72 THE SHE CELL TRANSFORMATION ASSAY - A PHARMACEUTICAL INDUSTRY PERSPECTIVE. Harvey JS[1]. [1]Safety Assessment, GlaxoSmithKline, Park Road, Ware, Hertfordshire, SG12 0DP, UK.

The Syrian Hamster Embryo (SHE) cell transformation assay is a short-term *in vitro* test for predicting rodent carcinogenicity. The available literature indicates that the SHE cell assay has a high level of concordance (81%) with the 2-year rodent bioassay. Recently the FDA has requested SHE assay data to assess the carcinogenic potential of New Chemical Entities (NCE's) that were positive in one or more standard *in vitro* genotoxicity tests. The re-emergence of the SHE assay has led to considerable debate regarding the suitability and relevance of the assay in the risk assessment of NCE's. For example, several technical issues still exist, such as a lack of objective criteria to define the end point of morphological transformation and the absence of an accepted regulatory assay guideline. Furthermore, the SHE cell assay has still not been subjected to an extensive validation exercise performed to ECVAM standards. More importantly, there is an absence of a mechanistic understanding of the processes that cause morphological transformation in SHE cells. This makes the interpretation of positive SHE cell assay data difficult in terms of carcinogenic risk assessment. For example, as some non-genotoxic, rodent non-carcinogens are positive in the SHE cell assay, there is concern that an NCE could be falsely implicated as a rodent carcinogen. More suitable approaches exist to evaluate the relevance of positive *in vitro* genotoxicity findings. Given the issues that surround the SHE cell assay it is premature to use a simple empirical correlation of the assay's concordance with the 2-year rodent bioassay alone as basis for regulatory decisions. Clearly more research is required before the role of the SHE cell assay in the drug development process can be accurately assessed.

73 NOVEL TRANSCRIPTIONAL REPORTERS FOR THE HUMAN *GADD45* GENE REVEAL THE CRITICAL IMPORTANCE OF DOWNSTREAM ELEMENTS IN MAXIMUM RESPONSE TO GENOTOXIC STRESS. Hastwell PW[1], Walmsley RM[1]. [1]Department of Biomolecular Sciences, The University of Manchester, PO Box 88, Manchester, M60 1QD, UK.

The *GADD45* alpha gene is transcriptionally induced in response to a wide spectrum of genotoxins. Regulation of the gene is complex and involves BRCA1 and both p53-dependent and –independent pathways. Gadd45 interacts with a number of important proteins, including PCNA, p21, core histones and Cdc2. The protein is thought to play a role in apoptosis and cell cycle checkpoints in the response to genotoxic stress and there is evidence that it is involved in global nucleotide excision repair. Studies of knockout mice confirm that GADD45 has an important role in the maintenance of genomic stability. *GADD45* promoter-reporter fusions (CAT/luc) constructed by other laboratories have been used to reveal that p53-independent induction is controlled via BRCA1 binding to the *GADD45* promoter. This region of the promoter has been shown to have a relaxed chromatin structure, poising the gene for transcriptional upregulation. A second region of relaxed chromatin structure is found around the third intron of the *GADD45* gene. This region contains a putative p53 response element. It has been shown that p53 can contribute to transcriptional activation at the *GADD45* promoter via interaction with WT1. It has been suggested that p53 may also bind directly to the third intron, though the consequence of this has not been explored. We have constructed novel *GADD45* reporters in which the promoter, in combination with various gene fragments downstream of 'start', controls expression of *EGFP*. GFP assays in two human lymphoblastoid cell lines (wt and mutant for p53) have revealed the essential role of the third intron for maximum transcriptional induction of *GADD45* following genotoxic stress. The simple assays provide a readout for GADD45 which have potential applicability in pre-regulatory genotoxicity screening.

74 A YEAST MODEL OF FRIEDREICH'S ATAXIA: GENOTOXICITY OF MITOCHONDRIAL IRON ACCUMULATION. Haugen AC[1], Karthikeyan G[1], Collins JB[2], Tucker CJ[2], Resnick MA[1], Van Houten B[1]. [1]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, U.S.A. [2]National Center for Toxicogenomics, Microarray Center, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, U.S.A.

Friedreich's Ataxia, an autosomal recessive disorder, results in neurodegenerative disease and cardiomyopathy. The *YFH1* gene is the Saccharomyces cerevisiae homologue of the human FRDA gene encoding the frataxin protein. Cells lacking *YFH1* exhibit 1) accumulation of iron, which cannot be extruded from the mitochondria; 2) oxidation of proteins; 3) oxidative DNA damage, which leads to petite colony formation with defects or loss of mitochondrial DNA and 4) nuclear chromosomal damage (Human Mol. Gen. 12:3331-3342, 2003). The cellular impact of mitochondrial iron overload in yeast was determined by global gene expression profiling in a *yfh1*α deletion mutant with defective mitochondrial function and no mitochondrial DNA (i.e., rho^0). Expression data mapped onto the yeast network of 20,985 protein-protein/protein-DNA interactions revealed YDR036C, Hap4, MRPS5, Cox9, and Cad1 as important centers of activity. The mostly downregulated cytochrome, heme, and iron/sulfur cluster assembly pathways, indicate that frataxin has a role in iron transport, iron/sulfur cluster biosynthesis, oxidative phosphorylation, and as an antioxidant. Interestingly, Grx5, a mitochondrial glutaredoxin involved in the synthesis of iron/sulfur clusters, was downregulated in the *yfh1*α rho^0 strain. In fact, the *yfh1*α rho^0 and *grx5*α expression profiles as compared to a rho^0 strain are comparable (J. Biol. Chem. 279:12386-95, 2004). Both profiles reveal the upregulation of the iron regulon as well as the downregulation of cytochromes and the Hap4 regulon. However, the *yfh1*α profile shows a large number of downregulated mitochondrial ribosomal proteins, not observed for the Grx5 mutant. Transcription profiling data for the gradual reduction of frataxin protein to levels comparable to human disease cells will also be presented.

75 HUMAN S CHECKPOINTS: MULTIPLE MECHANISMS INHIBIT REPLICON INITIATION AFTER DNA DAMAGE. Kaufmann WK[1], Heffernan T[1], Unsal-Kacmaz K[2], Heinloth A[3], Sancar A[2], Paules RS[3], Cordeiro-Stone M[1]. [1]Department of Pathology and Laboratory Medicine, UNC-CH, Chapel Hill, NC 27599. [2]Department of Biochemistry and Biophysics, UNC-CH, Chapel Hill, NC 27599. [3]National Institute of Environmental Health Sciences, RTP, NC 27705.

The S checkpoint is activated in response to diverse forms of DNA damage and slows the rate of DNA synthesis by inhibiting the initiation of new replicons. The ATM-dependent S checkpoint induced by ionizing radiation (IR) appears to inhibit replicon initiation by stimulating the proteolysis of the Cdk2-activating phosphatase Cdc25A. Previously, we have shown that the S checkpoint activated by ultraviolet radiation (UVC) is dependent on the transducer kinase ATR. Current studies were undertaken to characterize further the signaling pathway that inhibits replicon initiation in UVC-damaged cells. Immunocomplex kinase assays revealed a 50% inhibition of Cdk2/cyclin E activity when normal human fibroblasts were treated with a low dose of IR. The degree of inhibition correlated with a significant decrease in Cdc25A protein expression. In contrast, a sublethal dose of UVC (1 J/m2), which selectively inhibits replicon initiation by 50%, failed to inhibit Cdk2/cyclin E kinase activity or degrade Cdc25A. Pretreatment of cells with the proteasome inhibitor LLnL fully reversed the IR-induced S checkpoint, but had no effect on UVC-induced inhibition of DNA synthesis. Cdc25A degradation and marked Chk1 phosphorylation were observed after irradiating cells with a supralethal dose of 20 J/m2, suggesting that UVC-induced degradation of Cdc25A may be a high-dose phenomenon aassociated with activation of the replication checkpoint. Chk1 and Dbf4 were shown to physically interact *in vivo* and Dbf4 was a substrate for Chk1-dependent phosphorylation *in vitro*, implicating this essential kinase as a target of Chk1 in the UVC-induced S checkpoint. Current studies are focused on elucidating the significance of Chk1-dependent phosphorylation of Dbf4. Supported by NIH grants ES11012, CA55065 and ES10126.

76 A POLYMORPHISM IN THE DNA REPAIR GENE MGMT INCREASES HUMAN SENSITIVITY TO THE TOBACCO-SPECIFIC NITROSAMINE NNK. Hill CE[1], Affatato AA[1], Wolfe KJ[1], Galbert LA[1], Wickliffe JK[1], Abdel-Rahman SZ[1]. [1]Department of Preventive Medicine & Community Health, Division of Environmental Toxicology, University of Texas Medical Branch, Galveston TX, 77555-1110.

Efficient DNA repair is critical for the protection of cells from carcinogenic agents present in tobacco smoke. Many polymorphisms in several DNA repair genes have been associated with increased risk of cancer. O6-Methylguanine-DNA-Methyltransferase (MGMT) is a direct reversal DNA-repair protein that plays an important role in protecting the cell from mutations resulting from exposure to alkylating mutagens such as those in tobacco smoke. We propose that inherited polymorphisms in the coding region of MGMT, which result in amino acid substitutions, may significantly influence the level of smoking-induced genetic damage, a critical step in the cascade of events leading to cancer. We tested the hypothesis that the inheritance of the Leu84Phe polymorphism in MGMT is associated with increased genetic damage resulting from exposure to alkylating agents found in tobacco smoke. We used the mutagen sensitivity assay, with the tobacco-specific nitrosamine NNK as a model alkylating mutagen, to test this hypothesis. Lymphocytes obtained from 106 healthy volunteers were exposed *in vitro* to NNK and the genotoxic response was measured by assessing the increase in chromosome aberration (CA) frequency. A significant ($P=0.004$) increase in NNK-induced CA (total breaks/100 cells) was observed in cells from individuals with the Phe allele (2.29 ± 0.31) compared to cells from individuals homozygous for the wild-type Leu allele (4.0 ± 0.65). These data suggest that the inheritance of the Phe allele may affect the repair efficiency of genetic damage induced by NNK. These data may also provide a partial mechanistic explanation for previously reported findings that indicate an association between this polymorphism and increased risk of cancer. (Supported by an External Research Program grant from Philip Morris Inc)

77 TISSUE-SPECIFIC TIME COURSES OF SPONTANEOUS MUTATION FREQUENCY AND DEVIATIONS FROM THE CORE MUTATION PATTERN ARE OBSERVED IN MIDDLE TO LATE ADULTHOOD IN BIG BLUE® MICE. Hill KA[1,2], Farwell KD[1], Longmate J[3], Scaringe WA[4], Wang J[1], Sommer SS[1]. [1]Department of Molecular Genetics, City of Hope National Medical Center. [2]Department of Biology, The University of Western Ontario, London, ON Canada N6A 5B7. [3]Division of Information Sciences, City of Hope, National Medical Center. [4]Bioinformatics Group, Department of Molecular Diagnosis, City of Hope, Duarte, CA 91010.

To better define the time course of spontaneous mutation frequency in middle to late adulthood, we now report measurements at 10, 14, 17, 23, 25 and 30 months of age, in samples of adipose tissue, liver, neurons and male germline from Big Blue® mice. The data improve the resolution of the profile of mutation frequency in multiple tissues over the lifespan of the mouse and confirm (i) the previously observed occurrence of at least two tissue-specific profiles of spontaneous mutation frequency (constancy in neurons and male germ cells and elevation with age in liver and adipose tissue), (ii) a low mutation frequency in the male germline, and (iii) a constancy of mutation pattern with age within a tissue. These findings appear to extend to old age (30 mo). Additional findings include subtle, but highly significant differences in the mutation pattern between some tissues ($p=0.0002$), consistent with a minor effect of tissue-specific metabolism and larger inter-animal variation in spontaneous mutation frequency in liver and adipose tissues. The presumptive unaltered balance of DNA damage and repair with age in the germline has evolutionary consequences, is unexpected through old age, and is of particular interest given the controversy over whether or not increasing germline mutation frequency with paternal age is the cause of reports associating older fathers with a higher incidence of some types of genetic disease. These most detailed measurements available to date of the time course of spontaneous mutation frequency in individual tissues help to constrain hypotheses regarding the role of mutational mechanisms in DNA repair and aging.

78 PROTECTIVE EFFECT OF YEAST MAJOR AP ENDONUCLEASE APN1 EXPRESSION IN A MAMMALIAN NEURONAL CELL LINE. Ho R[1], Rachek LI[1], Xu Y[2], Kelley MR[2], LeDoux SP[1], Wilson GL[1]. [1]University of South Alabama, Mobile, AL 36688. [2]Indiana University, Indianapolis, IN 46202.

Reactive oxygen species (ROS) has been implicated in many types of cancer and neurodegenerative disorders. Mitochondria are the major source of endogenous ROS, and mitochondrial DNA (mtDNA) is prone to oxidative damage due to the lack of protective histones and its close proximity to the site of ROS generation. Our lab has previously shown that, by enhancing mtDNA repair in cells overexpressing certain glycosylases targeted to the mitochondrion, cell survival can be increased. Therefore, the purpose of this study was to enhance mtDNA repair and cell survival by targeting a different DNA repair enzyme to mitochondria. The yeast major apurinic/apyrimidinic (AP) endonuclease, Apn1, is a more efficient DNA repair enzyme than the major AP endonuclease in mammals. Apn1 was stably transfected into neurons derived from adult rat substantia nigra with or without a mitochondrial targeting signal (MTS). Cells were exposed to menadione for 1 hr and mtDNA and total DNA damage was analyzed by quantitative Southern blot and single cell gel electrophoresis (Comet assay), respectively. Short term (24 hr) and long term (10 day) cell survival was assessed also. Interestingly, our results showed that mtDNA repair was more efficient in cells expressing Apn1 compared with vector controls. Total DNA damage was less extensive in Apn1 expressing cells. Both Apn1 expressing cells also showed better cell survival than vector controls. These results demonstrate that targeting Apn1 to both the nucleus and mitochondria can enhance DNA repair and cell survival from oxidative stress.

79 INDUCTION OF A MUTATOR PHENOTYPE IN INFLAMED TISSUES. Hofseth LJ[1,2], Khan MA[1], Ambrose M[3], Kartalou M[3], Hussain SP[1], Samson LD[3], Harris CC[1]. [1]Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20892. [2]College of Pharmacy, University of South Carolina, Columbia, SC 29208. [3]Biological Engineering Division, MIT, Cambridge, MA 02139.

Chronic infection and associated inflammation is a key contributor to human carcinogenesis. Ulcerative colitis (UC) is an oxyradical overload disease and is characterized by free radical stress and colon cancer proneness. We examined tissues from non-cancerous colons of ulcerative colitis patients to determine (a) the activity of two base excision repair enzymes, AAG, the major 3-methyladenine DNA glycosylase, and APE1, the major apurinic site endonuclease, and (b) the prevalence of microsatellite instability (MSI). AAG and APE1 were significantly increased in the UC colon epithelium undergoing elevated inflammation, and microsatellite instability was positively correlated with their imbalanced enzymatic activities. These latter results were supported by mechanistic studies using yeast and human cell models in which over-expression of AAG and/or APE1 was associated with frameshift mutations and MSI. Our results are consistent with the hypothesis that the adaptive and imbalanced increase in AAG and APE1 is a novel mechanism contributing to MSI in patients with UC, and may extend to other chronic inflammatory diseases, or those with MSI of unknown etiology.

80 THE FATE OF CHROMOSOMAL DOUBLE STRAND BREAKS IN HUMAN CELLS. Honma M[1], Sakuraba M[1], Koizumi T[1], Hayashi M[1]. [1]National Institute of Health Sciences, Tokyo, Japan 158-8501.

We recently developed a system to trace the fate of chromosomal double strand breaks (DSBs) occurring in human genome (Honma et al., EMM 42, 288-298, 2003). The lymphoblastoid cell lines TSCE5 and TSCER2 are heterozygous (+/-) or compound heterozygous (-/-) for the thymidine kinase gene (TK), respectively, and were introduced an I-SceI endnuclease site into the gene. End-joining (EJ) for a DSB occurring at the I-SceI site results in TK-deficient mutants in TSCE5 cells, while the homologous recombination (HR) between the alleles produces TK-proficient revertants in TSCER2 cells. We demonstrated that almost all DSBs were repaired by EJ resulting simple deletions or DNA rearrangements, and HR rarely contributes to the repair of DSBs in this system. The phenotypic TK-selection may, however, be biased to detect large genomic changes and can not detect small deletion and small tract gene conversion. Introducing highly efficient DSBs into the cells using NucleofectorTM (Amaxa) made it possible to rescue mutants by the DSBs without the phenotypic selection. The TK-mutant frequency of TSCE5 in the new system was over 1%. We analyzed over 800 non-selected clones, and approximately 4% of the clones had a mutation involving I-SceIi site. Seventy percent of these mutations were small deletions ranging from 1 to 20bp, and others were over 20bp deletions and small DNA rearrangements. No gene conversion was observed. These results supports an idea that majority of DSBs were repaired by EJ resulting small deletion. Error-free EJ mechanism may be possible. Because this system can efficiently make a DSB at a specific site in human genome, it promises for tracing the fate of DSB at a cellular level.

