Poster Abstracts

DNA Repair and Damage Responses

P1

Double Strand Break Repair inHuman Mitochondrial Extracts. Hunter SE, Collins L, Van Houten B. National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States.

Mitochondrial DNA (mtDNA) is prone to double strand breaks (DSBs); however the nature of its repair in human cells is not understood. DSB repair was assayed in highly purified human mitoplasts using a newly developed quantitative PCR-based assay that measures ligation across a restriction site. DNA containing 5' or 3' overhangs is repaired more efficiently than blunt-ended DNA (13%, 8% and 3% repaired, respectively). Further investigation of the rejoining of PstI-generated DSBs revealed appreciable DNA processing, resulting in the loss of ~50 bases surrounding the PstI site. Sequence analysis of over 100 ligation products revealed several patterns of repaired DNA, most with deletions spanning 4-7 bp direct repeats. We hypothesize that broken DNA is resected to reveal regions of microhomology, allowing annealing and ligation. We propose that the 3'→5' resection is mediated by the exonuclease activity of DNA polymerase gamma and the 5'→3' exonuclease responsible for the resection is hSNM1B. hSNM1B has a predicted mitochondrial targeting sequence (MTS) with a putative cleavage site of 70 amino acids. Using immunofluorescence, hSNM1B-GFP is shown to be targeted to mitochondria. Furthermore, the hSNM1B MTS is both necessary and sufficient for mitochondrial localization. Studies are underway to determine the precise role of hSNM1B in mitochondrial DSB repair. There are several clinical syndromes associated with mtDNA deletions between direct repeats that may be associated with DSB repair. The study of mtDNA DSB repair will help clarify the underlying mechanisms of these diseases.

P2

Homologous Recombination Contributes Substantially to Base-Substitution Mutagenesis in Mammalian Cells. Hinz JM, Urbin SS, Thompson LH. Lawrence Livermore National Laboratory, Livermore, CA, United States.

In vertebrates the pathway of homologous recombination repair (HRR) includes five Rad51 paralogs (XRCC2-3, Rad51B-C-D), which are not absolutely required for cell viability, unlike Rad51. Mammalian Rad51 paralog mutants show broad-spectrum mutagen sensitivity and extreme sensitivity to DNA crosslinking. We reported that the *rad51d* null CHO mutant has a 12-fold elevated rate of spontaneous *hprt* mutations, 86% of which are deletions. To test whether HRR, often referred to as an "error-free" process, influences the rate of base substitution (BS) mutagenesis, we measured mutation rates at the *Na^+/K^+-ATPase* locus, which scores only BS events detected by ouabain resistance (Oua^R). In the *rad51d* mutant and its isogenic gene-complemented control, we measured both spontaneous Oua^R mutation rates by fluctuation analysis and ethylnitrosurea-induced mutant frequency. Intriguingly, in *rad51d* cells the spontaneous mutation rate was reduced ~2.5-fold, and induced mutations were 2-fold lower than in control cells. Importantly, we conclude that at least 50% of BS mutations in this model system arise through error-prone polymerase(s) acting during HRR-mediated restart of broken replication forks. This idea is consistent with recent findings that the phenotype of polζ null mouse cells resembles that of HRR mutants in terms of chromosomal instability and broad-spectrum mutagen sensitivity. In summary, the HRR pathway efficiently prevents chromosomal discontinuities at the minor expense of producing BSs. (Work performed under the auspices of the US DOE by LLNL under Contract DE-AC52-07NA27344; funded by NIH grant CA112566.)

P3

The Role of the DDB1-CUL4B^{DDB2} E3 Ubiquitin Ligase in Nucleotide Excision Repair. Kapetanaki MG[1,2], Guerrero-Santoro J[1,2], Hsieh CL[1,2], Gorbachinsky I[1], Levine AS[1,2], Rapic-Otrin V[1,2]. [1]Microbiology and Molecular Genetics, School of Medicine, Univ. of Pittsburgh, Pittsburgh, PA, United States, [2]Hillman Cancer Ctr., Univ. of Pittsburgh Cancer Institute, Pittsburgh, PA, United States.

