as well.

99 **COMMUNITY PARTICIPATORY-BASED REPRODUCTIVE EPIDEMIOLOGY USING DBCP EXPOSURE AS A MODEL.** Kim PJ[1], Rodriguez J[1], Lim KL[1], Robbins WA[1]. [1]UCLA SPH-EHS 56-070, Box 951772, Los Angeles, CA 90095-1772.

We explored the feasibility of conducting a retrospective epidemiological study of adult reproductive effects following childhood environmental exposure to DBCP (dibromochloropropane). DBCP is a male reproductive toxicant used extensively as a pesticide during the 1970s in the San Joaquin Valley of California. Although adverse effects for adult occupational exposures are clear, new evidence suggests pre-pubescent and pubertal males may also be sensitive. It is not known if levels of DBCP found in the environment might have effects on the developing male reproductive system. To investigate this, we compiled a detailed retrospective, school-based, DBCP exposure matrix using drinking water and pesticide usage data from nearby agricultural fields. Public and private sources were used and cross-referenced to establish validity of exposure data. We then constructed a database containing names and current addresses of males who attended schools with high DBCP exposure (≥ 4 ppb drinking water) versus low exposure (≤0.001 ppb drinking water) during 1970 through 1981. Focus groups were conducted with community members to determine the most socio-culturally sensitive techniques to encourage participation in a research study that would ask for reproductive history information and donation of semen. Surveys were sent to 200 men in both high and low exposed schools to determine willingness to participate in a reproductive study and to elicit information that would improve participation. A field test of 25 men was conducted to document participation rates for reproductive history and semen collection. DBCP exposure provided a natural experiment in which to test feasibility to integrate current biologic monitoring with retrospective exposure assessment in environmental epidemiology studies.

100 **RAD22 PROTEIN, A RAD52 HOMOLOGUE IN SCHIZOSACCHAROMYCES POMBE, BINDS TO DNA DOUBLE-STRAND BREAKS.** Kim WJ[1], Kim MJ[1], Park SD[1]. [1]School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea.

DNA double-strand breaks can be introduced by exogenous agents or during normal cellular processes. Genes belonging to *RAD52* epistasis group are known to repair these breaks in budding yeast. Among these genes, *RAD52* plays a central role in homologous recombination and DNA double-strand break repair. Despite its importance, its mechanism of action is not yet clear. It is known, however, that the human homologue of Rad52 is capable of binding to DNA ends *in vitro*. Herein, we show that Rad22 protein, a Rad52 homologue in the fission yeast *Schizosaccharomyces pombe*, can similarly bind to DNA ends at double-strand breaks. This end-binding ability was demonstrated *in vitro* by electron microscopy and by protection from exonuclease attack. We also showed that Rad22 specifically binds near double-strand break associated with mating type-switching *in vivo* by chromatin immunoprecipitation analysis. This is the first evidence that a recombinational protein directly binds to DNA double-strand breaks *in vivo*.

101 **REGULATION OF RRG1+ EXPRESSION BY GLUCOSE IN SCHIZOSACCHAROMYCES POMBE.** Kim MJ[1], Park SD[1]. [1]School of Biological Sciences, Seoul National University, Seoul 151-742, Korea.

The *rrg1+* (rapid response to glucose) was previously isolated as UV-inducible gene in *S. pombe*, designated as *uvi22+*. However, intensive studies with this gene revealed that transcription of this gene was not induced by DNA damaging agents, but by glucose. Glucose depletion from the growth medium led to a rapid decrease in the steady state level of the *rrg1+* mRNA within 10min. This effect was readily reversed upon readdition of glucose. In addition to glucose, various carbon sources, including sucrose, raffinose, fructose, maltose and ethanol could increase *rrg1+* mRNA level while other nutrients, such as ammonium and phosphate, and osmotic stress did not show any effect. To investigate the upstream signaling pathway for this glucose response of *rrg1+*, its mRNA level upon glucose availability was examined in the mutant cells of cAMP pathway and the treatment of rapamycin. The results showed that there was little change in the amount of *rrg1+* mRNA, suggesting that glucose-regulated expression of this gene would be controlled independently by cAMP pathway and TOR (target of rapamycin) pathway, which are well-characterized glucose signaling pathways in *S. cerevisiae*. Interestingly, the rapid decline of *rrg1+* mRNA by glucose-depletion resulted from reduction of the mRNA half-life. With all these results, it is the first report of glucose-regulated stabilization of mRNA in *S. pombe*.

102 **SIMULTANEOUS DETECTION OF APOPTOSIS, NECROSIS AND MICRONUCLEI IN A MICROSCALE MNT IN VITRO SCREENING ASSAY WITH L5178Y TK+/- CELLS.** Kirchner S[1], Albertini S[1], Chètelat AA[1], Gocke E[1], Muster W[1]. [1]Pharma Research Nonclinical-Development - Safety PRNS, F. Hoffmann La-Roche Ltd, CH-4070 Basel, Switzerland.

The MNT in vitro has been shown to be a valuable and sensitive method for the detection of chromosomal damage. Cellular stress like changes in the osmotic pressure or excessive cytotoxicity of a test compound are well known factors to influence chromosomal damage and can confound the interpretation of clastogenicity tests. The different modes of cell death may have variable impact on DNA cleavage or survival and therefore might influence the induction of MN at cytotoxic concentrations to a different extent. To investigate the impact of apoptosis and necrosis on the clastogenic response in a MNT in vitro screening assay we measured the induction of apoptotic and necrotic cells simultaneously with the formation of MN cells (by morphological criteria). All compounds tested so far revealed an unique pattern of apoptotic, necrotic and MN cells with decreased cell growth but could be classified into 3 categories. For the mutagen MMC the frequency of MN cells surpassed both the frequency of apoptotic and of necrotic cells. Compounds showing sizeable induction of MN only at cytotoxic concentrations (DMSO, $MgCl_2$) were characterized by increased apoptosis/necrosis ratios with MN cell frequencies clearly below apoptotic cell frequencies. Tetrachloroethylene and 4-nitrophenol showed no relevant induction of MN (growth reduction >50%) and revealed no significant increases of apoptotic and necrotic cells. The results suggest that impairment of the apoptotic pathway in relation to necrosis and cell proliferation plays an important role for the induction of MN at highly cytototoxic dose levels. The simultaneous detection of drug and dose dependent changes in the mode of cell death might provide useful additional information for the relevance and interpretation of positive findings in the MNT in vitro.

103 **DIRECT COMPARISON OF PLATE INCORPORATION AND PREINCUBATION METHODS FOR INITIAL MICROBIAL MUTAGENESIS SCREENING.** Kirsten NL[1,2], Quillen F[2], Noble C[2], Sina JF[2]. [1]SRI International, Biopharmaceutical Development Division, Menlo Park, CA 94025. [2]Merck Research Laboratories, West Point, PA 19486.