81 DEVELOPMENT OF MICROWELL SYRIAN HAMSTER EMBRYO (SHE) CELL MICRONUCLEUS (MN) ASSAY. Hu T[1], Gibson DP[1], Aardema MJ[1]. [1]Procter & Gamble, Miami Valley Laboratories, P.O. Box 538707, Cincinnati, OH 45252.

The SHE cell transformation assay has superior concordance for predicting rodent carcinogenicity of aromatic / nitroaromatic amines, while standard *in vitro* genotoxicity assays perform poorly with aromatic amines (AAs). For example, 71 % of non-carcinogenic AAs were positive in the Ames assay (Ashby and Tennant Mut. Res 257, 229-306 1991). The SHE cell transformation assay is limited in use due to the cost, time and technical training required to score morphological transformation. To address these issues, we are developing a microwell micronucleus assay in SHE cells to use as a screening assay. Use of microchamber slides will result in a faster, cheaper assay requiring less testing material, which is ideal for screening purposes. Treatment of SHE cells from passage 1 through 3 for 4 h with Mitomycin C (MMC) followed by 20 h recovery period induced a dose dependent increase in MN, that was similar in 8 well microchambers, 4 well microchambers and T25 flask. Benzo(a)pyrene and 2-Amino-4-nitrotoluene, an aromatic amine, also induced a significant increase in MN. For all test chemicals, results were similar in the presence and absence of 3 ug/ml Cytochalasin B. Further validation of the SHE microwell MN test is underway.

81A IRON OVERLOAD AS A RISK FACTOR AND MOLECULAR MECHANISM OF CARCINOGENESIS. Huang X[1]. [1]Department of Environmental Medicine, NYU School of Medicine, 550 First Avenue, New York, NY 10016

Iron is an essential element for human life. Iron deficiency anemia is a common condition known to the medical profession for centuries. In contrast, iron overload is mistakenly believed to be rare. Iron overload is an increase in total body iron generally exceeding 5 g. Iron overload primarily results from a deregulation of intestinal iron absorption, as in hereditary hemochromatosis, which is due to a point mutation in the HFE gene. Among Caucasians, prevalence of homozygotes carrying the HFE gene mutation is estimated to be 0.5-0.8%, while heterozygotes can be as high as 10%. Although iron stored in iron-containing proteins, such as ferritin, is not readily bioavailable to contribute to the adverse health effects, iron bound to low molecular weight (LMW) chelators, such as citrate and ATP, can have harmful consequences. In this presentation, I am going to show that LMW iron can induce an early signaling pathway that modulates the levels of activator protein-1 (AP-1), an important transcription factor in response to oxidative stress. Iron can stimulate mitogen-activated protein kinase (MAPK) family members of extracellular signal-regulated kinases (ERKs) and p38 MAPK but not c-jun NH2 terminal kinases (JNKs). Iron-induced AP-1 activation may be involved in the up-regulation of interleukin-6, a pro-inflammatory cytokine that can be both mitogenic (cell proliferation) and fibrogenic (extracellular matrix synthesis). I will also provide evidence showing that iron may be involved in hypoxia signaling. Because the murine HFE gene is structurally similar to the human HFE gene, the HFE knockout mice can exhibit relative sparing of iron loading and alteration in inflammatory cytokines. I will also present some evidence that iron overload can contribute to cancer development.

81B MUTAGENESIS VIA MISTRANSLATION. Humayun MZ[1]. [1]University of Medicine and Dentistry of New Jersey - New Jersey Medical School, Department of Microbiology and Molecular Genetics, International Center for Public Health E230P, Newark, NJ 07101-1709

Mutator phenotypes usually arise from defects DNA replication, repair, or recombination pathways. However, recent findings indicate that expression of a mutant glycine tRNA induces a strong mutator phenotype in *Escherichia coli*. Follow-up investigation showed that a number of genetic conditions, and transient environmental variables (such as antibiotic exposure) known to increase mistranslation also induce similar mutator phenotypes. These phenotypes are not mediated by known inducible pathways such as the SOS pathway, and their full expression appears to require *recABC*-mediated homologous recombination functions. The mutator phenotypes, termed TSM for translational stress-induced mutagenesis, appear to be mediated by a modified version of DNA polymerase III (pol-III). These findings imply the existence of previously unappreciated links between translation, replication, and recombination, and raise the possibility that mutator phenotypes with significance for cell survival, adaptation, cancer initiation and aging can arise through defects in a large repertoire of target genes, environmental conditions, and physiological states.

82 **RADICAL CAUSES OF HUMAN CANCER.** Hussain SP[1], Hofseth LJ[1], Wogan G[1], Wang XW[1], Harris CC[1]. [1]Laboratory of Human Carcinogenesis, CCR, NCI, NIH, Bethesda, MD 20892-4255 USA.

Free radicals are ubiquitous in our body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms. Because free radicals can also inflict cellular damage, several defenses have evolved both to protect our cells from radicals–such as the p53 pathway and antioxidant scavengers and enzymes–and to repair DNA damage. Free radicals can cause an adaptive increase in certain protective base excision repair enzymes. Paradoxically, if the increase in enzymes is imbalanced, e.g., the DNA glycosylase is increased more than the apurinic endonuclease, frameshift mutations occur as a novel etiology of microsatellite instability. Understanding the relationship between chronic inflammation and cancer provides insights into the molecular mechanisms involved. In particular, we highlight the interaction between nitric oxide and p53 as a crucial pathway in inflammatory-mediated carcinogenesis.

83 **MULTI-LABORATORY VALIDATION OF A FLOW CYTOMETRIC MICRONUCLEUS ASSAY: METHOTREXATE RESULTS: ADDITIONAL RAT PERIPHERAL BLOOD ANALYSIS IN STANDARD AND NON-STANDARD VEHICLES.** Hynes GM[1], Lynch AM[1], Torous DK[2]. [1]Genetic Toxicology Dept., GlaxoSmithKline, Park Rd, Ware, Herts, SG12 0DP. UK. [2]Genetic Toxicology Dept., GlaxoSmithKline, Park Rd, Ware, Herts, SG12 0DP. UK. [3]Litron Laboratories, Rochester, NY, USA.

An international, multi-laboratory research validation study is currently ongoing to validate the flow cytometry (FCM) Mn test. The goal of the trial is to support the validation and regulatory approval of the FCM method by ICCVAM. The results of Phase 1 of the collaborative trial were published (Torous et al, 2001) and confirmed FCM set-up and calibration parameters using a common biological standard and protocols. Here we report our contribution to Phase II, comparing Mn frequencies obtained in mouse peripheral blood by flow cytometry with manual scoring (using acridine orange staining). Mice were treated with methotrexate (0.5, 1, 2 and 4 mg/kg ip) or vehicle daily for 3 days and peripheral blood sampled 24 hours after the final dose to determine Mn frequency. Methotrexate induced a dose dependent increase in reticulocyte Mn frequencies by flow cytometry and by manual scoring and overall, there was good concordance between both methods of analysis. This data supports the conclusions of the Collaborative Study Group for the Micronucleus Test (Wakata et al, 1998) that the mouse peripheral blood micronucleus test may be integrated into routine toxicology assays which assess the acute toxicity of chemicals with appropriate dosing. In addition, we have evaluated reticulocyte Mn frequencies in rat peripheral bloods from animals treated with various standard and non-standard vehicles and compared the FCM results with manual scoring using AO staining. The results of our studies provide additional background data regarding the FCM Mn test.

84 **AN AUTOMATED APPROACH TO COMET ASSAY ANALYSIS.** Jackman SM[1]. [1]886 Chestnut Ridge Rd, 6th Floor, Morgantown, WV 26505.

The single cell gel electrophoresis assay (SCGE, comet assay) has been established as a direct visual and quantitative method for evaluation and detection of DNA damage in individual cell populations based on the extracellular extrusion of DNA fragments. Manual scoring of the cell populations is a labor intensive process that impacts the accuracy and reliability of the comet measurements. In an effort to increase throughput and repeatability of comet assay scoring, we previously developed the AutoComet™, a fully-integrated computer controlled optical microscopy system optimized for complete unattended scoring of comet assay parameters. The objective of the current study was to evaluate the capabilities, reliability and sensitivity of the AutoComet™ while demonstrating the benefits of utilizing an automated system for data acquisition. A series of *in vitro* (human peripheral lymphocytes and Chinese Hamster Ovary cells) and *in vivo* (male Wistar rats) experiments were performed with known genotoxic positive control agents (ethyl methanesulfonate, cyclophosphamide, hydrogen peroxide) for comparative analysis. No significant differences were found in the repeatability of the automated acquisition or in the sensitivity of scoring the comet assay parameters. A comparative analysis of the high-throughput AutoComet™ data acquisition and the data collected by visual classification scoring or computer-assisted scoring, demonstrated similar trends in analysis of comet assay parameters.

85 **THE ROLE OF THE SHE CELL TRANSFORMATION ASSAY IN DRUG DEVELOPMENT.** Jacobson-Kram D[1]. [1]Office of New Drugs, Center for Drug Evaluation and Research, FDA Rockville, MD 20852.

Results from carcinogenicity studies are generally not available for drugs until the time of approval. Many people, including healthy volunteers may have been exposed to pharmacologically active doses of the drug before carcinogenicity results are available. FDA's Center for Drug Evaluation and Research uses results of genetic toxicology studies as a surrogate for carcinogenicity during the drug development phase (clinical trials). A number of issues are considered in deciding whether drugs which give positive results in genetic toxicology studies can be given to subjects in clinical trials. This relates to the drug indication, the target population, duration of treatment and importance of the drug. In general, single dose clinical studies are permitted regardless of the genetox results. Some review divisions will require sponsors to perform a SHE cell transformation assay or a p53 carcinogenicity study prior to approving repeat dose clinical trials. The utility of the SHE cell transformation assay in drug development has been controversial. Published data on chemicals in general suggest a good correlation between results in the SHE assay and 2-year carcinogenicity studies in rats and mice. Results from an International Life Sciences Institute (ILSI) validation effort on human pharmaceuticals, suggested that the SHE assay is less predictive for human carcinogenic risk. The ILSI study found that the SHE assay had high sensitivity (83%) for detection of human carcinogens. However, its low specificity (15%) for prediction of "putative human noncarcinogens" led to a poor overall concordance of 37%. This symposium is designed to examine the utility of the SHE assay as a surrogate for carcinogenicity in the drug development process.

86 MODULATION OF CYP1A1 AND CYP1B1 EXPRESSION BY CHLOROPHYLLIN IN NORMAL HUMAN MAMMARY EPITHELIAL CELLS EXPOSED TO BENZO(A)PYRENE. John K[1,2], Keshava C[2], Divi RL[3], Whipkey DL[2], Poirier MC[3], Weston A[1,2], Nath J[1]. [1]Genetics & Development Biology Program, West Virginia University, Morgantown, WV 26506. [2]Toxicology & Molecular Biology Laboratory, NIOSH/CDC, Morgantown, WV 26505. [3]Carcinogen-DNA Interactions Section, NCI/NIH, Bethesda, MD 20852.

Benzo(a)pyrene (BP) is a ubiquitous procarcinogen that induces various cytochrome P450s including steroid hydroxylases. In human tissues it may perturb hormonal metabolism or be converted to metabolites that damage DNA. Using normal human mammary epithelial cells, we previously showed inter-individual variation for induction of CYP1A1 and CYP1B1 enzymes by BP. To investigate mitigation of CYP1A1 and CYP1B1 induction, a panel of 10 primary normal human mammary epithelial cell strains was exposed to BP in the presence of chlorophyllin. Cell strains were derived from tissue discarded at reduction mammoplasty obtained through the Cooperative Human Tissue Network (National Cancer Institute and National Disease Research Interchange sponsored). Each cell strain was subjected to four separate 24h treatment protocols: BP (4μM) alone, BP plus chlorophyllin (5μM), chlorophyllin pretreatment (24h) followed by BP, and chlorophyllin pretreatment followed by BP plus chlorophyllin. Total RNA extracted from each treatment group was reverse transcribed, and the expression levels of CYP1A1 and CYP1B1 were monitored using real time polymerase chain reaction (RT-PCR). Maximum induction of both CYP1A1 (7 to 96-fold) and CYP1B1 (8 to 43-fold) was observed in the group treated with BP alone. Minimal induction of CYP1A1 (2 to 54-fold) and CYP1B1 (3 to 39-fold) was observed in the group pretreated with chlorophyllin followed by BP plus chlorophyllin. Further studies will examine the impact of chlorophyllin treatment on BP-DNA adduct formation in normal human mammary epithelial cells. These studies show substantial inter-individual variations in CYP1A1 and CYP1B1 induction subsequent to BP exposure, as well as inter-individual variation in response to the chemopreventive agent chlorophyllin.

87 CHROMIUM PICOLINATE DOES NOT PRODUCE CHROMOSOME DAMAGE IN THE *IN VITRO* MAMMALIAN CHROMOSOME ABERRATION TEST WITH CHO CELLS. Juturu V[1], Slesinski RS[2], Gudi R[3], San R[3], Komorowski JR[1]. [1]Division of Technical and Scientific Affairs, Nutrition 21, Inc., Purchase, NY 10577. [2]Environ Health Sciences Inst., Arlington, VA 22203. [3]BioReliance, Rockville, MD 20850.

Chromium Picolinate (CrPic) is a dietary supplement in use for the past two decades that has shown clinical benefits for diabetes and associated risk factors. In this study, CrPic (Chromax®) was tested for its ability to induce chromosomal aberrations using Chinese hamster ovary cells (CHO). The cells were treated for standard time points of 4 and 20 hours in the absence of S9 activation and for 4 hours in the presence of S9 activation. CrPic was solubilized with dimethyl sulfoxide (DMSO) to attain the highest possible soluble test doses. The cells were treated with 96.5, 192.5, 385 or 770 μg/ml for 4 hours in the presence of metabolic activation and for 4 and 20 hours in the absence of metabolic activation. A distinct precipitate of CrPic was evident in the cell culture medium at 770 μg/mL. Higher doses were not tested according to recommendations in international guidelines. The data indicate that no statistically significant increase in structural or numerical chromosome aberrations was produced at any test dose level in the 4-hour treatments up to a precipitating dose of 770 μg/mL in both presence and absence of S9 metabolic activation systems, and up to 385 μg/mL (the maximum testable dose based upon observation of 52% mitotic inhibition) for 20 hours in the absence of S9 metabolic activation. The percentage of cells with structural or numerical aberrations in CrPic treated groups was not statistically different from controls at any dose level ($p<0.05$). These results demonstrate that Chromax® CrPic was negative for the induction of structural and numerical chromosome aberrations in CHO cells.

88 FUNCTIONAL POLYMORPHISMS IN WERNER SYNDROME PROTEIN. Kamath-Loeb A[1], Welcsh P[2], Loeb LA[1]. [1]Department of Pathology, University of Washington, Seattle, WA 98195-7705. [2]Dept. Medical Genetics, University of Washington, Seattle, WA 98195-7720.

The Werner syndrome (WS) gene product encodes a protein (WRN) of 1432 amino acids that belongs to the RecQ family of DNA helicases. In addition to its unwinding activity, WRN is unique among the RecQ helicases in that it also encodes a 3'->5' exonuclease. WRN interacts with a spectrum of proteins and these interactions may delineate its role in DNA metabolism. All known mutations in *WRN* generate stop codons or truncations that abolish protein expression. These mutations are causally associated with Werner syndrome (WS), a rare recessive disorder characterized by premature aging and genomic instability. In addition to WS-linked mutations, polymorphic amino acid substitutions have also been identified in the coding sequence of*WRN*. We have initiated studies to determine if any of the known polymorphisms affect the enzymatic activities of WRN. Two of the common poymorphisms, F1074L and C1367R, and two infrequent polymorphisms Q724L and S1079L, exhibit little change in activity relative to wild-type WRN; the polymorphism, T172P, shows a small but consistent reduction of activity. An infrequent polymorphism, R834C, in the consensus helicase motif V dramatically reduces both WRN helicase and exonuclease activity. By analogy with the crystal structure of *E. coli* RecQ, substitution of WRN R834 with cysteine could alter the hydrolysis of ATP; this has been confirmed experimentally. Genotypic analysis of DNA from over 1500 individuals indicates that R834C is preferentially present in individuals of Spanish ancestry. R834C is the first substitution described that nearly abolishes WRN activity while leaving protein expression largely unaffected.