The nucleotide excision repair pathway (NER) preserves the integrity of the genome by removing a variety of bulky lesions from DNA. The UV-damaged DNA-binding protein complex (UV-DDB) is involved in the recognition of chromatin-embedded UV-damaged DNA, and it is a component of the Cullin4A (CUL4A)-based ubiquitin ligase, DDB1-CUL4A^{DDB2}. We previously demonstrated that DDB1-CUL4A^{DDB2} ubiquitinates histone H2A at the sites of UV-lesions depending on functional DDB2. Consequently, in xeroderma pigmentosum group E (XP-E)cells DDB1-CUL4A^{DDB2} fails to bind UV-damaged chromatin. The fact that, in addition to CUL4A, Cullin4B (CUL4B) co-purifies with the UV-DDB complex prompted us to ask whether CUL4B had a role in NER as a separate E3 ubiquitin ligase. In this study, i) we gathered evidence that CUL4A and CUL4B form two individual E3 ligases, DDB1-CUL4A^{DDB2} and DDB1-CUL4B^{DDB2}, and ii) we investigated CUL4B's possible role in NER. For that purpose, we examined CULB's subcellular localization and found that a) in contrast to CUL4A, CUL4B is localized in the nucleus and facilitates the transfer of DDB1 into the nucleus independently of DDB2 and b) CUL4B binds to UV-damaged chromatin as a part of the DDB1-CUL4B^{DDB2} in the presence of functional DDB2. Furthermore, DDB1-CUL4B^{DDB2} is more efficient than DDB1-CUL4A^{DDB2} in mono-ubiquitinating histone H2A in vitro. Overall, this study suggests that DDB1-CUL4B^{DDB2} E3 ligase may have a distinctive function in modifying the chromatin structure at the site of UV-lesions to promote efficient NER.

P4

Hypersensitivity of Mitochondrial Mutant Cells to Ionizing Radiation, Phleomycin and Mitomycin C. Kulkarni RM, Reither A, Thomas RA, Tucker JD. Wayne State University, Detroit, MI, United States.

Oxidative phosphorylation is under control of the mitochondrial and the nuclear genomes. Many mitochondrial diseases result from mutations in mitochondrial-encoded genes involved in ATP synthesis. Leber optic atrophy and Leigh's syndrome are two such diseases and have phenotypes associated with energy deprivation. We hypothesized that when exposed to genotoxic agents, these mutant cells would exhibit reduced ability to repair nuclear DNA damage and have increased levels of chromosome damage and increased cell death. To test the hypothesis, we exposed normal and mitochondrial mutant cells to mitomycin C, phleomycin and ionizing radiation and measured chromosome damage and mitotic indices. Post exposure, the mutant cells showed increases in chromosomal aberrations and decreases in the mitotic indices compared to normal cells. In the mutant cells we observed a nearly complete lack of cells in metaphase exposed to doses above 2 Gy. After exposure to phleomycin above 2.5 µg/ml, none of the cells bearing the Leigh's mutation reached metaphase, and cells with Leber's mutation showed the same effect above 10 µg/ml. Exposure to mitomycin C showed a lack of cells in metaphase above 75 ng/ml in both cell lines. We conclude that when exposed to genotoxic agents, the mutant cells exhibit impaired ability to repair DNA damage. These observations may help explain the role of ATP in increased sensitivity of mitochondrial mutant cells to genotoxic agents, suggesting that individuals with mtDNA mutations may be at higher risk for cancer and other diseases that result from an accumulation of DNA damage.

P5

Age-Dependent Accumulation of Mitochondrial and Nuclear DNA Damage in Spermatogenic Cells Derived from APE1$^{+/-}$ Mice. Acevedo-Torres K[1], Walter C[2], Ayala-Torres S[1], Torres-Ramos CA[1]. [1]University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico, [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, United States.

Increased spontaneous mutagenesis has been demonstrated in spermatogenic cells obtained from old mice, similar to the human paternal age effect. Decreased expression of the base excision repair (BER) protein APE1 appears to at least partially mediate the increased mutagenesis in spermatogenic cells from old mice. Recent evidence has indicated that mitochondrial DNA (mtDNA) is a major target of oxidative stress, but the integrity of mitochondrial DNA in spermatogenic cells has not been well characterized. We sought to test two hypotheses: 1) that mtDNA damage increases in an age-dependent fashion in mouse spermatogenic cells and 2) that mice deficient in APE1 (heterozygous mice; APE1+/-) show accelerated damage in mtDNA. For this purpose, DNA from germ cells was obtained from 6-, 16- and 28-month- old wild-type (WT) and APE1+/- mice and mtDNA damage was measured by quantitative PCR. We detected an age-dependent increase in mtDNA lesions in both WT and APE1+/- mice. Interestingly, the 6- and 16-month- old APE1+/- mice show higher levels of mtDNA damage than the WT mice. We also measured levels of damage in the nuclear genome and our results show that the deficiency in APE1 also leads to an age-dependent increase in nDNA lesions in both WT and APE1+/- mice. However, only the 6- month-old APE1+/- mice exhibit higher levels of nDNA lesions as compared to WT. In summary, our results show that reduced APE1 leads to an age-dependent increase in mtDNA and nDNA damage and highlights the relevance of BER in the maintenance of mtDNA integrity in spermatogenic cells.

P6

Chromodomain Helicase DNA Binding Protein 2 and DNA Damage Response Signalling. Rajagopalan S[1], Nagarajan P[1], Mahadevan B[3], McDonald H[2], Matteson KJ[1], Venkatachalam S[1]. [1]University of Tennessee, Knoxville, TN, United States, [2]Oak Ridge National Laboratory, Oak Ridge, TN, United States, [3]Schering-Plough Research Institute, Summit, NJ, United States.