Two mutagenicity testing methods, plate incorporation and preincubation, were compared to determine which was more effective for mutagenesis screening. Eleven chemicals with and without structural alerts for mutagenicity were evaluated by both methods: N-nitrosodiethylamine, N-nitrosodimethylamine (DMN), ethylnitrosourea, phenanthrene, ethylmethanesulfonate, epichlorohydrin, cyclophosphamide, succinic anhydride, N-methylolacrylamide, dimethylaniline, and hydrazine dihydrochloride. *Salmonella typhimurium* strains TA1535, TA97a, TA98, and TA100 and *Escherichia coli* strain WP2 *uvr*A pKM101 were used with and without phenobarbital/beta naphthoflavone-induced rat liver metabolic activation. For the liquid preincubation phase, cultures were incubated with shaking at 37°C for 60 min before plating. The two methods showed equivalent overall detection of mutagenicity for 9 of the 11 chemicals. Only the preincubation method showed mutagenesis (≥2-fold increase) with DMN at ≥1000 µg/plate (*E. coli*), ≥6000 µg/plate (TA100), and 10,000 µg/plate (TA97a) and with N-methylolacrylamide at ≥3000 µg/plate (TA97a, TA100, *E. coli*). Toxicity, as evidenced by inhibition of the background lawn (no growth) and inhibition of revertants (≤2 standard deviations below control levels), was seen more often and at lower doses with the preincubation method. The increased exposure during preincubation appeared to result in a greater number of revertants induced by some mutagens, suggesting a greater sensitivity for weakly positive compounds, while the plate incorporation method provided a larger dynamic range for mutagen detection. Thus, our results did not provide clear evidence that one method is more effective for initial screening.

104 **RB-MEDIATED G1/S CHECKPOINT CONTROL.** Knudsen ES[1], Sever-Chroneos Z[1], Fribourg AF[1], Lan Z[1], Angus SP[1], Wang JYJ[2], Knudsen KE[1]. [1]Department of Cell Biology, University of Cincinnati, Cincinnati, OH 45267-0521. [2]Department of Biology, University of California San Diego, La Jolla, CA 92093.

The retinoblastoma tumor suppressor protein, RB, is a negative regulator of cellular proliferation that prevents inappropriate cell cycle progression. The importance of RB in this capacity is exemplified by the observation that RB is functionally inactivated at high frequency in human tumors. Recently it has been shown that RB also plays a role in the response to DNA damage, suggesting that RB loss in tumors may both yield a growth advantage and facilitate genomic instability. The mechanisms underlying these functions of RB are not clearly defined. Using constitutively active alleles of RB, we demonstrate that RB not only controls progression through G1, but also regulates progression through S-phase of the cell cycle by impacting the DNA replication machinery. Specifically, RB disrupts the stable recruitment of PCNA to sites of replication via attenuation of CDK2/Cyclin A activity, thus revealing a pathway through which RB acts to inhibit DNA-synthesis. We hypothesized that this inhibition of replication by RB may contribute to RB-dependent cycle arrest in response to DNA damage. We show that the chemotherapeutic agent cisplatin (CDDP) invokes separable G1 and S-phase cell cycle arrest that is RB-dependent, although CDDP-induced G2/M cell cycle arrest did not require RB activity. Furthermore, loss of this RB-dependent arrest results in DNA re-replication following CDDP treatment. Together, these data stress the importance of RB-mediated replication control and provide evidence that it is important for the maintenance of genomic integrity under conditions of genotoxic insult.

105 ***IN VIVO* GENOTOXICITY AND BIOAVAILABILITY OF 2,4,6-TRINITROTOLUENE AMENDED SOIL.** Kohan MJ[1], Bordelon NR[2], Brooks LR[1], Huggins-Clark G[1], Donnelly KC[2], George SE[1]. [1]ORD/NHEERL/ECD, US EPA, Research Triangle Park, NC 27711. [2]Texas A&M University, College Station, TX 77843-4458.

As chemicals age in soil, irreversible binding of chemical contaminants to organic components in the soil may occur, impacting the amount of chemical that is biologically available upon exposure. In this study, 10 week old Fischer 344 female rats were fed diets prepared with 2,4,6-trinitrotoluene (TNT) contaminated soil to determine if the bioavailablity of TNT is reduced when bound to soil as measured by excretion of mutagenic urine metabolites. Rats were provided food *ad libitum* prepared with TNT-contaminated soil (3% w/w soil, 1.6 mg TNT/g food), TNT (1.6 mg/g food), clean soil (3% w/w), or no additives for 14 days. On day 14, the rats were placed into metabolism cages, fed the same diets, and urine collected for 24 hours. Urine metabolites were deconjugated by enzymatic treatment (β-glucuronidase and sulfatase) or acid hydrolysis and concentrated. Mutagenic activity was determined in *Salmonella typhimurium* strains TA98 and TA1041. Animals received an average of 63.2 and 70.3 mg/kg/day of TNT in the presence and absence of soil. The presence of soil in the TNT diet reduced urine mutagenicity. Acid hydrolyzed urine from TNT- and TNT+soil-treated rats produced 120.0 and 36.0 TA98 revertants/µl urine equivalent and 505.5 and 124.0 TA1041 revertants/µl urine equivalent. Enzymatic deconjugated urine from TNT- and TNT+soil-treated rats generated 58.8 and 34.8 TA98 revertants/µl urine equivalent and 238.7 and 152.2 TA1041 revertants/µl urine equivalent. Reduction in urine mutagenicity suggests that the presence of soil renders TNT less available for metabolism and bioactivation *n vivo* resulting in reduced genotoxicity. [Abstract does not necessarily reflect EPA policy]

106 **THE FREQUENCY OF MICRONUCLEI, PLASMA MALONDIALDEHYDE AND BLOOD SEROTONIN AMONG CHILDREN AT RISK OF EXPOSURE TO SAW DUST.** Korraa SS[1], Iskander N[1], Shalaby H[1]. [1]National Center for Radiation Research and Teechnology - 3 Ahmed El Zomour St 8th Sector P.O.Box 29 Nasr City - Cairo Egypt.

The aim of this study was to evaluate the cytogenetic and health hazard effects induced in children at risk of exposure to sawdust. The frequency of micronuclei, plasma malondialdehyde, blood superoxide dismutase and blood serotonin were investigated in 40 delinquent children exposed to saw dust as part of their daily training in a handicraft training school at Giza District. Controls were obtained from 35 children attending regular school at the same district. The frequency of micronuclei, plasma malondialdehyde, blood serotonin levels were higher among children exposed to saw dust when compared with controls. These results indicate that exposure to saw dust jeopardize children to genotoxicant consequences and calls for protective measures to protect such children during their woodcraft lessons.

107 **STUDIES ON THE MUTAGENIC ACTIVITY OF SMOKELESS TOBACCO IN *LACZ* MOUSE ORAL TISSUES.** Kosinska W[1], von Pressentin MDM[1], Chen M[1], Guttenplan JB[1,2]. [1]N. Y. Univ. Dental College, Biological Sci./Biochem. Unit, New York, NY 10010. [2]N.Y. Univ. Medical School, Dept. Env. Medicine, New York, NY 10016.