89 NO MAJOR ROLE FOR 8-OXOGUANINE IN UVA-MUTAGENESIS. Kappes UP[1], Rünger TM[1]. [1]Department of Dermatology, Boston University School of Medicine, Boston, MA 02118.

Oxidative DNA damage, in particular 8-oxoguanine (8-oxoG), has been suggested to mediate mutation formation following exposure to long-wave ultraviolet light (UVA). It is processed primarily by base excision repair (BER). The initial repair step is the removal of 8oxoG by the damage-specific 8-oxoG DNA glycosylase (OGG1). In order to study the contribution of 8-oxoG to UVA-mutagenesis, we compared UVA- and UVB-induced mutation frequencies in mouse embryonal fibroblasts from OGG1-knockout mice and their OGG1-intact littermates, using the ouabain-mutagenesis assay. OGG1-knockout cells did not exhibit an increased frequency of UV-induced mutations, as compared to OGG1-intact cells, either with UVA, or UVB ($n=3$). This indicates that 8-oxoG, which is processed by OGG1, does not contribute significantly to either UVA- or UVB-mutagenesis. This is consistent with our results from sequencing 90 UVA- and UVB-induced hprt-mutations in primary human fibroblasts. There we find that G to T transversions, considered signature mutations for 8-oxoG, constituted only 13 % of UVA-induced point mutations. This low frequency is similar to what we found in UVB-induced mutations (15 %). Our data also shows that C to T transitions, including CC to TT tandem mutations, are the most common type of mutation not only with UVB (58 %), but also with UVA (48 %). These C to T transitions are considered signature mutations for pyrimidine dimers, which therefore appear to be major contributors not only to UVB-mutagenesis, as is well established, but also to UVA-mutagenesis. In addition, UVB- and UVA-induced C to T transitions were located at largely the same sites and hot spots, suggesting that UVA- and UVB-induced pyrimidine dimers are being formed through the same or similar mechanisms of DNA damage formation.

90 NICKEL-INDUCED CHROMATIN DAMAGE. Kasprzak KS[1].
[1]Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, MD 21702.

The molecular mechanisms of nickel carcinogensis involve both genotoxic and epigenetic effects. Over the last years we have been testing a hypothesis that the mechanisms would include damage to major chromatin components such as DNA, histones, and protamines, mainly through direct Ni(II) interactions with the latter two. Our investigations led to identification of Ni(II)-binding amino acid motifs: -CAIH- in histone H3, -TESHHK- in histone H2A, and RTH- in protamine P2. Using peptide models, we determined structural and redox properties of Ni(II) complexes with these motifs. The Ni(II)-CAIH complex was redox-active and enhanced promutagenic oxidative DNA damage. In contrast, the Ni(II)-TESHHK- complex lacked redox activity. However, Ni(II) in the latter mediated hydrolysis of the peptide bond between the Glu and Ser residues, yielding a new redox-active Ni(II)-SHHK- complex. The hydrolysis of H2A at the -TESHHK- motif, resulting in the truncation of its C-terminal tail, was also observed in Ni(II)-treated cells in culture. The binding to the N-terminal RTH- motif of protamine P2 enhanced Ni(II) capacity to mediate oxidative damage to the protamine itself and to DNA. In addition, we found a long-range structuring effect of Ni(II) coordinated by this motif on P2 folding that explained alterations in P2 association with DNA and site-specificity of Ni(II)-dependent P2 oxidation. In conclusion, Ni(II) may be bound and redox-activated by some chromatin proteins, or their degradation products, in somatic and sperm cells. Bound Ni(II) may also enhance peptide bond hydrolysis. These effects, damaging to the molecular components and structure of chromatin, may alter the fidelity of DNA replication and gene expression, and thus facilitate carcinogenesis, including paternally-mediated cancer.

91 DETECTION OF P53 AND K-RAS MUTATIONS IN SPUTUM OF NONSMOKING WOMEN EXPOSED TO SMOKY COAL COMBUSTION EMISSIONS IN XUAN WEI COUNTY, CHINA. Keohavong P[1], Lan Q[2], Gao WM[1], Zheng KC[1], Mady H[1], Melhem M[1], Mumford JL[3]. [1]University of Pittsburgh, Pittsburgh PA 15260. [2]National Cancer Institute, Bethesda, Maryland 20892. [3]U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Lung cancer mortality rates among Xuan Wei County population are among the highest in China and are associated with exposure to indoor emissions from burning of smoky coal. Previous studies of lung tumors from nonsmoking women and smoking men in this region showed high frequencies of mutations, mostly G to T transversions, in the p53 tumor suppressor gene and K-ras oncogene, suggesting these mutations were caused primarily by polycyclic aromatic hydrocarbons. In this study, sputum samples from 92 individuals with no evidence of lung cancer from Xuan Wei County were screened for p53 and K-ras mutations. Sputum cells were collected on a filter membrane by sputum cyto-centrifugation, stained, and histopathologically analyzed. Non-malignant epithelial cells were taken from each sputum sample, using a laser capture microdissection microscope, and molecularly analyzed. Epithelial cells taken from sputum of 15 (16.3%) individuals were mutation-positive, including thirteen (14.1%) individuals with each a p53 mutation, one (1.1%) individual with a K-ras mutation, and one (1.1%) individual with a p53 and a K-ras mutation. p53 mutations were found in epithelial cells taken from sputum of both individuals showing symptoms of chronic bronchitis (4 of 29, or 13.8%) and those without symptoms of this disease (10 of 63, or 15.8%). Therefore, mutations in the p53 gene and, to a lesser extent, the K-ras gene were present in non-malignant epithelial cells taken from sputum of individuals without evidence of lung cancer who were exposed to smoky coal emissions in Xuan Wei Couny and were at a high risk for developing the disease.

92 TRANSCRIPTIONAL RESPONSE TO DIESEL PARTICULATE EXTRACT (SRM1975) AND MODULATION BY CHLOROPHYLLIN IN NORMAL HUMAN MAMMARY EPITHELIAL CELLS USING DNA MICROARRAYS. KESHAVA C[1], WHIPKEY DL[1], WESTON A[1]. [1]Toxicology & Molecular Biology Laboratory, NIOSH/CDC, Morgantown, WV 26505.

Diesel particulate extract (DPE), a mixture containing polycyclic aromatic hydrocarbons, is a major environmental pollutant. Previously, we showed the existence of inter-individual variations in induction of CYP1A1 and CYP1B1 by the polycyclic aromatic hydrocarbon benzo[a]pyrene. Here we have investigated the ability of DPE to induce a variety of genes, including CYP1A1 and CYP1B1, in normal human mammary epithelial cells. Further, we investigated the ability of chrorophyllin to mitigate the effects of DPE. normal human mammary epithelial cells were subjected to four separate 24h DPE (200μg/ml, organic extract) treatments: DPE alone, DPE plus chrorophyllin (5μM), chrorophyllin pretreatment (24h) followed by DPE, and chrorophyllin pretreatment followed by DPE plus chrorophyllin. Following exposure, gene expression was monitored by DNA oligonucleotide microarrays (U133A, Affymetrix). Of the total 22,000 genes present on the array, exposure to DPE alone altered expression of 235 genes by at least 2 fold (111 genes induced and 124 genes reduced). In particular, CYP1A1 and CYP1B1 were induced by 10-fold and 6-fold, respectively ($r = 0.89$, $p<0.02$). Limited induction of both CYP1A1 and CYP1B1 was observed in the presence of all three chrorophyllin treatment regimens. Maximal mitigation of the effects of DPE was observed in the treatment group where cells were pretreated chlorophyllin followed by DPE plus chlorophyllin. (CYP1A1 52%, $p<0.02$; CYP1B1 38%, $p<0.03$). Also chrorophyllin mitigated the induction of several other DPE-induced genes (PTGS2, ENPP2, NCOA1, PLA2G4A, S100A8 and S100A9). Thus, chrorophyllin may be beneficial when used as a dietary supplement for individuals exposed to environmental and occupational chemical carcinogens.

93 EVALUATION OF THE PERFORMANCE OF A SMALL BATTERY OF *IN VITRO* TESTS IN DETECTING RODENT AND HUMAN CARCINOGENS. Kirkland DJ[1], Aardema MJ[2], Henderson L[3], Müller L[4]. [1]Covance Laboratories Ltd, Harrogate HG3 1PY, UK. [2]Procter & Gamble, Cincinnati, OH 45239 8707, USA. [3]Henderson Scientific Consultancy, Northwich CW8 2UT, UK. [4]Novartis Pharma AG, CH-4002 Basel, Switzerland.

The performance of a battery of 3 *in vitro* genotoxicity tests (Ames + MLA + *in vitro* MN) has been evaluated for its ability to detect rodent carcinogens, from the databases of Gold*et al*, NTP and IARC. Because there are so few published data on the *in vitro* MN, and the correlation between induction of MN and chromosomal aberrations [CA] *in vitro* is so high, *in vitro* CA data were also included as relevant. The sensitivity of the 3-test battery was high. Of the 422 carcinogens for which there were valid data, 409 (96.9%) gave positive results in at least 1 of the 3 tests. Of the 13 carcinogens that gave negative, equivocal or inconclusive genotoxicity results in a full 3-test battery, all were either non-genotoxic carcinogens (liver enzyme inducers, peroxisome proliferators, hormonal carcinogens), or were extremely weak (presumed) genotoxic carcinogens (e.g. N-nitrosodiphenylamine). The specificity of the 3-test battery was poor. We identified about 200 chemicals that were non-carcinogenic after testing in both male and female rats and mice. For the approximately 50% of non-carcinogens with relevant data, >80% gave positive genotoxicity results in 1 or more tests. Thus the specificity (ability to accurately detect non-carcinogens as negative) was <20%. Some of the positive non-carcinogens were aneugens or produced clastogenicity only at high osmolality, but a large number of these positives are unexplained. This performance highlights the importance of understanding the mechanism by which genotoxicity may be induced (whether it is relevant for the whole animal or human) and using weight of evidence approaches to assess the carcinogenic risk from a positive genotoxicity signal.

94 INVESTIGATING THE ROLE OF EXONUCLEASE I IN THE O^6-METHYLGUANINE-INDUCED APOPTOSIS. Klapacz J[1], Meira LB[1], Edelmann W[2], Samson LD[1]. [1]MIT, Cambridge, MA 02139. [2]Albert Einstein College of Medicine, Bronx, NY 10461.

Alkylating compounds occur endogenously and abundantly in our environment, and are also used to treat certain types of cancer. O^6-methylguanine (O^6MeG), comprising <8% of DNA alkylations, is considered to be one of the major mutagenic and cytotoxic lesions caused by alkylating agents. In some cells, S-phase checkpoint and apoptosis pathways were shown to solely depend on the absence of repair by O^6MeG DNA methyltransferase (MGMT) that demethylates O^6MeG to guanine. O^6MeG mispairs with T during DNA replication, generating G-to-A transitions. In the presence of the mismatch repair (MMR) pathway, the O^6MeG:T mispair is bound by the MSH2/MSH6 heterodimer (MutSá), and it is widely assumed that futile cycles of the O^6MeG:T mismatch repair, followed by the eventual formation of double-strand breaks, generates the signal for apoptosis.. Here, we explore whether the *Exo* I-mediated excision step of the MMR pathway is required to generate the signal for apoptosis in response to O^6MeG since such excision would be required for the futile cycles of mismatch repair. To this end, we have crossed *Mgmt* null mice (deficient in O^6MeG repair) with *Exo* I mice (deficient in the excision step of MMR). Our initial results demonstrate that the double mutant mice are viable and our ongoing studies to assess the alkylation sensitivity of the double mutant cells and animals will be reported at the meeting.

95 XPA AS A RATE-LIMITING FACTOR FOR UV SENSITIVITY AND NUCLEOTIDE EXCISION REPAIR. Köberle B[1], Roginskaya V[1], Wood RD[1]. [1]University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, Pa 15213.

The XPA protein is a core factor necessary for nucleotide excision repair (NER) in mammalian cells. Cells with inactivating mutations in the XPA gene have no capacity for NER and are hypersensitive to killing by UV. Testis tumor cell lines generally have lower levels of XPA than other tumor cell lines, which may be relevant for the unusual sensitivity of testis tumor cell lines to cisplatin (Welsh et al., IJC 110: 352-361, 2004). There is evidence, however, that even low expression of XPA protein in XPA-deficient cells is sufficient to confer normal UV resistance, and the extent to which XPA is rate limiting is not yet known. Using a mammalian expression vector for XPA cDNA and the doxycycline repressible tetracycline transactivator (tTA) we investigated UV sensitivity in relation to XPA molecules/cell. Stably transfected sublines of the XPA-deficient cell line XP12RO were isolated. Sublines, which differ in their level of XPA protein were selected. Comparing with a standard of purified XPA protein we calculated about 20 000 XPA molecules/cell for two sublines and 7 000 XPA molecules/cell for two other sublines. Long-term cultivation with doxycycline repressed XPA expression in all sublines. XPA expressing sublines showed UV resistance similar to that seen in repair proficient cell lines while cultivation with doxycycline rendered the sublines UV sensitive comparable to the parental cell line XP12RO. These data suggest that amounts of XPA protein as low as 7 000 molecules/cell are able to confer full UV resistance. We will present results regarding regulation of the levels of XPA further by doxycycline treatment for a short time to determine the impact on UV sensitivity and repair kinetics.

96 MULTIPLE DNA POLYMERASES INVOLVED IN CHEMICALLY-INDUCED FRAMESHIFT MUTAGENESIS IN *ESCHERICHIA COLI* AND*SALMONELLA TYPHIMURIUM*. Kokubo K[1,2], Yamada M[1], Kim SR[1], Gruz P[1], Shimizu M[1], Kanke Y[2], Nohmi T[1]. [1]National Institute of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan. [2]Otsuma Women's University, Chiyoda-ku, Tokyo 102-8357, Japan.

Translesion DNA synthesis is an important cellular mechanism to overcome replication block imposed by DNA damage. *Escherichia coli*possess five DNA polymerases, and the expression of *polB*, *dinB*and *umuDC* encoding DNA pol II, IV and V, respectively, is induced by DNA damage. To examine the relationship between DNA polymerases and DNA damage, we constructed derivatives of *Salmonella typhimurium* TA1538 expressing *E. coli* DNA pol II, pol IV or DNA pol RI, and compared the mutabilities against 30 mutagens. Strain TA1538 has a mutational hotspot of CGCGCGCG sequence, which is suitable to identify -2 frameshifts, and DNA pol RI is a homologue of *E. coli* pol V. Thirty mutagens were categorized into four classes. Class 1 includes chemicals such as 10-azabenzo-[a]pyrene (10-azaBP) whose mutagenicity was specifically enhanced by DNA pol IV. Class 2 includes chemicals such as 7,12-dimethylbenz[a]anthracene (DMBA) whose mutagenicity was enhanced by either DNA pol IV or DNA pol RI. Class 3 includes chemicals such as 1-nitropyrene (1-NP) whose mutagenicity was specifically enhanced by DNA pol RI. Class 4 includes chemicals such as Glu-P-1 whose mutagenicity was not enhanced by any of the three DNA polymerases. To further examine the relationship, we deleted genes encoding corresponding DNA polymerases in *S. typhimurium* TA1538. Deletion of $dinB_{ST}$ greatly reduced the mutagenicity of 10-azaBP. Interestingly, deletions of either $dinB_{ST}$ or $umuDC_{ST}$ diminished the mutagenicity of DMBA, and maximum mutagenesis by 1-NP required the presence of both $polB_{ST}$ and $umuDC_{ST}$. None of the deletions decreased the sensitivity to Glu-P-1. These results suggest that multiple DNA polymerases including DNA pol III holoenzyme play important roles in the frameshift mutagenesis by catalyzing erroneous DNA synthesis across DNA lesions.

97 MUTATIONAL SPECIFICITY OF N-NITROSONORNICOTINE IN LACZ MICE: MUTATIONS AT A:T BASE PAIRS. Kosinska W[1], Khmelnitsky M[1], Cote M[1], Guttenplan JG[1,2]. [1]Dept. Basic Sciences, New York Univ. Dental College, New York, NY 10010. [2]Dept. Environ. Medicine, New York Univ. Medical School, New York, NY 10016.

N-nitrosonornicotine (NNN) is the most abundant tobacco-specific nitrosamine. Its concentration in tobacco products is ca. 10 x higher than the potent carcinogen, NNK, and NNN is a potent carcinogen in a number of organs in rodents. We have reported that NNN is a more potent mutagen in lacZ mice than NNK in the mouth and esophagus, and less potent in lung and liver. As NNK is considered to be a potential human lung carcinogen, it seems reasonable to surmise that NNN is a potential human carcinogen in tobacco-related upper aerodigestive cancers. NNK induces mainly GC:AT transitions in several animal models, but its mutational specificity has not been reported. Here we report the mutational specificity of NNN in the cII gene of lacZ mice. Mice were given NNN in drinking water at a concentration of 0.1 mg/ml for two weeks, then 0.2 mg/ml for two weeks. Of the organs examined NNN induced the highest mutant fraction (MF) in liver (15.2 +/- 7.4 / 100,000 pfu) followed by esophagus, tongue, other pooled oral tissue and lung (all with MF's 9 - 11 / 100,000 pfu). The MF in control liver was 4.1 +/- 1.7. Thus far, 22 NNN-induced mutants have been sequenced and 86% of the mutations occurred at AT base pairs. Of these, 53% were AT:TA transversions, 26% were AT:CG transversions, and 10% AT:GC transitions and single-base deletions. The remaining mutations were GC:AT transitions. In control liver DNA 75% of the mutations were GC:AT transitions. Clearly the mutation specificity of NNN is different from that of NNK and spontaneous mutagenesis. The observation of two major mutations at AT base-pairs suggests that two major DNA adducts at AT base-pairs are the main precursors to mutagenesis induced by NNN. Supported by the Smokeless Tobacco Research Council Grant #0727.

98 CHRONIC EXPOSURE TO X-RAYS SUPPRESSES HOMOLOGOUS RECOMBINATION IN MICE. Kovalchuk O[2], Hendricks C[1], Cassie S[2], Engelward B[1]. [1]Biological Engineering Division, MIT, Cambridge, MA, 02139, USA. [2]Department of Biological Sciences, University of Lethbridge, Alberta, T1K 3V2, Canada.

Although homologous recombination helps somatic cells repair and tolerate DNA damage, mitotic recombination between misaligned sequences can lead to loss of genetic information (e.g. deletions, translocations and loss of heterozygosity). Given that such genetic changes may promote tumorigenesis, it is critical that we learn more about the genetic and environmental factors that modulate cellular susceptibility to recombination. We have engineered transgenic mice that allow for the quantification of homologous recombination events in primary somatic cells, both *in vitro* and *in vivo*. The Fluorescent Yellow Direct Repeat (FYDR) mice carry two different mutant copies of an expression cassette for enhanced yellow fluorescent protein (EYFP). Homologous recombination between these truncated sequences can restore expression of EYFP and thus yield a fluorescent phenotype. We have previously shown that recombination events that occurred *in vivo* can been detected directly in disaggregated epidermal cells. Here, we apply these "Recombomice" to studies of X-ray irradiation. We show that acute exposure to X-rays induces recombination *in vivo*. Strikingly, daily low-dose exposure results in a strong suppression of recombination events, both in animals and in cultured cells. This suppression is not due to silencing (shown by RTPCR), and may be due to an adaptive response. In line with this hypothesis, we show that low level irradiation induces increased levels of several proteins involved in non-homologous end-joining and base excision repair (Ku70, Polymerase beta, and AP endonuclease 1). Thus, the adaptive response appears to increase clearance of recombinogenic lesions *in vivo*.

99 RADIATION-INDUCED GEMONIC DNA METHYLATION CHANGES – THE BIOLOGICAL SIGNIFICANCE AND POSSIBLE MECHANISMS. Kovalchuk OV[1], Raiche JN[1], Slovack MK[1], Pogribny IP[2]. [1]University of Lethbridge, AB, Canada. [2]NCTR, FDA, Arkansas, USA.

Radiation is a well-known genotoxic agent that gives rise to a variety of long-term effects.One of the most analyzed, yet least understood long-term effects of radiation is transgenerational genomic instability. The inheritance of genomic instability suggests possible involvement of epigenetic mechanisms, such as changes of methylation of cytosine residues located within CpG dinucleotides. We pioneered the studies of the effects of whole body low dose irradiation(LDR)on global genome methylation in various tissues of male and female mice. We also evaluated changes in promoter methylation and expression of the tumor suppressor gene *p16* Text $_{INKa}$. We observed different patterns of radiation-induced global genome DNA methylation in the tissues of exposed males and females. We also found sex and tissue-specific differences in *p16* Text $_{INKa}$ promoter methylation. The aforementioned results warranted more studies in the area of radiation-induced genomic DNA methylation changes. As a next step we evaluated the effects of high dose irradiation(HDR)(5Gy)on genomic DNA methylation in spleen, liver, muscle and lung tissues of exposed male and female mice. We observed strong and significant HDR-induced DNA hypomethylation in liver and spleen, but not in lung tissue of exposed animals. We also confirmed that radiation-induced DNA methylation changes were persistent and were present up to 1 month following original exposure. We also evaluated the dose-dependence of radiation-induced methylation changes. The observed active radiation-induced DNA demethylation was a very interesting and puzzling finding. Possible mechanisms of the occurrence of this phenomemon, its relationship to DNA repair as well as the roles of DNA methyltransferases and 5 MeC DNA glycosylase are currently will be presented.

100 RELIABLE COMET MEASUREMENTS. Kumaravel TS. [1]Genetic and Molecular Toxicology, Covance Laboratories Limited, Harrogate HG2 1PY, UK.

Comet assay is being widely used to evaluate the genotoxic potential of chemicals, environmental contaminants and for environmental monitoring purposes. In recent years, there has also been some move to consider this assay as a valuable tool for regulatory studies. This assay commonly utilises commercially available software programmes to evaluate extent of genetic damage at single cell level. These programmes provides a large number of measurements (i.e. tail length, Olive tail moment, % tail DNA etc.) to evaluate the extent of DNA damage. At the moment, however, there is no general agreement with respect to most acceptable measurements or parameters. This study was carried out in order to establish, which measurement in the comet assay provides the most significant correlation with the DNA damage, which could be adopted for routine use. Pooled peripheral blood samples were irradiated with a range of doses of 137Cs gamma-radiation (unirradiated, 1, 2, 4 and 8 Gy). Following irradiation, adopting a standard protocol, comet assay was performed and different parameters were recorded using Komet 5.0 software (Kinetic Imaging Ltd., UK). Following statistical analyses, it emerged that, Olive Tail Moment (OTM) provided the most significant value (R-square= 0.9990) followed by Tail Extent Moment (R-square = 0.9982) and Tail DNA (%DNA) (R-square = 0.9910). Statistically, these values could not be considered significantly different from each other. Since both OTM and % DNA in the tail are most commonly used parameters, these two could continue to be used for routine use. However, since OTM provides the measurements in arbitrary unit, % DNA in the tail could be considered more robust. Other parameters might not be considered of significant use in genotoxicological studies.

101 HUMAN BREAST TUMORS MANIFEST BOTH HEREDITARY DEFICIENCY AND SOMATIC LOSS OF DNA (NUCLEOTIDE EXCISION) REPAIR. Latimer JJ[1], Johnson JM[2], Kelly CM[1], Grant SG[3], Vogel V[4], Brufsky AM[4], Kelley J[1]. [1]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA 15213. [2]Molecular Genetics and Biochemisrty Program, University of Pittsburgh, Pittsburgh, PA 15213. [3]Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA 15213. [4]Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15213.

Nucleotide Excision Repair (NER) is a complex DNA repair pathway activated by distortions in the DNA helix. NER deficiency is the basis of the disease Xeroderma Pigmentosum (XP), which has a high susceptibility to skin cancer. We hypothesized that NER also plays a role in protection against other tumor types. We have developed a novel method for reliable culture of normal Human Mammary Epithelial Cells (HMEC) and breast tumors. Using primary explant cultures from breast cancer patients and controls undergoing breast reduction mammoplasty, we applied the Unscheduled DNA Synthesis (UDS) assay, a functional measure of NER. We found that normal HMECs exhibit only ~25% of the NER capacity of skin fibroblasts. Early stage breast tumors were further reduced, on average exhibiting only 50% of normal activity ($P < 0.001$). Breast tissue samples with NER values of < 0.7 of normal have an odds ratio of 27:1 of being transformed. We have now completed analysis on HMECs derived from the Non-Tumor Adjacent (NTA) area of the original tumor block for a subset of our patients. These tissue samples are pathologically free of tumor cells. The average NER capacity of these samples falls between those of the normal and tumor samples, not significantly different from either. Comparing these results with those of the matched tumor, we find that both samples are NER deficient in 75% of cases, suggesting a systemic, probably hereditary deficiency (manifesting XP patients have exhibited as much as 60% of normal activity). In the remaining 25% of cases, there is a significant difference between the UDS results of the tumor and NTA ($P = 0.036$), evidence of loss of NER capacity during the development of the tumor. These data suggest that NER plays an important role in the etiology of breast epithelial tumors.

102 TARGETING DNA REPAIR ENZYMES TO THE MITOCHONDRIA. LeDoux SP. [1]Department of Cell Biology and Neuroscience, Univ. of South Alabama, Mobile, AL 36688.

Mitochondrial DNA (mtDNA) is a very sensitive target for many genotoxic agents. Mutations in and deletions of mtDNA have been causally linked to numerous diseases and to the normal process of aging. A key component in the cell's arsenal to maintain mtDNA integrity is efficient DNA repair. An understanding of the mechanisms by which mtDNA repair processes protect cells from genotoxic insults is only now beginning to be appreciated. Concurrent with the elucidation of DNA repair processes in the mitochondria was the discovery of the key role that mitochondria play in the process of apoptosis. The implication that the two processes are linked came during a series of experiments we conducted in which a correlation between increased induction of apoptosis and inefficient mtDNA repair was shown using primary cultures of CNS cells. In order to establish that mtDNA repair plays a key role in cell survival, we designed a strategy to alter the repair efficiency by targeting of DNA repair enzymes into the mitochondria. Targeting of recombinant OGG1 into mitochondria of either HeLa cells or primary cultures of oligodendrocytes resulted in increased mtDNA repair of oxidative damage and a concomitant decrease in induction of apoptosis. Conversely, targeting of Exo III to mitochondria of MDA-MB 231 breast cancer cells disrupted mtDNA repair which resulted in increased cell death. Additionally, data will be presented that demonstrates the effective use of protein transduction to target Exo III to mitochondria of MDA-MB 231 cells. These studies illustrate that the transient, direct mitochondrial delivery of specific recombinant proteins which are capable of enhancing or disturbing mtDNA repair represents a novel therapeutic strategy for protecting healthy cells or eliminating those that are abnormal.

103 MECHANISMS OF INHIBITION OF X-RAY-INDUCED MUTATIONS IN CHINESE HAMSTER G12 CELLS BY ANTIOXIDANTS. Leszczynska J[1], Lasano S[1], Klein CB[1]. [1]New York University School of Medicine, Dept. of Environmental Medicine, Tuxedo, NY 10987.

Antioxidants inhibit spontaneous or carcinogen-induced mutations. To study antimutagenesis mechanisms, we examined the mutant spectra of Chinese hamster G12 cells exposed to 100-750 cGy X-rays. We previously reported that ionizing radiation yields a combination of deletions, point mutations and epigenetic DNA methylation silencing of the *gpt* $^{+}$transgene. Analysis of mutants arising by 100, 250 and 750 cGy X-rays shows a *gpt* $^{+}$ deletion phenotype in 42% to 50% of those mutants. Pretreatment with vitamin E (5μM) or lycopene (5μM) reduced the number of mutants induced by 250 cGy X-rays by 60% and 56%, respectively. Surprisingly, these mutant spectra showed increases in the frequency of *gpt* $^{+}$ deletions, from 50% to 60% deletions in presence of vitamin E and from 50% to 80% deletions in presence of lycopene. Concurrently, the frequency of methylated mutants decreased. These data led to the suggestion that some antioxidants may not be able to suppress deletion mutations. To test this hypothesis further, we employed other oxidant scavengers N-acetylcysteine (NAC) and aspirin. Both 0.5 mM NAC and 0.5mM aspirin effectively reduced the G12 mutant yield induced by 250 cGy X-rays, as expected. Unlike the data obtained with vitamin E and lycopene, both NAC and aspirin inhibited the formation of deletion mutations. NAC was the more effective antimutagen, reducing the total *gpt* $^{+}$ mutant frequency by 50% and the deletion frequency from 50% to 17% (2/12). Aspirin was less effective, reducing the mutant frequency by about 30% while also inhibiting deletion mutations to 25% of the untreated total. Ongoing studies are examining the selectivity of lycopene's antimutagenic activity towards non-deletion mutations. Supported by CA73610, ES09845.

104 CHARACTERIZATION OF THE ROLE OF THE LOOP REGION OF DNA POLYMERASE BETA IN POLYMERIZATION FIDELITY. Lin GC[1], Sweasy JB[2]. [1]Yale University School of Medicine, Department of Genetics, New Haven, CT 06510. [2]Yale University School of Medicine, Department of Therapeutic Radiology and Department of Genetics, New Haven, CT 06510.

The loop region of DNA polymerase beta (pol beta) is located on the outskirt of the palm domain of the polymerase and it spans 14 amino acids. It is flexible and amorphous and therefore structural data have not been useful in assigning it a role in polymerization fidelity based on its shape and position. A screen conducted in our laboratory for AZT resistant pol mutants generated from random mutagenesis identified three AZT resistant mutants in this loop region. Two of these mutants were characterized kinetically and shown to be mutator mutants. Mutator mutants are pol mutants that are less discriminating than wildtype in terms of selecting the correct nucleotide for polymerization. My goal is to test the hypothesis that the loop region of pol beta is important for polymerization activity and fidelity and to elucidate its precise role in those processes. A second goal is to determine the nature of each of the amino acid residues of the loop that are important for activity and fidelity. The approach we have taken is to construct libraries of different pol beta mutants that contain alterations in the loop region. We have generated 14 libraries of pol beta variants with each library representing random amino acid substitutions at one of the fourteen loop residues. In addition, we have several mutant constructs with varying lengths of the loop. The resulting pol mutants are then assessed for activity and fidelity using genetic screens. Several of the mutants have been characterized and we have identified two mutator mutants thus far from the libraries, suggesting that the loop is important for accurate DNA synthesis. Biochemical characterization of the purified variants will assist in the elucidation of the role of the loop domain in accurate DNA synthesis and is currently underway.

105 EVALUATION OF CELLOMICS MICRONUCLEUS BIOAPPLICATION -AN AUTOMATED SCORING SYSTEM. Lu S[1], Khoh-Reiter S[1], Lee M[1], Jessen B[1], Stevens G[1]. [1]WW Safety Sciences La Jolla Laboratories, Pfizer Inc, San Diego, CA.

Evaluation of the genotoxicity of compounds is an important aspect of safety testing during drug discovery and development. The *in vitro* micronucleus assay is a standard genotoxicity assay used to assess the potential for compounds to produce chromosomal aberrations. Manual scoring of the micronucleus assay is extremely labor intensive and therefore automation of this task is desired. The Cellomics Micronucleus Bioapplication software allows for automated and rapid quantification of binucleated cells and micronuclei required for the assay. The goal of this evaluation was to compare the Cellomics Micronucleus Bioapplication assay results with those generated by manual scoring. Freshly passaged CHO-WBL cells were plated on collagen-coated plates and were incubated overnight. Cells were first stained with a dye to readily locate the cytoplasm and then treated for 24 hours with test compounds to evaluate micronuclei formation. The cells were then fixed and stained with DNA/nuclear dye for identification of micronuclei and assessment of total nuclear count. The plates are scanned in the Arrayscan HCS Reader using the Micronucleus Bioapplication. Known clastogens including cyclophosphamide, 2-nitrofluorene, dimetylbenaznthrance (DMBA), mitomycin C (MMC), and 5-FU 5-fluorouacil (5-FU), as well as eight proprietary Pfizer compounds were tested. Although different quantitative results (i.e., percentage of binucleated cells containing micronuclei) were obtained with automated and manual scoring, both methods generated same genotoxicity calls for all the compounds. The present results demonstrate that the new cellomics micronucleus bioapplication is likely to become a valuable tool for improved micronucleus testing.

106 CIGARETTE SMOKE INDUCES ANAPHASE BRIDGES AND CHROMOSOMAL INSTABILITY IN NORMAL CELLS. Luo LZ[1], Werner KM[1], Gollin SM[2], Saunders WS[1]. [1]Department of Biological Sciences, University of Pittsburgh, The Oral Cancer Center of Discovery, and the University of Pittsburgh Cancer Institute, Pittsburgh, PA 15260. [2]Department of Human Genetics, University of Pittsburgh, The Oral Cancer Center of Discovery, and the University of Pittsburgh Cancer Institute, Pittsburgh, PA 15261.

Exposure to cigarette smoke has long been linked to carcinogenesis, but the emphasis has been placed on mutational changes in the DNA sequence caused by the carcinogens in smoke. Here, we report an additional role for cigarette smoke exposure in contributing to genomic instability in cells. We have found that cigarette smoke condensate (CSC) induces anaphase bridges in cultured human cells, which in a short time lead to genomic imbalances. The frequency of the induced bridges within the entire population decreases with time, and this decrease is not dependent upon the p53-mediated apoptotic pathway. Additionally, we show that CSC induces DNA double stranded breaks (DSBs) in cultured cells and purified DNA. The reactive oxygen species (ROS) scavenger, 2' deoxyguanosine 5'-monophosphate (dGMP) prevents CSC-induced DSBs, anaphase bridge formation and chromosomal instability (CIN). Therefore, we propose that CSC induces bridges and CIN via DNA DSBs. Furthermore, since the amount of CSC added to the cultures was substantially less than that extracted from a single cigarette, our results show that even low levels of cigarette smoke can cause irreversible changes in the chromosomal constitution of cultured cells.