Smokeless tobacco products are carcinogens in the oral cavity, but attempts to demonstrate carcinogenicity in rodents have been equivocal. In this study we administered extracts of smokeless tobacco in the drinking water to lacZ mice. The extracts consisted of mixtures of an aqueous extract and an ethanol extract of the leaves. 6 female mice/group were given increasing doses (from 2 to 12 mg/ml) of smokeless tobacco extract in drink over 12 weeks as a dose-finding study and single sentinel mice were taken at three time points before the final three mice were sacrificed. There was no trend towards an increased mutant fraction (MF). However, there was a marginal increase in the(MF) relative to that in untreated controls at longer exposures in both tongue and other pooled oral tissues. After exposure to an additional average of 12 mg/ml snuff extract per day for 7 weeks, the MF in tongue and other pooled oral tissue (OT) resp. was (in mutants/100,000 pfu) 6.4 ± 3.2 and 6.8 ± 2.9 for moist snuff, and 7.6 ± 3.6 and 5.5 ± 2.3 for dry snuff. The corresponding values for controls were 4.1± 2.9 and 4.6 ± 2.3. The effect of moist snuff extract on mutagenesis by NNK (also given in drink) was also examined, and the MF for tongue and OT was between 10 and 13 for the NNK alone and NNK + snuff extract indicating there was no demonstrable effect of the extract on mutagenesis by NNK. This study showed snuff extracts are weakly mutagenic at best under the conditions described here, and also that ingestion of the snuff extract had no apparent deleterious effects on health. The dose given here extrapolates to a similar daily dose in human snuff dippers. Possibly exposure to higher doses would be necessary to observe mutagenesis in mice. Supported by Smokeless Tobacco Res. Coun. Grant # 0727-01.

108 **TRANSGENIC P53 KNOCKOUT MICE AS AN ALTERNATIVE MODEL FOR CARCINOGEN BIOASSAY: RESULTS WITH CYCLOPHOSPHAMIDE.** Krishna G[1], Urda G[1], Tefera W[1], Reindel J[1]. [1]Department of Drug Safety Evaluation, Pfizer Global Research & Development, Ann Arbor Laboratory, Ann Arbor, MI 48105.

Transgenic mice have shown promise as an alternative model for a 2-year mouse carcinogen bioassay. These models seem to save time and reduce the number of animals needed in carcinogenicity testing. Thus, International Conference on Harmonization guidelines includes a provision for the optional use of alternative, short-term in vivo carcinogenicity studies. The present study was designed to evaluate the effects of cyclophosphamide (CP) at the human therapeutic dose range (1 to 5 mg/kg/day, orally) in p53 heterozygous (p53+/-) and wild type (p53+/+) mice. Groups of 20 male p53 heterozygous or wild type mice were orally dosed by gavage daily with either vehicle (saline) or 5-mg/kg CP for up to 24 weeks. Interim sacrifices at Weeks 6 and 12 were also included to evaluate possible early diagnostic indicators of tumor formation. DNA sequencing of p53 gene, micronucleus evaluation and histopathology were also included in select tissues. There were no biologically significant drug-related clinical signs, body weight changes, or gross pathology at either interim or terminal sacrifice. In heterozygous p53 knockout mice, 2 skin sarcoma tumors, 1 in each of vehicle controls and CP-treated, were noted. No tumors were identified in wild type mice. An evaluation of bone marrow micronuclei, as a measure of chromosome damage, and mutations in select p53 exons revealed no obvious biologically significant differences between vehicle control and CP-treated animals at any of the sacrifices. These data suggest that CP, at a therapeutic dose of 5 mg/kg/day given orally by gavage for up to 24 weeks, was not carcinogenic in transgenic p53 knockout mice. In the literature, however, CP at higher doses with a different treatment regimen, has been shown to cause elevated incidence of tumors in transgenic mice.

109 **ROLE OF INSOLUBLE METAL IONS IN AIRBORNE PARTICULATE INDUCED DISEASE.** Kristovich R[1], Long JF[1], Waldman WJ[1], Williams MV[1], Dutta PK[1]. [1]The Ohio State University, Columbus, OH 43210.

Epidemiological studies have demonstrated that there is a positive correlation between the inhalation of airborne particulate matter and increased morbidity and mortality. However, the mechanism(s)by which these airborne particulates contribute to human disease is unknown. It has been suggested that the biological effects of these particulates are due to their size and to soluble metal ions complexed to the particulates. Conflicting results have been obtained with regards to the role of metal ions in this process. To investigate the possibility that insoluble metal ions may contribute to the oxidative stress induced by fine (<2.5 µm) particulates we developed a procedure, using zeolite Y as a manifold, that allows for the synthesis of carbon particles in the size range of 0.05 to 1 µm containing various metal ions incorporated into the carbon framework. Using a human umbilical vein endothelial cell (HUVEC) and human macrophage culture system we have demonstrated that exposure of HUVEC to carbon-iron particulates (1 µm; 0.5-2.5 µg) for 24 hr results in the modest enhancement of expression of endothelial adhesion molecule ICAM-1, and that this response was potentiated by treatment of HUVEC with supernatants from macrophages exposed to the particulates. Using the transgenic Chinese hamster ovary cell line AS52, we demonstrated that the cytotoxicity of these carbon/Fe particulates was dose dependent (14-60 µg/ml) and that the exposure of AS52 cells to these particulates resulted in a increase in the mutation frequency when compared to controls. These results demonstrate that insoluble metal ions complexed to airborne particulates may contribute to the biological effects of the particulates.

110 **INDUCTION OF MICRONUCLEI BY ALKYLATING AGENTS IN P53 HEMIZYGOUS AND WILD TYPE MICE.** Krsmanovic LS[1], Gudi R[1], Jacobson-Kram D[1]. [1]Department of Genetic Toxicology, BioReliance, Rockville, MD 20850.

The p53 gene is frequently found to be mutated in a variety of human tumors. Its function is thought to be critical in regulating cell replication and inducing apoptosis after DNA damage. p53 homozygous and hemizygous knockout mice develop tumors with greatly reduced latency period compared to wild type mice. A micronucleus study was conducted in p53 hemizygous and wild type (C57) mice with alkylating agents to investigate the incidence of micronuclei with single or multiple dose administrations. Mice were treated with cyclophosphamide (CP) at 50 mg/kg or mitomycin (MMC) at 5 mg/kg by oral gavage with a single administration or by two administrations separated by 24 hours. Bone marrow cells were collected at 24 hours after final treatment. Substantial reductions in polychromatic erythrocyte (PCE) to total erythrocyte ratio relative to the solvent control were seen in males and females with both dosing regimens in both p53 and wild type mice. Statistically significant increases in micronucleated PCEs in treated groups relative to the respective vehicle control groups was observed in male and female mice with both dosing regimens in both strains ($p>0.05$, Kastenbaum-Bowman). These results suggest that: 1) baseline frequencies of micronuclei are the same in transgenic and wildtype mice 2) no apparent difference in the incidence of micronuclei was observed between p53 and wild type mice. We conclude that mice hemizygous for the gene p53 process alkylating agents induced DNA damage similarly to wild type mice and that experiencing a single large dose of an alkylating agent does not affect the transgenic animals ability to process a second dose.