107 INTEGRATION OF CHROMOSOMAL DAMAGE ASSESSMENT WITH ROUTINE TOXICITY TESTING USING A FLOW CYTOMETRIC ASSAY FOR MICRONUCLEATED RETICULOCYTES. MacGregor JT[1,2], Bishop ME[3], Dertinger S[4], McNamee J[5], Harper S[6], Hotchkiss C[3], Hayashi M[7]. [1]FDA Natl. Center for Toxicolocal Res., Rockville, MD 20857. [2]Toxicology Consulting Services, Arnold, MD 21012. [3]FDA Natl. Center for Toxicological Res., Jefferson, AR. [4]Litron Laboratories, Rochester, NY. [5]Health Canada, Ottawa, Ontario, Canada. [6]FDA Center for Food Safety and Applied Nutrition, Laurel, MD. [7]Natl. Inst. of Health Sciences, Tokyo, Japan.

We have evaluated a flow cytometric method (Dertinger et al., Mutat. Res. 542: 77-87, 2003) that allows assessment of micronucleated reticulocytes (RETs) in μl quantities of peripheral blood (PB) from the rat, dog, rhesus monkey, and human. Young RETs are labeled with FITC-anti-CD71 (transferrin receptor) and micronuclei with propidium iodide (with RNase treatment). We compared this method with microscopic scoring using standard or supravital acridine orange staining, using samples from normal and splenectomized Sprague-Dawley rats treated with cyclophosphamide (CP), cis-platin or vinblastine, in normal dogs and rhesus monkeys using CP, and in humans receiving radio- or chemotherapy. The data demonstrate the sensitivity and reproducibility of the flow cytometric assay in the Sprague-Dawley rat, beagle dog, rhesus monkey, and human. In rats, the flow method did not eliminate the spleen effect on induced MN-RET frequency, but spontaneous MN RETs were relatively insensitive to the spleen effect. Splenic selection in dogs appears similar to, or slightly stronger than in, rats. The data shows that effects on splenic efficiency would not lead to false positive responses in the rat, and are unlikely to do so in the dog. In normal (eusplenic) animals the dynamic range of induced effects was smaller in PB than in bone marrow, but the flow method had superior counting statistics, lower variability, and higher sensitivity than manual scoring. MN-RETs derived from fragments or whole chromosomes (CP- vs. vinblastine-induced) behaved similarly. The data suggest that flow cytometric assessment of micronucleus induction can be integrated into routine toxicity testing, eliminating the need for a separate bioassay, and can also be applied in human and non-human primate studies.

108 ATPASE ACTIVITY OF MSH2 PROTEINS FROM TRYPANOSOMA CRUZI CORRELATES WITH DIFFERENCES IN MISMATCH REPAIR EFFICIENCY OBSERVED AMONG VARIOUS PARASITE STRAINS. Machado-Silva A[1], Augusto-Pinto L[1], DaRocha WD[1], Pena SDJ[1], Teixeira SMR[1], Machado CR[1]. [1]Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Av Antônio Carlos 6627, 31270-901, Belo Horizonte, MG, Brazil.

Recent studies from our group based on *MSH2* SNPs showed that the *Trypanosoma cruzi* species can be divided into three haplogroups, denominated A, B and C. Furthermore, studies of microssatelite instability and cisplatin resistance assays indicated that strains belonging to MSH2 haplogroup A have a more efficient MMR repairing activity than haplogroup B and C strains. PCR amplification of a multi copy *T. cruzi* antigen, TcAG48, showed that haplogroup B and C strains display greater TcAG48 genetic variability when compared to haplogroup A strains. It should be noted that haplogroup B and C strains are responsible for the chronic cases of Chagas disease in Brazil. Since MSH2 plays a central role in MMR, we decided to investigate whether amino acid differences between the haplogroups could result in functional differences in this protein. Hence, the *MSH2* of two *T. cruzi* strains, Colombiana (haplogroup A) and CL Brener (haplogroup B) was amplified and sequenced. We found that 10% of the amino acid substitutions are located in regions described as important for the protein structure or its functional activity. Hence, recombinant MSH2 from both strains was expressed and purified from *E. coli* cultures and their *in vitro* activity was compared using ATPase assays. Our results suggest that the MSH2 from Colombiana possesses a higher intrinsic ATPase activity compared to the CL Brener isoform, indicating that the differences in MMR efficiency found between *T. cruzi* haplogroups are related to differences in MSH2 ATPase activity. These findings are consistent with our hypothesis which predicts that polymorphisms in the *MSH2* gene may determine metabolic differences in the MMR pathway among *T. cruzi* strains and that this could be an important source of antigenic diversity in the parasite.

109 REDUCTION IN TAMOXIFEN METABOLIC ACTIVATION AND GENOTOXICITY BY ANTISENSE TECHNOLOGY. Mahadevan B[1], Arora V[2], Schild LJ[3], Keshava C[4], Cate ML[2], Iversen PL[2], Poirer MC[3], Weston A[4], Pereira, C[5], Baird WM[1]. [1]Oregon State University, Corvallis, OR, 97331. [2]AVI BoiPharma, Corvallis, OR, 97331. [3]National Cancer Institute, NIH, Bethesda, MD, 20829. [4]National Institute for Occupational Safety and Health, CDC, Morgantown, WV, 26505. [5]Department of Statistics, Oregon State University, Corvallis, OR, 97331.

Tamoxifen (TAM) is widely used in the treatment and prevention of breast cancer. There is clear evidence that cytochrome P450 (CYP) 3A enzymes play an important role in TAM metabolism, resulting in metabolites that lead to formation of TAM-DNA adducts. We have investigated the effect of CYP 3A2 antisense (AVI-4472) exposure on CYP 3A2 enzyme activity and protein levels, as well as TAM-DNA adducts, in livers of rats administered TAM (50 mg/kg body weight [bw]/day) for 7 days, and 0, 0.5, 2.5, or 12.5 mg AVI-4472/kg bw/day for 8 days, beginning 1 day before TAM exposure. The specific activity of CYP 3A2 was increased after TAM administration, and decreased significantly (~ 70%) in the presence of 12.5 mg AVI-4472. CYP 3A2 protein levels, determined by immunoblot analysis, showed a similar pattern. Hepatic TAM-DNA adduct levels were measurable in all TAM-exposed groups. However, rats co-treated with 2.5 and 12.5 mg AVI-4472/kg bw/day showed statistically significant (~50%) reductions in TAM-DNA adduct levels (2.0-2.8 adducts/108 nucleotides) compared to rats treated only with TAM (5.1 adducts/108 nucleotides). Analysis of gene expression patterns using the rat toxicology U34 array (Affymetrix) containing *MT*850 genes revealed down regulation (≥2 fold) of several CYP genes and a dose dependent suppression of CYP3A9 in the TAM-exposed rats co-treated with AVI-4472. These results are being validated by RT-PCR. Overall the data confirm the usefulness of antisense technology to redirect TAM metabolic activation and lower TAM genotoxicity in rat liver.

110 EVALUATION OF MUTAGENICITY IN BIG BLUE (BB) MICE ADMINISTERED ACRYLAMIDE (AA) AND GLYCIDAMIDE (GA) IN DRINKING WATER FOR 4 WEEKS. Manjanatha MG[1], Aidoo A[1], Shelton SD[1], Bishop ME[1], McDaniel LP[2], Doerge DR[2]. [1]FDA/NCTR, Division of genetic and Reproductive Toxicology, 3900 NCTR Rd., Jefferson, AR-72079. [2]FDA/NCTR, Division of Biochemical Toxicology, 3900 NCTR Rd., Jefferson, AR-72079.

The recent discovery of AA, a probable human carcinogen, in a variety of fried starch-based food products has drawn attention to its genotoxicity and carcinogenicity. GA, the epoxide metabolite of AA, is believed to be associated with genotoxic effects of this compound. In order to evaluate the genotoxicity associated with exposure to AA and its metabolite GA, groups of male and female BB mice were administered 0, 10 or 50 ppm of AA and GA in drinking water for 4 weeks. Micronucleated reticulocytes in the peripheral blood were assessed by flow cytometry within 24 hr of the last treatment, and lymphocyte *Hprt* and liver *cII* mutagenesis assays were conducted 21 days following the last treatment. Except for the AA-treated females, the frequency of micronucleated reticulocytes for the high dose of AA and GA from both sexes was significantly (2-4-fold) higher than the control ($P \leq 0.01$; control 0.26%). The low and high doses of AA and GA produced significant increases in the lymphocyte *Hprt* mutant frequencies (MFs) that were 3-24-fold higher than the respective control ($P \leq 0.01$; control MF 2.1 $\pm$0.3 X 10^{-6}). At the high dose, AA and GA displayed significant increases in the liver *cII* MFs compared to control ($P \leq 0.05$). These results suggest that AA and GA are genotoxic in BB mice. DNA sequence analysis of mutants is underway to determine the molecular mechanism associated with AA and GA genotoxicity in BB mice.

111 ACB-PCR MEASUREMENT OF RARE K-RAS CODON 12 MUTATIONS IN LIVER OF N-HYDROXY-2-ACETYLAMINOFLUORENE-TREATED BIG BLUE RATS®. McKinzie PB[1], Chen T[1], Heflich RH[1], Parsons BL[1]. [1]Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, FDA, Jefferson, AR 72079.

Mutations in the K-*ras* gene are important in the etiology of a number of different cancers. K-*ras* codon 12 GGT to GAT and GGT to GTT mutations are the most frequently observed K-*ras* point mutations in human and rodent tumors. Consequently, these mutations may be useful biomarkers for evaluating the cancer risk associated with chemical exposures. Allele-specific competitive blocker PCR (ACB-PCR), a sensitive DNA-based method already established for the measurement of human K-*ras* codon 12 mutations, has now been developed for the measurement of rat K-*ras* codon 12 GGT to GAT mutation. The method that was developed utilizes a set of mutation fraction standards to quantify mutant fractions as low as 10^{-5}. In an initial application of the ACB-PCR method, liver tissues of male Big Blue® rats treated with *N*-hydroxy-2-acetylaminofluorene (*N*-OH-AAF) or with the vehicle-control were analyzed for K-*ras* mutation. The *N*-OH-AAF was administered in 4 doses of 25 mg/kg body weight at 4-day intervals and livers were harvested 10 weeks after initial dosing. The ACB-PCR assay was sensitive enough to detect K-*ras* mutation in control as well as *N*-OH-AAF-treated samples. Thus, this method can be used to quantify an important, cancer-associated biomarker in normal appearing liver as an early event in carcinogenesis. This ACB-PCR assay will be used in future studies to investigate the correlation between K-*ras* mutant fraction and tumor development.

112 FUNCTIONAL GENOMIC STUDIES OF HUMAN PEROXIDASES. McLachlan JJ[1], Josephy PD[1]. [1]Microbiology Dept., University of Guelph, Guelph, Ont., Canada N1G 2W1.

Heme-containing peroxidases are enzymes which use hydrogen peroxide to oxidize organic or inorganic substrates. Eosinophil peroxidase (EPX), lactoperoxidase (LPO), and myeloperoxidase (MPO) constitute a family of enzymes which are closely related in DNA/amino acid sequence. All three genes are closely linked at human chromosomal position 17q23.1. Neutrophils are the major blood cell type expressing MPO. LPO is an important enzyme of human milk and other secretions. Both MPO and LPO function to kill bacteria, by catalyzing the formation of reactive cytotoxic oxidants, such as HOCl and HOSCN. These enzymes can also catalyze the oxidative bioactivation of environmental mutagens, such as aromatic amines and polycyclic aromatic hydrocarbons. Human genetic variation in the coding sequences of these enzymes might be associated with differences in susceptibility to environmental mutagenesis. Several coding sequence variants of LPO have been reported on the Environmental Genome Project database (www.niehs.nih.gov/envgenom/). We are exploring the functional significance of these polymorphisms. Three of the LPO single nucleotide polymorphisms (SNPs) occur in sequences that are highly conserved among the three human peroxidases: R414Q, V421M, and R514Q. We are using the baculovirus/ insect cell system to express the wild-type and variant forms of LPO and also the corresponding mutations in MPO. Site-directed mutagenesis was performed successfully and recombinant baculovirus has been prepared. We plan to examine the effects of the SNPs on recombinant protein expression, secondary structure, and stability; enzyme activity; and capacity to activate chemical carcinogens. (Supported by NSERC Canada).

113 RIDDELLIINE-INDUCED MUTATIONS IN THE LIVER *cII* GENE OF TRANSGENIC BIG BLUE RATS. Mei N[1], Heflich RH[1], Chou MW[2], Fu PP[2], Chen T[1]. [1]Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, FDA, Jefferson, AR 72079. [2]Division of Biochemical Toxicology, NCTR/FDA, Jefferson, AR 72079.

To examine the mutagenicity of riddelliine, female transgenic Big Blue rats were gavaged with riddelliine for 12 weeks. The liver DNA was isolated for analysis of the mutations in the transgenic *cII* gene. A significant dose-response in MF was found, increasing from 30 x 10^{-6} in the control animals to 47, 55 and 103 x 10^{-6} in the low-, middle- and high-dose treatment groups, respectively. Molecular analysis of the mutants by sequencing indicated that there was a statistically significant difference between the mutational spectra from the riddelliine-treated and control rats. G:C to T:A transversion was the major type of mutation in rats treated with riddelliine, whereas G:C to A:T transition was the predominant mutation in the controls. In addition, mutations from the riddelliine-treated rats included an unusually high frequency of tandem base substitutions of GG to TT and GG to AT. Since riddelliine mainly induces liver hemangiosarcomas, we further evaluated the cell-specificity of riddelliine mutagenicity in rat liver. The liver parenchymal and endothelial cell fractions were isolated and purified. While there was no difference in the *cII* MFs of liver parenchymal cells between control and riddelliine-treated rats, the *cII* MF of liver endothelial cells from fromtreated rats was significantly greater than the *cII* MF of endothelial cells fromfrom control rats. DNA sequencing of the mutants indicated that the frequency of G:C to A:T transitionitio was reduced and the frequency of G:C to T:A transversion was increased in endothelial cells from the treated rats. These results indicate that riddelliine is a genotoxic carcinogen in rat liver, mainly targeting the endothelial cells. It induces a specific mutational spectrum, including a potential signature mutation, tandem base substitution.

114 DEFECTIVE REPAIR OF ALKYLATION DNA DAMAGE IN MICE. Meira LB[1], Pease K[1], Kerrison F[1], Dong M[1], Fry R[1], Dedon P[1], Samson LD[1]. [1]Div. of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

Alkylating agents are an important class of damaging agents because they can be found in the environment and are formed during normal cellular metabolism besides being used as chemotherapeutic agents. Alkylated DNA bases can be cytotoxic, mutagenic and ultimately carcinogenic. Three DNA repair pathways deal with alkylated bases in DNA: (i) the base excision repair pathway (BER), initiated when the damaged base is removed by a DNA glycosylase; (ii) direct reversal of base damage wherein the alkyl group on O6-methylguanine is transferred to a methyltransferase, inactivating it and restoring the normal base, and (iii) oxidative demethylation of base damage by action of *AlkB* homolog genes. We have exploited two mouse models previously generated in our lab, defective in the repair of alkylation DNA damage; the 3MeA DNA glycosylase knock-out (*Aag*null mice), and the O^6-MeG DNA repair methyltransferase knock-out (*Mgmt*null mice). We have combined traditional mouse genetics approaches with microarray technology in an attempt to determine the importance of both *Aag* and *Mgmt* in protecting a mammalian organism from spontaneous and/or alkylation-induced damage. A long term study has been initiated to look at the cancer predisposition of our *Aag* null and *Mgmt* null mice to alkylating agents. Histopathological analysis of a large cohort of treated animals has revealed new phenotypes for treated *Aag* and *Mgmt* null animals. In addition, wild-type and repair deficient mice exposed to different alkylating agents were analyzed for their short term responses to drug treatments. Both wild type and DNA repair deficient mice elicit a robust transcriptional response at different time points after drug treatment, and we will present a dissection of the specific contributions of 3MeA and O^6-MeG to this response.

115 A CAENORHABDITIS ELEGANS MODEL OF FRIEDREICH'S ATAXIA SHOWS IRON SENSITIVITY, MITOCHONDRIAL DNA DAMAGE, AND ALTERED GENE EXPRESSION. Meyer JN[1], Boyd WA[2], Haugen AC[1], Freedman JH[2], Van Houten B[1]. [1]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA. [2]Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA.

Frataxin is a mitochondrial protein involved in iron-sulfur protein synthesis that, when present in reduced amounts (30-50%) in humans, causes Friedreich's ataxia. Among the symptoms of this disease are high levels of mitochondrial iron and oxidative damage to mitochondrial protein and DNA. However, the precise mechanism by which reduced frataxin causes illness is not clear. In order to study the effects of frataxin reduction and consequent mitochondrial iron overload in a multicellular eukaryote, we are characterizing the effects of frataxin (frh-1) deficiency in the Caenorhabditis elegans mutant strain VC389 as well as an RNAi knockdown population (null mutants are embryonic lethal). VC389 nematodes are maintained as a population heterozygous for deletion of frataxin (frh-1 +/-) and show reduced frataxin expression as measured by semiquantitative RTPCR. They are also more sensitive than wildtype C. elegans to iron toxicity, as measured by reduced growth and fecundity. Furthermore, preliminary data indicates that VC389 nematodes show increased mitochondrial DNA damage as compared to wildtype nematodes as assessed by a long PCR-based assay we have recently adapted for use in this organism. We will present the details of the development and validation of this assay and its use in measuring nuclear and mitochondrial DNA damage and repair. Finally, we are currently comparing gene expression in VC389, RNAi knockdown, and wildtype C. elegans, and will present these results as well as a comparison of the differences observed in nematodes with those observed in frataxin knockdown versus normal yeast.

116 MITOCHONDRIAL UPTAKE OF AP-ENDONUCLEASE, A BASE EXCISION REPAIR ENZYME. Mitra S[1,2], Chattopadhyay R[1], Wiederhold L[1], Szczesny B[1], Hazra TK[1,2], Izumi T[1,2]. [1]Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555. [2]Department of Human Biological Chemistry and Genetics.

Reactive oxygen species (ROS), generated predominantly in the mitochondria (mt) of eukaryotic cells which is repaired via the DNA base excision repair (BER) pathway. Contrary to other BER proteins, the mitochondria-specific AP-endonuclease (APE), has not been characterized in mammals. In an attempt to definitively characterize mitochondrial APE, we extensively fractionated beef liver mitochondria, and then purified the active enzyme via multiple FPLC steps. MS analysis showed that the mitochondrial APE is derived from APE1 by deletion of 33 N-terminal amino acids. The N-terminal 61 residues are dispensable for APE activity, but include the nuclear localization signal (1-20 residues) and sites of acetylation important in APE1's role as a transcriptional regulator. The mitochondrial extract generates the identical truncation product. The mitochondrial APE has 3- fold higher specific activity than the full-length APE1. We have created mouse embryo fibroblast lines which lack endogenous APE1 alleles but contain a "floxed" human APE1 gene. Deletion of the APE1 transgene due to Cre expression induces apoptosis in 18-24 h with activation of caspase 3, which is prevented by coexpression of wild-type APE1, but not the repair-defective mutant. These results indicate that, unlike in *E. coli* and yeast, APE activity is essential for survival of mammalian cells. Because the nuclear genome damage could be alternatively repaired via the PNK-dependent, APE-independent BER pathway (likely to be absent in the mitochondria), it appears that the repair of spontaneous damage to the mitochondrial genome is essential in mammalian cells. (Research supported by USPHS grants R01 CA53791, R01 ES08457, P01 AG10514, and T32-ES07254.)

117 LIVER *cII* MUTANT FREQUENCY CORRELATES WITH TUMORIGENICITY IN FEMALE BIG BLUE MICE AND RATS FED MALACHITE GREEN AND LEUCOMALACHITE GREEN. Mittelstaedt RA[1], Mei N[1], Shaddock JG[1], Dobrovolsky VN[1], McGarrity LJ[1], Greenlees KJ[2], Heflich RH[1]. [1]Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079. [2]Center for Veterinary Medicine, U.S. Food and Drug Administration, Rockville, MD 20855.

Malachite green (MG), a triphenylmethane dye used in aquaculture as an antifungal agent, is rapidly reduced *in vivo* to leucomalacite green (LMG). Both chemicals have been found in fish intended for human consumption, indicating a potential for consumer exposure. A recent NTP study found some evidence that LMG was carcinogenic in the female mouse liver, but only equivocal evidence of carcinogenicity in female rat liver. There was no evidence that MG was carcinogenic in female mice. Both compounds have displayed relatively weak genotoxic responses in short-term *in vitro* assays. We fed female Big Blue B6C3F1 mice and Fischer 344 rats doses of MG and LMG used in the carcinogenicity bioassay and evaluated genotoxicity after 4 and 16 weeks of treatment. Neither compound increased the peripheral blood micronucleus frequency or the *Hprt* lymphocyte mutant frequency. The 16-week treatment with LMG, however, significantly increased the liver *cII* mutant frequency and produced a unique spectrum of mutations. Increases in liver *cII* mutant frequency were not seen in the mice treated for 16 weeks with MG or in rats treated for 16 weeks with LMG. These results indicate that LMG is an *in vivo* mutagen in female mouse liver and that the liver *cII* mutagenicities of MG and LMG correlate with their respective tumorigenic potential in mice and rats. The lack of increased micronuclei frequencies and lymphocyte *Hprt* mutants in female mice treated with LMG suggests that its genotoxicity is targeted to the tissue at risk for tumor induction.

118 P53 HAS A DIRECT APOPTOGENIC ROLE AT THE MITOCHONDRIA. Moll UM[1]. [1]Stony Brook University, Department of Pathology, Stony Brook, NY 11794.

We are interested in the mechanisms by which p53 kills cells. While p53 can induce apoptosis by target gene regulation, evidence for a p53 transcription-independent action had previously received little attention and a mechanism for the latter was completely unknown. We recently discovered that a fraction of induced wildtype p53 rapidly translocates to mitochondria after multiple apoptotic stimuli in malignant and immortal cells as well as in primary cells *in vivo*. p53 translocation precedes changes in mitochondrial membrane potiential, Cytochrome C release and caspase 3 activation. Of note, targeting p53 directly to mitochondria of p53-null cells is sufficient to launch effective apoptosis from this platform. We show that the p53 protein can directly induce permeabilization of the outer mitochondrial membrane by forming inhibitory complexes with the protective BclXL and Bcl2 proteins, and by inducting Bak oligomerization that in turn induces the penultimate Cytochrome C release. p53 binds to BclXL via its DNA binding domain. Purified p53 releases cytochrome c from liver mitochondria in a dose-dependent manner. Thus, p53, by moving rapidly to the mitochondria, effectively 'jump starts' and amplifies its transcription-dependent effect in apoptosis. We probe the significance of mitochondrial p53 in cancer and show that tumor-derived transactivation-deficient mutants of p53 concomitantly loose the ability to interact with BclXL and promote Cytochrome C release. This opens the possibility that mutations might represent 'double-hits' by abrogating the transcriptional and mitochondrial apoptotic activity of p53.

119 *IN VITRO* EVALUATION OF GLYPHOSATE-INDUCED DNA DAMAGE IN FIBROSARCOMA CELLS HT1080 AND CHINESE HAMSTER OVARY (CHO) CELLS. Monroy CM[1], Cortes AC[1], Sicard DM[1], Plewa MJ[2], Groot H[1]. [1]Laboratorio de Genetica Humana, A. A. 4976, Universidad de los Andes, Bogota, Colombia. [2]Department of Crop Sciences, 1101 West Peabody Drive, University of Illinois at Urbana-Champaign, Illinois,USA.

Glyphosate is a broad–spectrum nonselective herbicide, used to eliminate unwanted weeds in agricultural and forest settings. Herbicide action is achieved through inhibition of aromatic amino acid biosynthesis in plant cells; since this is not a conserved mechanism between human and plant cells, glyphosate is considered to be a low health risk substance for humans. However, there is controversy about possible unwanted side effects of glyphosate use; toxicity and genotoxicity studies indicate that it is not harmful, yet recent investigations suggest that it can alter various cellular processes in animals. This might be a health and environmental risk factor in areas where glyphosate is widely used. The objective of the present study was to evaluate glyphosate cytotoxic and genotoxic effects in CHO cells and HT1080 cells.Acute and chronic cytotoxicity were determined through exposure of cultured cells to different concentrations of glyphosate, and cell viability analysis was performed with Crystal Violet and Trypan Blue staining. Genotoxicity was determined using the comet assay and data analysis was performed using the one-way ANOVA Dunnet's test.For chronic cytotoxicity there was a clear dose-dependent effect both in CHO and HT1080 cells after treatment with 900-3000 μM. In the acute cytotoxicity study, CHO cells exposed to 1.0-7.0 mM and HT1080 cells exposed to 4.5-5.75 mM, had cell viability counts of >80%. Genotoxic effects were evidenced in CHO cells at glyphosate concentrations of 5.5-7.0 mM and in HT1080 cells at concentrations of 5.25-5.75 mM. Data were analyzed using the tail moment for the CHO cells and DNA migration and cell morphology was used for the HT1080 cells. There was no significant difference in the results in the two cell types, nevertheless the HT1080 cells were more sensitive.

120 DEVELOPMENT OF FTC ANTIOXIDANT MICROPLATE ASSSAY AND THE ISOLATION OF ANTIOXIDANTS FROM AGRICULTURAL BY-PRODUCTS. Muellner MG[1], Rundell MS[1], Vaughn SF[2], Berhow MA[2], Wagner ED[1], Plewa MJ[1]. [1]University of Illinois at Urbana-Champaign, Urbana, IL 61801. [2]U.S. Department of Agriculture, NCAUR, Peoria, IL 61604.

Oxidative damage to DNA is mutagenic and over time contributes to cancer induction and other diseases. Antioxidants play a role in protecting cells from oxidative stress. We are identifying agents with antioxidant activity from commercial corn and soybean processing by-products. The widely used ferric thiocyanate antioxidant assay (FTC) is time consuming and requires large amounts of sample. Our modified FTC microplate assay uses a standard 96-well microplate and allows for 8 replicates per concentration with 1l different concentrations of sample. The soybean processing by-product, phytochemical complex (PCC) contains a high level of soy isoflavones; PCC and the individual isoflavones, genistin, genistein, daidzin, daidzein, glycitin, and glycitein were analyzed using the modified FTC assay to quantify their antioxidant activity. The concentration preventing 50% of the oxidation of linoleic acid (I50) was calculated for each isoflavone. The I50 values were the following: glycitein = 30.4 μM, daidzein = 157.6 μM, genistein = 190.6 μM, daidzin = 213.9 μM, and genistin = 1437 μM. This antioxidant assay is very sensitive and can detect differences among structurally related compounds. Research funded by C-FAR grant IDACF 01I-2-4 CS and the Illinois Corn Marketing Board.

121 INTRA- AND INTER-LABORATORY VARIABILITY DOES NOT PRECLUDE IDENTIFICATION OF HYDROXYUREA MOLECULAR SIGNATURE. Muller A[1], Boitier E[1], Hu T[2], Carr G[2], Lefevre AC[1], Aardema M[2], Thybaud V[1]. [1]Aventis Pharma, CRVA, 13 Quai Jules Guesde, BP14, 94403 Vitry sur Seine, France. [2]Procter & Gamble, Cincinnati, OH 45252, USA.

To determine the parameters driving the development of a biologically meaningful gene expression database generated through multi-institutional collaborations, we assessed the gene expression profiles of mouse lymphoma L5178Y cells treated with Hydroxyurea (HU). Cells were dosed for 4 hrs and harvested immediately or left for a 20hr-recovery period. Cytotoxicity was evaluated by cell count and genotoxicity by *in vitro* micronucleus assay at 24hrs. Gene expression profiling was performed using Affymetrix mouse MG-U74A GeneChips. Three independent studies with similar experimental designs in two laboratories were carried out. Results of the gene expression analysis between the three studies included overlap and differences of gene lists found to be significant within a study and a joined statistical analysis taking into account the study as an experimental factor. Despite the differences in gene modulation between the studies at either time point, we could reliably identify 173 genes modulated by HU treatment at 4hrs and 608 genes at 24hrs after merging datasets from the 3 studies. These modulated genes shared core biological processes with the intersections (55 and 108 genes at 4 and 24hrs, respectively) of 3 separate analyses, but also gave insight into other modulated pathways that could not be identified from the separate analyses. After 4hrs, there was a clear response towards cell cycle regulation, mitosis, cytokinesis, DNA repair and induction of apoptosis, whereas after 20hrs the response was mainly characterized by lymphocyte specific activities and apoptosis. Our results showed that, despite intra- and inter-laboratory variability, merging the three studies provided information that was relevant to HU biology and that could therefore be used to populate a database of reference toxicants.

122 BIOMARKERS OF OXIDATIVE STRESS ARE ELEVATED AMONG AGRICULTURAL WORKERS. Muniz J[1], Kisby GE[1], Lasarev M[1], Koshy M[2], Kow YW[2], Li X[1], McCauley L[3]. [1]CROET, Oregon Health & Sciences University, Portland, OR 97239. [2]Dept Radiation Oncology, Emory University, Atlanta, GA 30322. [3]School of Nursing, University of Pennsylvania, Philadelphia, PA 19104.

Few studies are designed to increase our understanding of the biological mechanisms linking pesticide exposure and health outcomes. Organophosphate pesticides (OPs) have been found to induce oxidative stress, thus we conducted a cross-sectional investigation of the relationship between OP exposure and biomarkers of oxidative stress. Pesticide applicators (n = 12), farmworkers who do not apply pesticides (n = 10), and controls (n = 9) participated in the study. Work histories and life-style factors were assessed by questionnaire. We analyzed urine for dialkylphosphate pesticide metabolites (DAP) and 8-oxodG. Lymphocytes were analyzed for BER activity and protein levels (i.e., AP endonuclease, APE). Plasma was analyzed for malondialdehyde (MDA). General linear models were used to assess the significance of these measurements among the three groups while controlling for age, smoking, dietary intake and creatinine. The lowest APE activity was observed in the control group (median = 0.53), and higher activity observed in the farmworkers (median = 4.09), and applicators (median = 6.83). A similar pattern was observed for protein levels of APE and for plasma levels of MDA. Other components of the BER pathway were also perturbed in the lymphocytes of applicators and pickers. Controls had the lowest 8-oxodG urinary levels (estimated median = 22.6) compared to applicators (median 85.3) and farmworkers (median 193.3). Though the highest levels of DAP were observed in the agricultural groups, no significant patterns of association between the urinary OP metabolites and biomarkers of oxidative stress were found. In summary, these preliminary results are striking and point to the need for further investigation of the underlying mechanism of oxidative stress in pesticide-exposed populations.

123 EFFECTS OF SOYBEAN PROCESSING BY-PRODUCT ON SPONTANEOUS MUTATION IN MISMATCH-REPAIR DEFICIENT CELLS. Mure K[1], Plewa MJ[2], Takeshita T[1], Rossman TG[3], Klein CB[3]. [1]Dept. of Public Health, Wakayama University School of Medicine, Wakayama, Japan. [2]Dept. of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL. [3]Dept. of Environmental Medicine, New York University School of Medicine, Tuxedo, NY.

It was previously shown using Comet assays and flow cytometry that the extracts from a commercial soybean processing by-product protected Chinese hamster lung (CHL) or ovary (AS52) cells against direct DNA damage, clastogenic damage, and point mutations induced by 2-acetoxyacetylaminofluorene. To further valuate the effects of soy compounds on spontaneous mutagenesis in human cells, studies were conducted to investigate whether soybean processing by-products are antimutagenic (*hprt*locus) in human mismatch repair deficient (*hMLH1*$^-$) HCT116 colon cancer cells that have previously been useful in antimutagenesis studies by lycopene, ascorbate, vitamin E, and epigallocatechin gallate. We tested two soybean by-products, refined soy (Central Soya Company, Fort Wayne, IN) and Nova soy (Archer Daniels Midland Co, Decatur, IL). Prior to mutation testing, the toxicity of the soys were tested by long-term clonal survival assays, and non-toxic (10 μg/ml) and minimally toxic (20 μg/ml) doses were chosen. In support of the Chinese hamster studies, the soybean processing by-products effectively reduced spontaneous mutation rates in HCT116 cells. The refined soy reduced spontaneous mutagenesis rates over three weeks by 13% (10 μg/ml) to 20% (20 μg/ml). In contrast, the Nova soy has a much stronger effect on spontaneous mutagenesis, reducing the spontaneous mutation rate by 32% (10 μg/ml) and 62% (20 μg/ml). These data show that soybean processing by-products have protective antimutagenic potential in human cells. The mechanisms of antimutagenesis will be further elucidated through analysis of mutation spectra as well as by analysis of the effects of refined soy and Nova soy in Comet and other DNA damage analysis studies. Supported in part by CA89732.

123A SHE CELL TRANSFORMATION: PERFORMANCE OF THIS *IN VITRO* MODEL FOR PREDICTING RODENT CARCINOGENICITY. Myhr B[1]. [1]Covance Laboratories Inc.