111 **MUTAGENICITY IN SALMONELLA AND DNA DAMAGE IN THE CHO/COMET ASSAY INDUCED BY NITROHALOMETHANES, A NOVEL CLASS OF DRINKING WATER DINSINFECTION BY-PRODUCTS.** Kundu B[1], Warren SH[2], DeMarini DM[2], Richardson SD[3], Wagner ED[4], Plewa MJ[4]. [1]Dept. Environ. Sci. & Engin., UNC, Chapel Hill, NC 27599. [2]US EPA, NHEERL, RTP, NC 27711. [3]US EPA, NHEERL, Athens, GA. [4]Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801.

Halomethanes are a class of drinking water disinfection by-products (DBPs) whose genotoxicity has been studied extensively, with most halomethanes inducing mutagenic, clastogenic, and carcinogenic effects. Recently, a related class of DBPs, nitrohalomethanes (NHMs), has been identified in drinking water. These NHMs are predicted to have potential adverse health effects and are currently part of a nationwide occurrence study of DBPs in drinking water. Although they have been predicted to be toxic, no genotoxicity data had yet been generated for these compounds. NHMs are similar in chemical structure to well-studied halomethanes, with the addition of a nitro-group (NO2). We examined the mutagenicity of NHMs in Salmonella strains TA98, TA100, TA104, and RSJ100 and their DNA damaging ability in a single-cell gel electrophoresis (Comet) assay with Chinese hamster ovary (CHO) cells. The NHMs induced revertants in all Salmonella strains, about three-fold above background levels. In the Comet assay, these DBPs showed a DNA-damaging capability of over one order of magnitude above the drinking water contaminant MX, a potent genotoxicant. Though there is agreement with the qualitative results from the two systems, the potency of the NHMs differs in each system. In Salmonella, NHMs were generally weak mutagens and not as potent as their analogous halomethanes (minus the NO2 group). The addition of a nitro-group to the halomethanes reduces the base-substitution mutagenicity of the NHMs. The NHMs were potent inducers of DNA damage in mammalian cells and may reflect high risk to humans. Future research will be focused on analyzing Salmonella revertants induced by NHMs using colony probe hybridization and PCR/DNA sequence analysis. [Abstract does not necessarily represent the policy of the US EPA.]

112 **REGULATION OF *NFS*A EXPRESSION BY SOXS IN *ESCHERICHIA COLI*.** Lambert IB[1], Paterson ES[1], Boucher S[1]. [1]Carleton University, Ottawa, On, Canada, K1S5B6.

The mutagenic and antibacterial activity of nitroaromatic and nitroheterocyclic compounds is mediated by short-lived metabolic intermediates formed during the reduction of these compounds by bacterial nitroreductases. In *Escherichia coli*, two oxygen-insensitive nitroreductases, NfsA and NfsB, have been characterized. The *nfs*A gene encodes the major oxygen-insensitive nitroreductase in *E. coli*. Recently, *nfs*A was shown to be upregulated by the redox-cycling compound paraquat, suggesting that it is part of the *sox*RS regulon which includes several genes that encode products involved in the *E. coli* oxidative stress response. The SoxS protein is a positive regulator that binds to a conserved 'soxbox' sequence overlapping, or directly upstream of, the promoter sequences of these genes. Using primer extension, we have located the transcriptional start site of *nfs*A 292 bp upstream of its start codon suggesting that *nfs*A is transcribed as part of an operon that includes a small ORF (*ybj*C) of unknown function. Translational fusions with *lac*Z suggest that *ybj*C is expressed *in vivo* and that both it and *nfs*A are positively regulated by SoxS following paraquat challenge. Mutational disruption of *ybj*C does not alter the inducibility of *nfs*A-*lac*Z fusion constructs by paraquat, suggesting that the *ybj*C gene product does not play a role in transcriptional regulation of *nfs*A. Using gel-shift assays, we have demonstrated that purified SoxS-His_6 protein binds to a DNA fragment containing the *ybj*C-*nfs*A promoter region. Deletion of the region directly upstream of the -35 sequence of the *ybj*C-*nfs*A promoter eliminates SoxS-His_6 binding and prevents induction of *lac*Z fusions by paraquat.

113 FANCONI ANEMIA FANCG/XRCC9 PARTICIPATES IN SENSITIVITY OF CHO CELLS TO MULTIPLE TYPES OF DNA DAMAGING AGENTS. Lamerdin JE[1], Dias J[1], George JW[1], Souza B[1], Christian AT[1], Liu N[1], Thompson LH[1]. [1]Biology and Biotechnology Research Program, Lawrence Livermore Natl. Laboratory, P.O. Box 808, Livermore, CA 94551.

We cloned the human *XRCC9* gene based on the ability of the cDNA to complement the mitomycin C (MMC) sensitivity of the CHO UV40 mutant (Liu et al. 1997, PNAS 94: 9232-9237). *FANCG* was cloned independently by cDNA-complementation of human Fanconi anemia (FA) group G cells under MMC selection and proved to be identical to *XRCC9*. We found that another CHO mutant, NM3, was also complemented by *FANCG*. FISH chromosome analysis shows that CHO cells have a single copy of *FANCG*. Both UV40 and NM3 carry *FANCG* frame-shift mutations that severely truncate the proteins in exons 1 and 3, respectively. Based on MMC sensitivity of *FANCG* transformants of mutant NM3, we found full correction with the gene, but not with the cDNA, which produced high levels of FANCG protein that may interfere with normal function. NM3 cells are ~5-fold sensitive to MMC and ~3-fold sensitive to MMS and UV radiation. The MMS and UV sensitivities were also corrected in transformants. Sensitivity to mutagens other than DNA crosslinking agents has not been a recognized feature of FA cell lines in any of the FA complementation groups. It is uncertain whether this difference is attributable to this particular gene, species differences, or the lack of sufficiently quantitative data on FA cell lines. Since normal human cells vary in sensitivity due to genetic heterogeneity, studying isogenic mutant and gene-transformant pairs should help answer this question. Toward this end, we have corrected the MMC sensitivity of EUFA143 lymphoblasts by transfection with the *FANCG* gene. Our current working model is that FANCG participates in a regulatory pathway that promotes homeostasis of reactive oxygen species (ROS). (Work was done under the auspices of the US DOE by LLNL under contract No.W-7405-ENG-48).