Many short-term tests (SSTs) have been developed to aid in the identification of potential carcinogens. However, all SSTs currently required or recommended by the regulatory organizations are actually tests for mutational events or DNA damage. Because some compounds are able to cause tumor development without directly altering DNA, it is not surprising that the performance of the commonly used SSTs (with an overall concordance of only about 60% relative to rodent bioassay) is far from what was hoped. On the other hand, cultured mammalian cells can respond to both genotoxic and so-called non-genotoxic carcinogens by undergoing transformation through various stages. Regardless of what mechanism(s) may be involved, cell transformation assays have the potential to identify carcinogens with a higher degree of confidence than relying strictly on genotoxicity assays. The morphological transformation of SHE cells *in vitro* by chemicals has had a long, tortuous history of some 40 years in discovering efficient methods of conducting assays. Many assays failed to give responses to carcinogens. With the recent discovery that a slightly acidic culture medium (pH 6.65 to 6.75) greatly improved the repeatability between trials, much of the operational impediment to routine testing was eliminated. Now, with 100 chemical agents tested (for which rodent bioassays showed to include 68 carcinogens and 32 noncarcinogens), the low pH SHE cell transformation assay yielded a concordance of 81% with the rodent bioassay results, while maintaining a sensitivity of 85% and a specificity of 72%. Among ten aromatic amines tested (5 carcinogens and 5 noncarcinogens) the assay was 100% correct. The results showed that the SHE cell assay is superior to all current genotoxicity tests for predicting tumor induction in rodents. Such results indicated that SHE cell transformation assays can contribute meaningfully to drug development programs, even though very little has yet been learned about what critical molecular changes are responsible for the observed morphological changes in colony formation. The results obtained to date appear to justify renewed efforts to understand the mechanisms involved in morphological transformation and to develop an image analysis system to reduce the labor, training, and variability in scoring colonies.

124 DNA REPAIR PATHWAYS IN THE HEMATOPOIETIC SYSTEM. Nattamai KJ[1], Daria D[1], Geiger H[1]. [1]Division of Experimental Hematology, Cincinnati Children's Hospital Medical Center and Department of Pediatrics, University of Cincinnati Medical Center, Cincinnati.

We hypothesize that aging of stem cells is due to a loss of somatic DNA integrity. It is thought that aging of stem cells leads to reduced renewal and thus reduced tissue homeostasis in aged animals. Faithful repair of DNA damage such as chromosomal double strand breaks (DSBs) is critical to preserve genomic integrity. Aberrant DSB repair might result in chromosomal rearrangements, including translocations that in turn promote mutagenesis, senescence or cell death. The role of DNA repair processes in the maintenance of the stem cell phenotype has been poorly evaluated. We chose 3 distinct approaches to further characterize DNA repair pathways in hematopoietic (stem) cells. Our analyses revealed: 1) Hematopoietic stem cells show an at least 2-3 fold higher expression of several DNA repair/checkpoint control genes (like Rad1, Rrm2b DDB2, Rad52, Rad50) compared to mature hematopoietic cells. We thus conclude that hematopoietic stem cells use DNA repair pathways in a fashion that is different from mature cells. 2) Hematopoietic cells show a higher ratio of genomic deletions/translocation events over point mutations compared to other tissues, which might explain the high frequency of deletions/ translocations seen in hematopoietic malignancies. These data was generated using a murine transgenic mutation indicator strain (pUR288),.3) Over-expression of XRCC4 in murine hematopoietic stem cells, which is a gene involved in the non-homologous end-joining pathway of DNA repair, revealed a *in vivo* radio-protective role for hematopoietic stem cells for this protein by a not yet characterized mechanism.

125 HEMATOPOIETIC AGING IN CROSSLINK REPAIR-DEFICIENT *ERCC1*$^{-/-}$ MICE. Niedernhofer LJ[1], Prasher J[2], Lalai A[3], Touw I[2], Hoeijmakers JHJ[3]. [1]Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA 15213, USA. [2]Department of Hematology, Erasmus Medical Center, 3000 DR Rotterdam, NL. [3]Department of Cell Biology and Genetics, Erasmus Medical Center, 3000 DR Rotterdam, NL.

The ERCC1-XPF heterodimer is a substrate-specific endonuclease essential for nucleotide excision repair, DNA interstrand crosslink repair and some types of mitotic recombination. Mice deficient in ERCC1 (*Ercc1*$^{-/-}$) die in the third week of life with a number of symptoms that could be interpreted as highly accelerated, segmental premature aging. Hematopoietic function is commonly affected in mammalian aging resulting in anemia and decreased stress hematopoiesis, but the reason for this functional decline is unclear. To further characterize the premature aging features of *Ercc1*$^{-/-}$ mice and to determine if unrepaired DNA damage contributes to loss of hematopoietic function, we compared hematopoiesis in *Ercc1*$^{-/-}$, young and old wild type mice. Three week old *Ercc1*$^{-/-}$ mice exhibited multi-lineage cytopenia with marked thrombocytopenia. Histologic examination of the femoral bone marrow space revealed extensive fatty replacement in both the *Ercc1*$^{-/-}$ and aged mice compared to young wt mice. Both the number of bone marrow progenitors and their proliferative capacity were significantly diminished in *Ercc1*$^{-/-}$ mice compared to control littermates. Expansion of fetal liver progenitors *in vitro* demonstrated diminished stress erythropoiesis due to an inability of the cells to proliferate rather than differentiate. Thus the hematopoietic system of *Ercc1*$^{-/-}$ mice is prematurely aged by multiple criteria. Additionally, this phenotype is highly reminiscent of Fanconi anemia, a rare genetic disorder diagnosed by the exquisite sensitivity of patients' cells to DNA interstrand crosslink damage. This similarity strongly supports a causal role for unrepaired spontaneous interstrand crosslinks in the decline of hematopoietic function observed in *Ercc1*$^{-/-}$ mice, Fanconi patients and possibly natural aging.

126 MICROARRAY DATA INFORMATION DEPENDS ON VALIDATION CRITERIA: HOW LOW OR HIGH STRINGENCY METHODS LEADS TO DIFFERENCES IN PROBE LEVEL INFORMATION. N'jai AU[1], Means JC[1]. [1]Chemistry Department/ Great Lakes Center for Environmental and Molecular Sciences.

Gene expression profiling is increasingly used in risk assessment and is useful in determining biomarkers of exposure to specific contaminants. Traditional studies that defined molecular indicators of exposure and health effects were limited to assaying changes in a limited number of proteins or mRNAs. With the advent of DNA microarrays, thousands of genes or transcripts can be assayed simultaneously. Another great advantage of microarray technology is the ability to determine relationships among genes and biological pathways that underlie subtle changes in physiology or health. However, microarray data is usually voluminous and interpretation of gene expression data is often complicated with problems related to validation criteria. Here we show through differential analysis of microarray data presented the potential for variation in the probe level information due to level of stringency applied as a filter. For instance, using 3-fold signal log intensity and a count-percentage of 75-100, we observed significant differences in differentially expressed transcripts or genes when compared to 2-fold signal log intensity and a count-percentage of 75-100. Differences in validation criteria applied may lead to a gain or loss of information and therefore may cause shifts or skew in gene expression profile of compounds or disease.

127 ANALYSIS OF CHANGES IN GENE EXPRESSION IN RAT LIVER AFTER BENZO(A)PYRENE EXPOSURE ASSESSED BY AFFYMETRIX MICROARRAY AND REAL-TIME PCR. N'jai AU[1], Means JC[1]. [1]Department of Chemistry/Great Lakes Center for Environmental and Molecular Sciences (GLEAMS), Western Michigan University, Kalamazoo, MI 49008.

Benzo (a) pyrene (BaP), an environmental contaminant produced from diesel exhaust and organic matter combustion, is associated with a variety of adverse health effects, including cancer. In this study, we used Real-Time quantitative (Taqman) PCR (RT-PCR) to validate the expression of 6 target genes of interest (Cyp1a1, Cyp1a2, GST-yc2, p53, Bcl-2, IL-1â) previously identified from our Affymetrix microarrays global transcript profiling experiment of BaP induced gene expression changes. Adult male F344 rats were exposed to BaP through the diet for either 2 weeks (0.1 or 0.01mg/g-diet) or 3-months (0.01, 0.1 or 1.0mg/g-diet). Gene expression levels in liver tissue were analyzed using Affymetrix microarrays. Differential expression of a large number of genes in response to BaP was observed at both acute and chronic exposures (N'jai and Means, 2004). Subsequent RT-PCR analysis of our target genes of interest revealed significant differences ($p < 0.05$) between the control and treated groups, and confirms our initial findings from the Affymetrix microarray data. Our candidate genes for RT-PCR were selected based on their association with health effects or involvement in disease-related pathways. Furthermore, we observed significant differences in tissue and body weights of rats between treatment groups. In addition, liver tissue was analyzed by LC/MS for BaP metabolites. Functional analysis of the genes differentially expressed by BaP reveals the regulation of several biochemical pathways such as Ah receptor signaling, Apoptosis, immune, metabolic and Redox pathways.

128 EFFECT OF POLYCYCLIC AROMATIC HYDROCARBON ON HUMAN PROSTATE CARCINOMA CELL LINE (LNCAP). Nwagbara OF[1], Gragg RD[1], Reed SF[1]. [1]Florida A&M University, Environmental Sciences Institute, Tallahassee, FL 32307.

Polycyclic aromatic hydrocarbons (PAHs) such as benzo-[a]pyrene (B[a]P) and its metabolites (e.g.B[a]P-7,8-diol) are known to act through the aryl hydrocarbon receptor to elicit a variety of cancer and noncancer endpoints in exposed animal and cellular model, and humans. In addition, B[a]P modulates various endocrine functions by enhancing ligand metabolism, altering hormone synthesis, downregulating receptor levels, and interfering with gene transcription. Recent evidence suggests that aryl hydrocarbon receptor (AhR) agonists (TCDD, PAHs) can suppress androgen receptor (AR) activation and prostate cell growth. Cell viability studies show that treatment with B[a]P-7,8-diol alone had no effect on cell viability, while treatment with the same concentrations of B[a]P-7,8-diol in the presence of R1881 had a decreasing effect on cell viability. Also, cell cycle distribution results indicate that exposure to increasing concentrations of B[a]P-7,8-diol alone did not affect cell cycle distribution, while exposure to these same concentrations in the presence of R1881 induces G1 delay/or apoptosis and S phase arrest at 50uM of B[a]P-7,8-diol. These results show that the effects of B[a]P-7,8-diol are concentration-dependent in the inhibition of cell cycle progression from G1 to S phase in the presence R1881. Further studies are being carried out in our laboratory to examine the relationship between the AhR, AR and pRB protein, p21cip1, and p27kip1 in B[a]P and B[a]P-7,8-diol induced apoptosis and cell cycle arrest on LNCaP cells.

129 METABOLIC ACTIVATION OF 3-NITROBENZANTHRONE BY THE HUMAN RECOMBINANT CYTOCHROME P450 AND ACETYLTRANSFERASE. Oda Y[1], Watanabe T[2], Hirayama T[2]. [1]Osaka Prefectural Institute of Public Health, Higashinari-ku, Osaka 537-0025. [2]Kyoto Pharmaceutical University, Yamashita-ku, Kyoto 607-8414.

3-Nitrobenzanthrone (3-NBA), which exists in a diesel exhaust particle or an airborne particulate matter, is a powerful mutagen and suspected carcinogen. In this study we investigated what human P450 (CYP) isozymes and *N,O*-acetyltransferases (NATs) are involved in the genotoxic activation of 3-NBA using SOS/*umu* strains expressing human NAT1 and NAT2, and the newly developed *umu* tester strains *Salmonella typhimurium* OY1022/1A1, OY1022/1A2, OY1022/1B1, OY1022/2C9, OY1022/2D6, OY1022/2E1, OY1022/3A4, and OY1022/2A6, which express respective human CYPs and NADPH-cytochrome P450 reductase. Induction of *umuC* gene expression by 3-NBA was measured by beta-galactosidase activity. 3-NBA showed stronger cytotoxicity and *umuC* induction in NAT2-expressing strain rather than NAT1-expression strain, indicating that NAT2 is involved to metabolic activation by 3-NBA. Among eight OY strains used, 3-NBA was found to show the strongest *umuC* induction in OY1022/1A1 and OY1022/3A4 strains, and subsequently it was the order of CYP1A2>1B1>2E1=2C9=2D6=2A6. These results suggest that the genotoxic activation of 3-NBA is due to the reduction of NADPH-cytochrome P450 reductase, the oxidation reaction by human CYP1A1 or CYP3A4, and the following *O*-acetylation reaction by human NAT2.

130 CLASTOGENICITY AND MUTATIONAL SPECIFICITY OF AN N-HYDROXY METABOLITE OF AMINOPHENYLNORHARMAN. Ohe T[1], Mizuno T[1], Totsuka Y[2], Takamura T[2], Oda Y[3], Wakabayashi K[2]. [1]Kyoto Women's University, Kyoto 605-8501, Japan. [2]Natl. Cancer Center Res. Inst., Tokyo 104-0045, Japan. [3]Osaka Pref. Inst. of Pub. Health, Osaka 537-0025, Japan.

Aminophenylnorharman (APNH) is a newly identified mutagenic heterocyclic amine formed by norharman with aniline in the presence of S9 mix. APNH shows potent mutagenic activity towards *Salmonella typhimurium* TA98 and YG1024 with S9 mix, and induces SCEs and chromosome aberrations in Chinese hamster lung(CHL) cells at concentration less than 10 ng/ml in the presence of S9 mix. It is supposed that APNH is metabolized to the *N*-hydroxyamino derivative by metabolically activation of the exocyclic amino group by CYP1A1 and 1A2, and the *N*-acetoxy form produced by the action of *O*-acetyltransferase to induce mutations in *Salmonella* strains. In the present study, we examined the ability of *N*-hydroxy-aminophenylnorharman(*N*-OH-APNH), a *N*-hydroxy metabolite of APNH to induce SCEs and chromosome aberrations in CHL cells *in vitro* and the mutational specificity of *N*-OH-APNH using *Escherichia coli lac*Z reversion assay. The positive result was accompanied by a statistically significant induction in SCEs and chromosome aberrations at concentrations less than 25 ng/ml of *N*-OH-APNH both with and without S9 mix. We further compared mutational spectrum induced by *N*-OH-APNH with that by APNH in the *lac*Z gene of *E. coli* expressing bacterial *O*-acetyltransferase. The mutational spectrum induced by *N*-OH-APNH consisted mainly of –2 (C · G - G · C) frameshift mutations and to a lesser extent, -1 (G · C) frameshift mutations both with and without S9 mix, as in the case for APNH with S9 mix. These findings indicate that *N*-hydroxymetabolite of APNH plays an important role in mutagenic process of APNH.

131 GENOTOXICITY AND CELL CYCLE GENE EXPRESSION CHANGES INDUCED BY NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTIS). Olivero OA[1], Tejera AM[1], Das SA[1], Divi RL[1], Poirier MC[1]. [1]Carcinogen-DNA Interactions. National Cancer Institute, NIH. Bethesda, MD 20892.

The antiretroviral NRTIs, are genotoxic due to drug-DNA incorporation and DNA chain termination. Current clinical protocols for HIV-1 treatment include 2 NRTIs, but the genotoxic effects of drug combinations have rarely been studied. Here we report genotoxicity and changes in gene expression in human cervical epithelioid carcinoma (HeLa) cells exposed simultaneously to 125, 250 and 500 uM Zidovudine (AZT) combined with 10-fold lower doses of Lamivudine (3TC). A dose-related increase in percentage of cells in S, from 9.5 to 36%, with a concomitant decrease in % of cells in G1, from 82.6 to 58.5% was observed by flow-cytometry in cells exposed to AZT plus 3TC for 24 hours. Changes in gene expression were analyzed by cDNA microarray (Microarray Facility, NCI) and Superarray cell cycle specific pathway membranes, using RNA from cells exposed to 500 uM AZT /50 uM 3TC. Alteration of cell cycle/DNA damage/nucleic acid synthesis genes was consistent with the observed accumulation of cells in S-phase. For example, a 4-fold up-regulation was observed for DDIT3 a stress-response gene, that blocks G_1/G_2 proliferation and induces apoptosis. A second gene, Cyclin D1, responsible for G_0/G_1 progression, was up-regulated 2-fold. Cyclin D2, which assists in the G_0/G_1 transition, was upregulated and CDKN1A (P21), a check point that arrests cells to allow repair was downregulated. All of these events would accelerate the passage of cells into S phase. The major changes in gene expression observed by microarray were confirmed using real time RT-PCR technology, and the values obtained correlated well with initial the gene expression observations. Overall the study demonstrates that gene expression data may assist in the prediction of genotoxicity, during clinical use of NRTI drug combinations.

132 THE WERNER SYNDROME PROTEIN AND DNA REPAIR PATHWAYS AT TELOMERIC DNA. Opresko PL[1], Otterlei M[2], Fan J[1], Kolvraa S[3], Wilson DM[1], Seidman MM[1], Bohr VA[1]. [1]Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, MD 21224. [2]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, N-7489 Trondheim, Norway. [3]Institute of Human Genetics, University of Aarhus, 8000 Aarhus C, Denmark.

We are examining roles for the Werner syndrome (WS) protein, a RecQ helicase, in recombination repair at telomeres. WS is characterized by cellular defects associated with telomere dysfunction including genomic instability, premature senescence, and accelerated telomere erosion. In yeast the RecQ helicase Sgs1p acts in an alternative lengthening of telomeres (ALT) pathway via recombination. We found the WRN protein associates with telomeres when dissociation of telomeric D-loops is likely during replication and recombination. In human ALT cells WRN associates directly with telomeric DNA. The majority of TRF1/PCNA co-localizing foci contained WRN in live S-phase ALT cells, but not in telomerase positive HeLa cells. Biochemically, the WRN helicase and 3' to 5' exonuclease act simultaneously and cooperate to release the 3' invading tail from a telomeric D-loop *in vitro*. The telomere proteins TRF1 and TRF2 limit digestion by WRN. We propose roles for WRN in dissociating telomeric structures for ALT pathways. We are also examining base excision repair (BER) at telomeres. Oxidative stress was found to induce telomere dysfunction and erosion by unknown mechanisms (von Zglinicki, 2002). One possibility is that oxidative damage in telomeres interferes with binding by the telomere maintenance proteins TRF1 and TRF2, and thus disrupts telomere structure/function. We found site specific 8-oxo-guanine and abasic lesions in telomeric DNA interfere with TRF1 and TRF2 binding *in vitro*. Thus, repair of modified bases in telomeres is likely important for telomere function. Consistent with this, we found TRF2 interacts with proteins involved in BER, including FEN-1 and DNA polymerase Beta. We are studying roles for BER and TRF2 protein interactions in preserving telomere function.

133 VALIDATION OF A MODIFICATION OF THE SYRIAN HAMSTER EMBRYO (SHE) CELL TRANSFORMATION ASSAY AT PH 6.7 USING DIFFERENT CELL ISOLATES AND THE RODENT CARCINOGEN 2,4-DIAMINOTOLUENE. Pant K[1], Reece JD[1], Gibson DP[2], Aardema M[2], San R[1]. [1]BioReliance, Rockville, MD 20850. [2]The Proctor & Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45252.

The Syrian hamster embryo (SHE) cell transformation is used as *in vitro* screening test for carcinogenesis. In this study, the standard test procedures were used with the following modification. Target cells were grown for only five hours instead of usual 24 hours (H. Zhang, Mutat. Res. 548, 1-7, 2004) prior to detaching from the flask and seeding in the plates. Using this modification and three different cell isolates, reproducible results were obtained with the promutagen benzo(a)pyrene. Traditionally, chemicals have been tested in this assay using a 24-hour and/or 7-day exposure. With certain chemicals, the length of exposure has been observed to impact the outcome of the assay and may have mechanistic implications (LeBoeuf et al., Mutat. Res. 356, 85-127, 1966). 2,4-DAT, a known rodent carcinogen, has previously been shown to be positive in this assay using a 7-day exposure (ibid.). In the present study, 2,4-DAT was tested using a 24-hour dosing regimen in a preliminary toxicity determination with concentrations ranging from 25 μg/mL to 1250 μg/mL. Based on the toxicity profile, the transformation assay was performed at concentrations of 50 to 1250 μg/mL. The concentrations of 50, 100, 250, 400 and 500 μg/mL were scored for morphological transformation. The morphological transformation frequency for these concentrations (1.5% to 5.02%) was well above that of the solvent control (0.68%). Thus, 2,4-DAT gave a positive response in the SHE cell transformation assay with 24-hour exposure regimen.

134 DETECTION OF MRNA *IN SITU* USING ROLLING CIRCLE AMPLIFICATION. Petibone DM[1], Thomas RA[1], Itoh S[1], Tucker JD[1]. [1]Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

The cytoplasm and nuclei of eukaryotic cells contain a myriad of mRNA species, some of which produce proteins involved in cell-cycle regulation and DNA repair. While some mRNAs are expressed at relatively high levels, and are detectable by conventional *in situ* detection methods, characterizing the temporal and spatial presence of rare mRNAs in tissue samples remains problematic. Identifying changes in gene expression levels in response to adverse environmental conditions in a cell specific manner may help determine key players in these responses. RCA is a ligation dependent isothermal polymerization reaction that generates a linear single-stranded nucleic acid concatemer from a ssDNA circularized padlock. If labeled nucleotides are incorporated into the polymerization reaction, the resulting DNA product can be quantified directly or indirectly through fluorescently conjugated antibodies. The labeled RCA product is capable of generating a large signal from low-copy mRNA that can, in turn, be visualized *in situ*. Here we have used the RCA technique as a method for *in situ* detection of GAPDH gene expression and semi-quantitative analysis of changes in induced IL-2 gene expression levels in human lymphoblastoid cell lines. Padlock oligonucleotides specific for GAPDH and IL-2 mRNA were compared to a padlock negative control (null) with an artificial ligation target. Un-ligated and null padlocks were removed from the cells prior to setting up polymerization reactions. Gene expression levels show increases over the null control of up to ~10-fold. Potential applications for RCA *in situ* include a determination of cell specific changes in cell-cycle regulation, DNA repair gene expression in response to mutagenic agents, and analysis of gene expression to unravel pathways involved in tumorogenesis.

135 THE GENOTOXICITY OF THE DRINKING WATER DISINFECTION BY-PRODUCT IODOACETIC ACID IS REDUCED BY MODULATORS OF OXIDATIVE STRESS. Plewa MJ[1], Cemeli E[2], Anderson D[2], Wagner ED[1]. [1]University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA. [2]University of Bradford, Bradford, UK.

During the twentieth century the disinfection of drinking water was an outstanding achievement in protecting the public health against water borne diseases. An unintended consequence of chemical disinfection of water is the generation of drinking water disinfection by-products (DBPs). Recently a U.S. EPA study reported the presence of iodoacid DBPs in finished drinking water in Region 6 of the U.S. We recently reported that iodoacetic acid (IA) expressed the highest genotoxic potency of any DBP analyzed in a mammalian cell system. One possible mechanism of this genotoxic damage is via the induction of oxidative stress. We used nontoxic concentrations of catalase and butylated hydroxyanisole (BHA) to determine if these agents could affect the generation of IA-induced genomic damage in Chinese hamster ovary (CHO) cells. The SCGE (comet) assay was used as the genotoxic indicator test. IA at a concentration of 25 μM did not induce cytotoxicity in exposed CHO cells during the 4-h treatment time. Concentrations of catalase from 100-500 U/ml or BHA from 10-100 μM were not cytotoxic or genotoxic in treated CHO cells. IA at a concentration of 25 μM induced a significant increase in the SCGE tail moment of CHO cells treated for 4 h. Exposing CHO cells to 500 U/ml catalase and 25 μM IA resulted in a 42% reduction in DNA damage as compared to the cells treated with IA alone. BHA concentrations of 10, 50 and 100 μM caused a concentration-dependent reduction of IA-induced DNA damage of 75%, 83% and 87%, respectively. These data indicate that agents that repress oxidative stress-induced DNA damage also repress the genotoxic potency of iodoacetic acid.

136 FURTHER DEFINING PROTEIN-PROTEIN INTERACTIONS BETWEEN TWO MULTI-FUNCTIONAL HUMAN DNA REPAIR PROTEINS, XERODERMA PIGMENTOSUM-G AND POLY(ADP-RIBOSE) POLYMERASE. Pluth JM[1], Zahed Karagaran H[1], Campeau E[1], Cooper PK[1]. [1]Lawrence Berkeley National Lab, Berkeley, CA 94720.

Recently we determined that three XPG-deficient patient cell lines show decreases in total PARP protein levels as compared to a number of normal fibroblast lines. This observation led us to investigate a possible interaction between these proteins in normal cells. Immmunoprecipitations from nuclear extracts indicate that XPG and PARP interact. We investigated possible functional interactions between these proteins by measuring PARP activity in the presence of XPG in combination with various DNA substrates. PARP auto-ribosylation was stimulated by XPG with all DNA substrates tested, but most significantly with Holliday junction substrates. Using isogenic XPG-complemented and non-complemented hTERT immortalized lines we have determined that complementation of cells with XPG does not restore PARP levels prior to damage, but after damage (H_2O_2 or IR), XPG-complemented lines show greater PARP protein accumulation as compared to non-complemented cells. Our observation that XPG complementation reduces UV sensitivity but not H_2O_2 sensitivity may be related to the very low levels of PARP in the XPG-deficient cell lines. We hypothesize that during the establishment of XPG-deficient cell lines, changes are selected for, which allow for optimal survival, one of which may be lowered PARP levels. We are in the process of complementing XPG-deficient lines with PARP to determine whether this will rescue the H_2O_2 sensitivity observed in both the XPG-complemented and non-complemented lines. In addition, we are performing studies using siRNA to determine what the immediate and long-term effects of XPG loss may be in a cell. These studies should help to define a genetic and possibly functional interaction between PARP and XPG in genomic maintenance.

137 CHARACTERISTICS OF INDUCED INSTABILITY AT A MOUSE TANDEM REPEAT LOCUS. Polyzos AA[1], Parfett CL[1], Healy C[1], Yu T[1], Douglas G[1], Yauk CL[1]. [1]Environmental Health Centre, Health Canada, Tunney's Pasture, 0803A, Ottawa, ON, K1A 0L2, Canada.

Expanded simple tandem repeat (ESTR) loci are non-coding DNA region in the mouse genome that show a high level of instability resulting from loss or gains of repeat units but with several characteristics differing from other classes of tandemly repeated DNA. The frequency and extent of both induced and spontaneous mutations at ESTR loci such as *Ms6-hm* have facilitated their detection using PCR methods. Analysis of pedigrees or direct amplification of single DNA molecules (individual ESTR alleles) has previously indicated that *Ms6-hm* is unstable in both mouse germline and somatic cells. Mutation levels increase when premeiotic and meiotically active germ cells are treated to genotoxic insults (such as ionizing radiation or direct DNA alkylation (using ENU)) resulting in common profiles of size alterations to those of uninduced mutations. This might be indicative of the participation in induced instability of a non-meiotic mitotically associated process such as replication slippage. The link between these effects and genotoxicity is indirect or epigenetic. ESTR instability in cultured murine cells from both genotoxic and non-genotoxic carcinogens (ionizing radiation, DNA alkylation (ENU), oxidative stress (xanthine oxidase generated oxygen radicals) and TPA) has been investigated to probe induced mutation in cells not undergoing meiosis.

138 SEMI-QUANTITATION OF POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DNA ADDUCTS IN HUMAN CERVIX BY IMMUNOHISTOCHEMISTRY AND THE AUTOMATED CELLULAR IMAGING SYSTEM (ACIS). Pratt MM[1], Castle PE[2], Schiffman M[2], Glass AG[3], Scott DR[3], Rush BB[3], Olivero OA[1], Poirier MC[1]. [1]Carcinogen-DNA Interactions Section, Center for Cancer Research, NCI, NIH, Bethesda, MD. [2]Division of Cancer Epidemiology and Genetics, NCI, NIH, DHHS, Bethesda, MD. [3]Northwest Kaiser Permanente, Portland, OR.

Epidemiological studies have shown that, among women with cancer-associated HPV-infection, smoking may increase the cervical cancer risk an additional 2-4-fold (Castle et al., JNCI, 94:1406, 2002). It is therefore of interest to examine human cervical tissue for PAH-DNA adduct formation. Previously, antiserum elicited against DNA modified with anti (+/-)r-7,t-8-dihydroxy-t-9,10-epoxy-benzo-[a]pyrene (BPDE) was validated for semi-quantitative immunohistochemistry (IHC) of human esophagus using the ACIS (van Gijssel et al., CEBP 11:1622, 2002). Here we report optimization of IHC conditions for PAH-DNA adduct visualization in human cervical biopsy tissue. In order to prepare an internal standard, a low-dose BPDE-exposed human keratinocyte standard curve was generated using *in vitro* cultured human cervical keratinocytes exposed for 1 hr to 0, 0.053, 0.153, or 0.310 uM BPDE. Keratinocytes showed parallel dose-dependent increases in BPDE-DNA adducts by IHC/ACIS analysis and quantitative chemiluminescence immunoassay (CIA). In a pilot study, the presence of nuclear PAH-DNA staining was demonstrated in 2 human cervical biopsy samples from HPV-infected women. In these samples the PAH-DNA staining, visible with BPDE-DNA antiserum, was reduced 82 and 89% in parallel sections stained with specific antiserum that had previously been absorbed with the BPDE-DNA immunogen. ACIS values for specific antiserum staining were 12.2 and 6.9 arbitrary units (AUs)/nucleus, compared to 1.4 and 1.2, respectively, for parallel sections stained with absorbed serum. Therefore, the observed nuclear staining is specific for PAH-DNA adduct formation. Further studies will explore the usefulness of PAH-DNA adduct formation as an indicator of cervical cancer risk in smoking women with cancer-associated HPV infection.

139 INHIBITION OF INDUCIBLE GENE EXPRESSION BY CHROMIUM. Puga A[1]. 1Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0056.

Co-contamination with complex mixtures of carcinogenic metals, such as chromium, and polycyclic aromatic hydrocarbons is a common environmental problem with multiple biological consequences. Chromium exposure alters inducible gene expression, forms chromium-DNA adducts and chromium-DNA cross-links, and disrupts transcriptional activator/coactivator complexes. We have previously shown that exposure of mouse hepatoma Hepa-1 cells to chromate inhibits the induction of the *Cyp1a1* and *Nqo1* genes by dioxin. We have tested the hypothesis that chromium blocks gene expression by interfering with the assembly of productive transcriptional complexes at the promoter of inducible genes. To this end, we have studied the effects of chromium on the expression of genes induced by B[a]P, another aryl hydrocarbon receptor (AHR) agonist, and characterized the disruption of Cyp1a1 transcriptional induction by chromium. Gene expression profiling using high density microarray analysis revealed that the inhibitory effect of chromium on B[a]P-dependent gene induction was generalized, affecting the induction of over 50 different genes involved in a variety of signaling transduction pathways. The inhibitory effect of chromium on Cyp1a1 transcription was found to depend on the presence of promoter-proximal sequences and not on the cis-acting enhancer sequences that bind the AHR complex. Using transient reporter assays and Chromatin immunoprecipitation analyses, we found that chromium prevented the B[a]P-dependent release of histone deacetylase-1 from Cyp1a1 chromatin and blocked p300 recruitment. These results provide a mechanistic explanation for the observation that chromium inhibits inducible, but not constitutive gene expression.

140 CONDITIONAL EXPRESSION OF HOGG1 IN MITOCHONDRIA IMPROVES MITOCHONDRIAL REPAIR OF NO-INDUCED DNA DAMAGE AND ENHANCES CELL SURVIVAL. Rachek LI[1], LeDoux SP[1], Wilson GL[1]. 1Department of Cell Biology and Neuroscience, College of Medicine, University of South Alabama, Mobile, Alabama 36688.

Nitric oxide (NO), a free radical inorganic gas, has been shown to have a biphasic action on cell killing with low concentrations exhibiting a protective effect against cell death, whereas higher concentrations are toxic. There is good evidence that NO is genotoxic, i.e. it can cause DNA damage and mutations. Recently, it has been reported that NO can be generated by mitochondria. Mutations in mitochondrial DNA (mtDNA) have been associated with a variety of diseases including diabetes, ischemic heart disease, Parkinson's disease, Alzheimer's disease, and the normal process of aging. These mutations could be partially the result of either increased damage to mtDNA or decreased repair of this damage, or a combination of both factors. It has been shown that NO damages mtDNA to a greater extent than nuclear DNA. Previously, we have reported that conditional targeting the DNA repair protein hOGG1 to mitochondria augmented mtDNA repair of oxidative damage in this organelle and enhanced cellular survival. In the current studies we used HeLa TetOff/MTS-OOG1 transfected cells that conditionally expressed OGG1 in mitochondria in the absence of the doxycycline (Dox-) to evaluate the effect of additional hOGG1 expression on the repair of NO-induced mtDNA damage and cell survival. Dox ± HeLa TetOff/MTS-OOG1 and vector transfectants were exposed to NO produced by the rapid decomposition of 1-propanamine, 3-(2-hydroxy-2-nitroso-1-propylhydra-zino) (PAPA NONOate) and the resultant damage to mtDNA was determined by quantitative Southern blot analysis. Functional studies revealed that cells expressing recombinant OGG1 were more proficient at repairing NO-induced damage in their mtDNA, and this increased repair led to increased cellular survival following NO-exposure.