114 CHARACTERIZATION OF A DNA DAMAGE-INDUCIBLE GENE FROM A FLUORESCENT *PSEUDOMONAS* SP. STRAIN YP. Lee JS[1], Park Y[1], Park JK[2], Park SD[3]. [1]Division of Biological Sciences and Research Center for Proteineous Materials, Chosun Univeristy, Kwang-ju 501-759, Republic of Korea. [2]Division of Life Science and Medicinal Resources Research Center, Wonkwang University, Iksan 570-749, Republic of Korea. [3]School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea.

The *recA* gene plays a central role in genetic recombination and SOS DNA repair in *Escherichia coli* (*E. coli*). In the present study, a 42 kDa RecA-like protein inducible to a variety of DNA damages was identified and a gene encoding the protein was cloned by immunological screening with polyclonal anti-*E. coli* anti-RecA antibodies as a probe. It was shown by dose-response and time-course experiments that the cellular level of RecA-like protein was increased to 3-8 times by DNA damaging agents such as nalidixic acid (NA) and mitomycin-C (MMC), compared to those of controls. The most effective doses of NA and MMC for the induction of RecA-like protein were 30 μg/ml and 0.3 μg/ml at the treatment time points of 150 min, respectively. The enhanced level of the RecA-like protein was gradually decreased to control level for 10 hr. The *recA*-like gene was also cloned and characterized from the *Pseudomonas* cells. Southern blot analysis showed that *recA*-like gene was located in the 3.2 kb *Eco*RI fragment of *Pseudomonas* chromosomal DNA. In addition, the *recA*-like gene was transcribed into approximately 1.1 kb RNA transcript, as judged by Northern blot analysis. The cellular level of RNA transcript of *recA*-like gene was increased to an average of 5.15-fold upon treatment with DNA damaging agents, including ultraviolet-light, NA, methyl methanesulfonate, and MMC. These results suggest that the *recA*-like gene is inducible to DNA damage. However, it seemed that the cloned gene did not restore the DNA damage sensitivity of *E. coli recA* mutants. The results presented by this study suggest that a typical SOS function driven by RecA-like protein is highly conserved in *Pseudomonas* cells like in *E. coli*.

115 TRANSCRIPT PROFILING OF A CARCINOGEN/NON-CARCINOGEN PAIR IN A 14 WEEK RAT STUDY. Li B[1], Flick L[1], Phelps JB[1], Newton RK[1], Li Q[1], Sullivan J[1], Watson DE[1]. [1]Lilly Research Labs, Greenfield, IN 46140.

Methapyrilene (MP) and pyrilamine (PYR) are H1 receptor antagonists developed as sedatives and allergy medications. They are close structural analogs but differ dramatically with respect to their tumorigenic potential. Whereas PYR appears to be not carcinogenic to rats at 3000 ppm in the diet for 104 weeks, MP causes hepatocellular carcinomas after as little as 26 weeks at 1000 ppm. This difference can not be explained by a direct genotoxic effect: neither MP nor PYR are genotoxic in the majority of standard gentox assays. To further understand the differences in chemical carcinogenesis displayed by these compounds, we investigated histopathological and gene expression changes in the livers of rats exposed to MP or PYR in the diet for up to 14 weeks. Exposure times (4, 14, 28, 99 days) and dietary concentrations of MP (50, 250, 1000 ppm) and PYR (1000, 3000 ppm) were identical to previously published experiments. At 99 days there was clear evidence of neoplastic transformations in the livers of animals exposed to the highest dose of MP for 99 days. RNA was processed and hybridized to Affymetrix rat genome (U34A) arrays. Gene expression data were filtered: difference call I or D; fold change greater than 2; and min avg diff intensity greater than 250. The genes that met these criteria (1163 genes) at the 99 day time point were used for hierarchical clustering. There was high correlation between transcript profiles of different treatments (0.94 or greater) except for the highest dose of MP (0.49). Expression changes at the highest MP dose occurred for genes involved in cell differentiation and proliferation, transcription factors, seven transmembrane/G protein-coupled receptors, serine/threonine kinases, growth factors, and other genes that may be involved in cellular transformation.

116 EXPRESSION OF HMMS2 ANTISENSE IN HUMAN CELLS DECREASES THE FREQUENCY OF RECOMBINATION AND INCREASES THE FREQUENCY OF INDUCED MUTATIONS. Li Z[1], Xiao W[2], McCormick JJ[1], Maher VM[1]. [1]Michigan State University, East Lansing MI. [2]University of Saskatchewan, Saskatoon, Canada.

In *S. cerevisiae* yeast, *MMS2*, coding for ubiquitin conjugating enzyme-like protein, has been shown to be involved in an error-free post-replication repair pathway (Broomfield et al., 1998). To determine the role of the human homolog, *hMMS2*, in cells dealing with DNA damage, we transfected *hMMS2* antisense into MSU-1.2 cells that are capable of detecting mutation induction and also homologous intrachromosomal recombination. Transfectants expressing high level of *hMMS2* antisense were selected and used to determine the effect of antisense on UV-induced mutagenesis and recombination. The results showed that the transfectants expressing *hMMS2* antisense were not more sensitive to the cytotoxic effect of UV than the controls (parental cells or non-expressing vector-control transfectants). However, expression of antisense *hMMS2* significantly reduced the frequency of UV-induced intrachromosomal recombination and in the same experiments doubled the frequency of UV-induced 6-thioguanine resistant mutants. The data support the hypothesis that *hMMS2* is involved in an error-free damage avoidance pathway, in which blocked forks use undamaged homologous DNA as a template to avoid translesion synthesis. (Supported by DHHS grant ES09822 and NIH GM21858.)

117 **EXPOSURE SCHEMES AND P53 STATUS AFFECT RADIATION-INDUCED IN VIVO LOSS OF HETEROZYGOSITY.** Liang L[1,2], Mendonca MS[2], Shao C[1], Deng L[1], Stambrook PJ[3], Tischfield JA[1]. [1]Rutgers University, Piscataway, NJ 08854. [2]Indiana University School of Medicine, Indianapolis, IN 46202. [3]University of Cincinnati, Cincinnati, OH 45267.

Ionizing radiation induces loss of heterozygosity (LOH) in cultured cells. Using mice heterozygous at the adenine phosphoribosyltransferase (*Aprt*) locus that were whole-body irradiated with X-rays, however, we demonstrated that single or daily-fractionated doses of X-rays did not yield changes in the frequency of in vivo LOH although these schemes slightly changed the mechanisms resulting in LOH. In contrast, 4 Gy X-rays fractionated as 1 Gy/week doses over 4 weeks, generated increased frequencies of intragenic alteration and interstitial deletion/gene conversion that were the main causes of an about 3-fold increase in the frequency of LOH. Our results strongly correlate with the observations by Kaplan *et al* in 1952 that weekly-fractionated doses of X-rays was more effective in induction of lymphoma than single or daily-fractionated doses of X-irradiation. Moreover, in p53-deficient mice, a 4 Gy single dose of X-rays produced about an 8-fold increase in the frequency of in vivo LOH that was mainly caused by interstitial deletion/gene conversion and mitotic recombination. These results suggest that radiation-induced LOH in vivo depends on the way in which the radiation is delivered and on cellular responses to radiation, such as p53-dependent cell cycle arrest and apoptosis. Our studies of animals that have been exposed to X-rays are critical to our understanding of how radiation can contribute to various diseases including cancers and to the setting of practical guidelines for radiation exposure.

118 **INDUCTION OF OXYRADICALS BY ARSENIC: IMPLICATION FOR MECHANISM OF GENOTOXICITY LIU SX, HEI TK,CENTER FOR RADIOLOGICAL RES., COLUMBIA UNIVERSITY. NEW YORK, NY. 10032.** Liu SX[1], Hei TK[2]. [1]Center for Radiological Res., Columbia University.New york, NY.10032. [2]Center for Radiological Res., Columbia University.New york, Ny.10032.

Arsenic is a well-established human carcinogen, the mechanisms by which it induces cancer remains poorly understood. We previously showed arsenite to be a potent mutagen in human-hamster hybrid (AL) cells and that it induces predominantly multilocus deletions (Hei et al., Proc. Natl. Acad. Sci., 95: 8013, 1998). It has been suggested that increase in superoxide-driven hydroxyl radical productions induced by arsenic mediates the genotoxicity in mamma lian cells. We show here by confocal scanning microscopy with the fluorescent probe CM-H2DCFDA that arsenite induces, within 5 min after treatment, a dose dependent increase of up to 3-fold in intracellular oxyradical production. Concurrent treatment of cells with arsenite and the radical scavenger, DMSO (dimethyl sulfoxide), reduced the fluorescent intensity to control levels. Electron spin resonance (ESR) spectroscopy using TEMPOL-H as a probe in conjunction with superoxide dismutase and catalase to quench superoxide anions and hydrogen peroxide, respectively, indicates that arsenite increases the levels of superoxide-driven hydrxyl radicals in these cells. Furthermore, reducing the intracellular levels of non-protein sulfhydryls (mainly glutathione) in AL cells with buthionine S-R-sulfoximine (BSO) increases the mutagenic potential of arsenite by more than 5-fold. The data provide convincing evidence that reactive oxygen species, particularly hydroxyl radicals, play a important causal role in the genotoxicity of arsenical compounds in mammalian cells. (Supported in part by NIH grant: ES08821 &ES10349)

119 **RNA POLYMEARASE II AS A SENSOR AND INTEGRATOR OF CELLULAR STRESS.** Ljungman M[1], O'Hagan H[1], Yanamadala S[1], Tenbroeke ML[1]. [1]Department of Radiation Oncology, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI 48109-0936.

We have previously shown that certain DNA-damaging agents, such as UV light and cisplatin, may induce p53 and apoptosis by inhibiting transcription. We here show that blockage of elongation of transcription by UV light or camptothecin leads to the accumulation of nuclear p53 that is phosphorylated on the ser15 site and acetylated at the lys382 site of p53. However, inhibition specifically of the transcription initiation stage by the kinase inhibitors DRB or H7 resulted in the nuclear accumulation of unmodified p53. For ionizing radiation (IR) we did not find a significant nuclear accumulation of p53 but the p53 proteins induced were modified at ser15. Interestingly, when combining low doses of DRB with IR a high level of modified p53 was found in the nucleus and this lead to a super-induction of the p53-inducible p21/WAF1 gene. These results suggest that DRB trapped p53 in the nucleus while IR activated p53 as a transcription activator. Using DNA repair-deficient human fibroblasts we found that both the accumulation and modification of p53 following UV-irradiation was triggered by UV-induced lesions specifically in the transcribed strand. Finally, we show that mismatch repair proteins may trigger induction of p53 and apoptosis by blocking transcription in cell treated with alkylating agents. We propose that cells may use the elongating transcription machinery to sense DNA damage either directly or indirectly through the formation of mismatch repair complexes. The blockage of the transcription machinery then triggers the inducion of stress signal transduction pathways leading to the induction of p53 and apoptosis.

120 **ASSESSING THE REPAIR OF BIOLOGICALLY SIGNIFICANT LEVELS OF DNA ADDUCTS IN HUMAN CELLS.** Lloyd DR[1], Hanawalt PC[1]. [1]Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020.

We have developed an experimental approach for studying global nucleotide excision repair of DNA adducts at biologically significant levels in human cells. By exploiting the sensitivity of the ^{32}P-postlabelling assay, a technique commonly used for the detection of DNA adducts in human tissues, it has been possible to monitor cellular excision repair of such lesions at levels comparable to those documented in certain human populations as a result of environmental exposures. We have demonstrated that the efficient global repair of the major *N*2-guanine adduct formed by the carcinogen benzo(*a*)pyrene diol epoxide (BPDE) is dependent upon the tumour suppressor protein p53. However, this p53-dependent repair mechanism functions specifically at the low levels of DNA adducts (around 1 adduct per 10^7 nucleotides) that are present in certain individuals (e.g. smokers) who have been exposed to enhanced levels of DNA-damaging agents. The repair of low levels of DNA adducts formed benzo(*g*)chrysene diol epoxide (B(*g*)CDE), a related carcinogen that forms DNA adducts with both guanine and adenine bases, was also found to be p53-dependent. However, the repair of the (-)-*anti*-B(*g*)CDE-adenine adduct was particularly dependent upon p53 function, suggesting that recruitment of the p53-dependent repair pathway is related to adduct structure or conformation. The manner in which p53 regulates the removal of biologically significant levels of these DNA lesions from the genome is currently being investigated. In addition, we are continuing to exploit the versatility and sensitivity of the ^{32}P-postlabelling assay to study repair of different types of DNA damage, and at levels that are significant in terms of human environmental exposure and carcinogenesis.

121 DEFECTIVE PROCESSING OF HYDROGEN PEROXIDE-INDUCED OXIDATIVE DNA DAMAGE IN PREMATURE CATARACT PRONE MOUSE LENS EPITHELIAL CELLS. Lohani A[1], Kumaravel TS[1], Mambo E[1], Egwuagu C[2], Evans MK[1]. [1]Laboratory of Molecular Genetics, National Institute on Aging, Baltimore, Maryland 21224, USA. [2]Laboratory of Immunology, National Eye Institute, Bethesda, Maryland 20892, USA.

To assess the possible role of oxidative DNA damage and repair in premature cataractogenesis, we examined the cellular response of the premature cataract prone Nakano mouse lens epithelial cells (NKR11) to hydrogen peroxide-induced oxidative stress. NKR11 cells are more sensitive to 50-400μM doses of H_2O_2 than normal mouse epithelial cells on the MTT-based cellular proliferation and viability assay. NKR11 cells also showed more apoptosis at 24 hrs after H_2O_2 treatment (50-150μM) than the control cells. To examine whether this hypersensitivity is due to defective removal of DNA lesions induced by H_2O_2, we investigated the rate of removal of H_2O_2 induced DNA lesions in NKR11 and control cells, using the single cell gel electrophoresis (comet assay). NKR11 cells removed DNA lesions induced by 50-100μM H_2O_2, as efficiently as the control cell lines, however they were deficient in the removal of lesions induced by 150μM H_2O_2. The comet Assay used with DNA repair enzymes as damage specific probes showed that NKR11 cells were defective in removal of both Endo III and fpg sensitive sites, suggesting that NKR11 cells may have defects in the base excision repair (BER) pathway involved in the removal of these H_2O_2 induced lesions. Defects in BER may play a mechanistic role in cataractogenesis seen in the Nakano mouse.

122 DNA REPAIR GENE EXPRESSION IN MOUSE OOCYTE, ZYGOTE, AND TWO-CELL EMBRYO. Mabery SL[1], Schahin-Reed D[1], Marchetti F[1], Coleman MA[1], Wyrobek AJ[1]. [1]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94551.

DNA repair gene expression during early development is critical to the survival of the embryo. The capacity of oocytes to repair DNA damage in its genome as well as that of the fertilizing sperm is essential to the production of a normal zygote. In mice, the first cell cycle after fertilization is controlled by maternal messages that are accumulated in the egg before fertilization, while during the two-cell stage, embryonic genome activation occurs, both stages are susceptible to DNA damage. The objective of this study is to investigate the expression of DNA repair genes during these early embryonic stages. This study investigates homologous (RAD54 and RAD51) and nonhomologous (Ku80, Ku70, and DNA-PKcs) double-strand break repair genes because of their importance in preventing chromosomal aberrations. We used semi-quantitative RT-PCR to determine relative transcript levels of DNA repair genes in oocytes, zygotes, and 2-cell embryos. Transcripts for RAD54 and RAD51 were detected in all three cell types. The relative levels of transcripts were approximately 10-fold higher in occytes than in zygotes or 2-cell embryos. However, Ku80, Ku70, and DNA-PKcs were detected in two-cell embryos but not in oocytes and zygotes. These findings suggest that there is differential regulation of the expression of repair genes involved in doble-strand break repair in the early stages of mammalian development. [Work performed under the auspices of the U.S. DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48 with support from NIH ES09117-03 and California TRDRP 7RT-0073.]

123 RPA FOCI IN IRRADIATED CELLS AS AN INDICATOR OF UNREPAIRED DNA DAMAGE. MacPhail SH[1], Olive PL[1]. [1]British Columbia Cancer Research Centre, Vancouver, B.C., Canada V5Z 1L3.

Replication Protein A is a single-strand DNA binding protein involved in replication, nucleotide excision repair, mismatch repair, and recombinational repair. We are examining the possibility that RPA foci formation after X-irradiation may be useful as an indicator of the presence of unrepaired DNA damage, and may correlate with loss of cell function. Using fluorescence tagged antibodies, diffuse staining was observed in undamaged nuclei, but with time after irradiation, large discrete complexes were visualized and counted using the particle counting feature of our image analysis software. Exposure of SiHa cervical carcinoma cells to X-rays resulted in a proportional increase in number of RPA foci per nucleus as well as an increase in the percentage of cells with foci, with maximum numbers (up to 150 foci/cell after 50 Gy) observed 4-8 hours after irradiation. Doses as low as 50 cGy produced measurable numbers of RPA foci, and rate of foci development was faster for cells in S phase than cells in G1 phase. After 24 hours, foci numbers began to decrease to background levels (< 4 foci/cell) as cells underwent division. In the first divisions after irradiation, micronuclei were highly enriched for RPA foci, consistent with the idea that foci represent sites of unrepaired DNA damage. In addition, with time after exposure to 4 Gy, RPA foci reappeared in some nuclei. After 3 days, 77% of colonies contained, on average, 20% cells with 40 foci/nucleus, and by 7 days, 90% of colonies contained cells with foci. Development of foci in cells that survived initial damage may be indicative of genomic instability. If foci reappear only in doomed cells, they may also help explain the small colony size observed after exposure to ionizing radiation and the phenomenon called delayed reproductive cell death.

124 MUTATIONS INDUCED BY (-)-ANTI-11R,12S-DIHYDRODIOL, 13S,14R-EPOXIDE OF DIBENZO[A,L]PYRENE IN THE CODING REGION OF THE HPRT GENE IN CHINESE HAMSTER V-79 CELLS. Mahadevan B[1], Dashwood WM[2], Doehmer J[3], Baird WM[1]. [1]Dept. of Environmental & Molecular Toxicology, Oregon State University. [2]Linus Pauling Institute, Oregon State University. [3]Institute of Toxicology & Environmental Hygiene, Technical University of Munich, Germany.

Dibenzo[a,l]pyrene (DB[a,l])P is a polycyclic aromatic hydrocarbon which is an exceptionally potent carcinogen. The secondary metabolite of DB[a,l]P, the fjord region (-)-anti-11R,12S-dihydrodiol, 13S,14R-epoxide (DB[a,l]PDE) was used to investigate the mutations induced in the coding region of the *hprt* gene in chinese hamster V-79 cells. These cells were exposed to 1, 2, 5, and 10 nM DB[a,l]PDE and mutant clones resistant to 6-thioguanine were selected. RNA was isolated from these clones and cDNA was generated for PCR amplification of the *hprt* gene. The nucleotide sequence of the amplified clones were then analyzed for any base changes in the 657 bp fragment. At all doses, base substitutions were the most prevalent mutations observed followed by frame shift mutations and exon deletions. Tandem mutations were mainly observed at 5nM DB[a,l]PDE while a higher frequency of frame shift mutations was found in the 10nM treatment group. There appears to be an increasing frequency of A to T transversions with increasing dose of DB[a,l]PDE. A dose dependent increase of DNA adducts with increasing concentrations of DB[a,l]PDE was also noticed. Thus, the mutational profile at lower concentrations of 2 and 5 nM exhibited a wide spectrum of mutations compared to the higher 10 nM concentration of DB[a,l]PDE.

125 **DNA AND CHROMOSOME DAMAGE BY CHLOROHYDROXYFURANONES IN MAMMALIAN CELLS.** Mäki-Paakkanen J[1], Laaksonen M[1], Komulainen H[1], Vartiainen T[1,2], Hakulinen P[1], Munter T[3], Kronberg L[3]. [1]Division of Environmental Health, National Public Health Institute, P.O. Box 95, FIN-70701 Kuopio, Finland. [2]Department of Environmental Sciences, University of Kuopio, P.O. Box 1627 FIN-70211 Kuopio, Finland. [3]Department of Organic Chemistry, Åbo Akademi University, Biskopsgatan 8, FIN-20500 Turku, Finland.

Chlorohydroxyfuranones (CHFs) are disinfection by-products which may contribute to the cancer risk associated with chlorinated drinking water. Three CHFs, 3,4-dichloro-5-hydroxy-2(5H)-furanone (MCA), 3-chloro-4-(chloromethyl)-5-hydroxy-2(5H)-furanone (CMCF) and 3-chloro-4-methyl-5-hydroxy-2(5H)-furanone (MCF) were studied for the induction of DNA and chromosome damage. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a known genotoxin in bacteria and mammalian cells, was used as a reference substance. The compounds were tested in the alkaline single cell gel electrophoresis (SCGE or Comet) assay for DNA damage, and in the sister-chromatid exchange (SCE) and chromosome aberration (CA) tests using Chinese hamster ovary (CHO) cells. The exposure was one hour in PBS without metabolic activation. All compounds induced DNA damage, SCEs and CAs in a dose-related manner. MCA was, however, a weak inducer of SCEs. The lowest positive dose in inducing DNA damage was 5 μg/ml, 5 μg/ml and 8 μg/ml for CMCF, MCA and MX, respectively. Much, ten times or more, higher concentrations of MCF were needed for a comparable positive Comet response. MX induced SCEs at 0.25-0.75 μg/ml, CMCF at 1-4 μg/ml, and MCF at 22-66 μg/ml. MCA caused a statistically significant increase of SCEs at 1.5 μg/ml. CAs induced by the CHFs were mainly chromatid-type breaks and exchanges. MX caused severe, but still analysable, chromosome damage at ≥1 μg/ml, MCA at about ≥2 μg/ml, CMCF at ≥6 μg/ml, and MCF at about ≥150 μg/ml. The data indicate that MX, MCA, CMCF and MCF all are genotoxic in mammalian cells but there are differences in their potency of genetic activity.

126 **LOCALIZATION OF DOUBLE STRAND BREAK REPAIR PROTEINS DURING THE EARLY STAGES OF MOUSE DEVELOPMENT.** Marchetti F[1], Hwang M[1], Wyrobek AJ[1]. [1]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore,CA 94550.

Analyses of chromosomal aberrations in mouse zygotes have shown that the majority of chromosomal aberrations produced after paternal exposure are of the chromosome-type rather than of chromatid-type, regardless of the mutagen used. This suggests that the double strand repair (DSB) pathway may be involved in the processing of sperm DNA damage. The purpose of this research is to characterize the protein expression of genes belonging to non-homologous end joining (NHEJ) and homologous recombination (HR) pathways during the first two cell cycles of mouse development using immunocytochemistry. B6C3F1 female mice were superovulated and mated with B6C3F1 males. Zygotes and 2-cell embryos were collected 25 h and 44 h after the induction of ovulation, respectively. Strong nuclear staining was observed in zygotes and 2-cell embryos for both Ku80 and Ku70 antibodies (NEHJ pathway). This staining disappeared as the cells neared metaphase. Antibodies for RAD51 (HR pathway) showed a clear nuclear staining in 2-cell embryos, while very little or no staining in zygotes. These results suggest that DSB repair related genes are developmentally regulated, with only genes belonging to the NHEJ pathway being expressed immediately after fertilization, while genes belonging to the HR pathway may not be expressed until the second cell cycle when embryonic genome activation takes place. Investigating the DNA repair capacity of early embryos and the role of specific DNA repair pathways in response to paternal DNA damage is important for understanding the mechanisms that cause abnormal reproductive outcomes. Work performed under the auspices of the U.S. DOE by the LLNL under contract W-7405-ENG-48 with support from NIH ES 09117-03.

127 **GENERATION OF DNA REPAIR-DEFICIENT CHO CELLS EXPRESSING ADH4, THE ESOPHAGEAL ALCOHOL DEHYDROGENASE.** Marietta CA, Brooks PJ. [1]Section on Molecular Neurobiology. [2]Laboratory of Neurogenetics. [3]National Institute on Alcohol Abuse and Alcoholism, NIH. [4]12420 Parklawn Drive. [5]MSC 8110. [6]Bethesda, MD 20892-8110.

According the recently released 9th Report on Carcinogens (USPHS, NTP), alcoholic beverage consumption is now classified as a known human carcinogen, causally related to an increased risk of cancers of the upper gastrointestinal (GI) tract. The mechanisms by which alcoholic beverage consumption increase upper GI cancers are unknown. Cells of the upper GI tract express a specific form of alcohol dehydrogenase called ADH4, which is highly catalytically active in ethanol (EtOH) oxidation. Our hypothesis is that conversion of EtOH to acetaldehyde, a known carcinogen, by ADH 4 contributes to the carcinogenic effect of ethanol consumption. To address this hypothesis, we have generated recombinant CHO cells lines that overexpress the human ADH4. Plasmids encoding the human ADH4 under the control of the CMV promoter and a neomycin resistance cassette were transfected into wild type (AA8) and NER deficient (UV5) CHO cells and clones selected in G418. Several clones of each cell line were isolated and assayed for ADH 4. Transfected clones had levels of ADH 4 in the range of 20-30 micromoles/min/mg protein using 200mM EtOH as a substrate. These values are approximately 10 fold higher than the minimal levels measured in non-transfected parental lines, and are comparable to the activity measured in rat stomach homogenates under the same conditions. Preliminary results to date indicate that UV5 cells expressing ADH4 are more sensitive to the cytotoxic effects of 200mM EtOH than control cells. Ongoing experiments are investigating the mutagenic effects of EtOH in these lines. The ultimate goal is to determine whether intracellular metabolism of EtOH by enzymes expressed in esophageal cells can produce mutagenic damage in mammalian cells.

128 **DATABASE MINING AND GENE-SPECIFIC PCR TO IDENTIFY DNA REPAIR GENES EXPRESSED IN EARLY DEVELOPMENT.** Marsh BJ[1], Mabery S[1], Coleman MA[1], Wyrobek AJ[1]. [1]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA. 94551.

The wealth of public gene expression information has made database mining a potentially valuable tool for characterizing genes and pathways. We conducted searches of the NCBI Expressed Sequence Tag (EST) and the NCI Library Differential Display databases to investigate genes involved in base-excision repair (BER), nucleotide-excision repair (NER), homologous recombination repair (HRR), and non-homologous recombination repair (NHRR) in 2-cell, 8-cell and blastocyst cDNA libraries. Across these databases, genes were inconsistently reported as present or absent and no clear pattern of DNA repair gene expression was apparent for the 2-cell through the blastocyst stages. To address these inconsistencies, we performed gene-specific PCR on 2-cell, 8-cell and blastocyst cDNA libraries. RAD51 and RAD54 transcripts, involved in the HRR pathway, were detected at each cell stage. In contrast, Ku70, Ku80, and DNAPKcs transcripts, involved in the NHRR pathway, were detected in only the blastocyst library. XPA and XRCC1 transcripts, involved in the BER and NER pathways respectively, were detected in each stage tested. Our findings suggest that PCR was more sensitive than database mining for identifying specific genes involved in DNA repair and oxidative stress response and for characterizing the developmental progression of DNA repair pathways that are modulated during early cell stages. [This work was conducted under the auspices of DOE and LLNL under contract W-7405-ENG-48 with support from NIH (ES09117-02) and DOE (KP110202).]