Eukaryotic cells respond to DNA damage by activating DNA repair or apoptosis related genes. Chromatin modifiers play an important role in this process by bringing about alterations in chromatin structure to allow access to various nuclear factors. Chromodomain helicase DNA binding proteins (CHD) are highly conserved chromatin remodeling factors that share sequence motifs and functional domains. To better understand the functional role of Chromodomain helicase DNA binding protein 2 (Chd2) in mammals, we have generated a mutant mouse model. Phenotypic analysis of the mutant model indicates that the Chd2 protein plays a critical role in tumour suppression as the heterozygous mutant mice develop lymphomas. Chd2 deficient cells generated from the homozygous mutant embryos, exhibited growth defects, accumulated higher levels of γH2AX, and exhibited an aberrant DNA damage response after X-ray irradiation. In addition, Chd2 is phosphorylated after DNA damage and Chd2 mutant cells exhibit increased chromosomal aberrations after treatment with low doses of X-ray irradiation (2 Gy). Experiments are underway to further determine the role of Chd2 in the repair of X-ray induced DNA strand breaks using Comet assays. To get additional insights on the molecular functions of Chd2 we have also performed experiments to identify Chd2 associated proteins via tandem affinity purification followed by mass spectrometric analysis. Experiments are being performed to identify Chd2 interacting proteins and determine the role of Chd2 in DNA damage responses.

P7

Use of a Werner Helicase Knockdown Cell Line to Investigate the Role of *WRN* in Mammalian Recombination. Rahn JJ, Della-Coletta L, Lowery M, Limanni T, Adair G, Nairn R. UT MD Anderson Cancer Center, Science Park Research Division, Smithville, TX, United States.

Werner syndrome in a rare autosomal recessive condition resulting in premature aging, overall genetic instability and predisposition to cancer. Werner syndrome patients have mutations in the Werner helicase gene (*WRN*), which encodes a member of the RecQ helicase family. While all the functions of *WRN* in humans are not precisely understood, its potential yeast homologs Sgs1 and Srs2 have been shown to be involved in recombinational repair of double-strand breaks by suppressing crossovers and processing non-homologous ends. In order to investigate the potential role of *WRN* in mammalian cell mitotic homologous recombination, a stable shRNA *WRN* knockdown CHO cell line was established. A previously developed gene targeting assay system at the hemizygous *APRT* locus was employed using insertion-type vectors with long and short end-blocking 3'-OH nonhomologies. This assay examined the effects of *WRN* knockdown on recombination efficiency and the distribution of crossover vs. conversion recombinants. Both targeted and untargeted recombination rates were slightly reduced in *WRN* knockdown cell lines compared to the parental cell line. However, recombinants obtained from the *WRN* knockdown cell line were predominantly gene conversions, in marked contrast to parental recombinant clones, which reflected a variety of events (gene conversions, targeted integrations and vector corrections). These data suggest a potential role for *WRN* in the synthesis-dependent strand annealing pathway analogous to that of yeast Srs2. Supported by CA097175.

P8

Limited Base Excision Repair in *C. elegans*. Lehmann DW, Asagoshi K, Prasad R, Wilson SH, Van Houten B. National Institute of Environmental Health Sciences, Durham, NC, United States.

Caenorhabditis elegans, an invaluable laboratory species in recent years, has a largely undefined DNA repair system. Searches for homologous genes between mammals and nematodes show a high degree of conservation. Mismatch repair, nucleotide excision repair, homologous repair all show high degrees of homology (100%, 93%, and 89% respectively), however, base excision repair (BER) only has 53% homology to known BER features in the nematode. The existence of uracil glycosylase (UDG) and two AP endonucleases (APE) have been previously identified. We have found proteins, based on Western blot analysis, that cross-react with mammalian directed antibodies for UDG, ligase 1 (lig1), XRCC1, and DNA polymerase beta (polß). Using nicking assays, we have attempted to identify the base removal activity of thymine glycol (TG), 8-oxoguanine (8oxoG), and uracil. For the first time we are able to demonstrate that extracts prepared from L1 nematodes are capable of conducting uracil-mediated base excision repair reaction, where subsequent to actions of UDG and APE, *C. elegans* DNA polymerase(s) was able to fill a single-nucleotide gap in the presence of 5'terminal deoxyribose phosphate flap. We are currently cloning putative polypeptides identified via conserved protein sequence homology searches in an attempt to verify which are involved in this reaction. In addition, we demonstrate *in vivo* repair kinetics against hydrogen peroxide, juglone, and MMS in wild-type and knockout mutants. The presence of a fully intact BER system in the nematode model is evident.

P9

hMSH6: DNA Mismatch Repair or Damage Signaling? Kaliyaperumal S, Williams K. University of Toledo Health Science Campus, Toledo, OH, United States.

The DNA Mismatch Repair (MMR) pathway accurately repairs base mismatches and insertion-deletion loops (IDLs) to help maintain genomic integrity. MMR proteins also recognize specifically damaged bases after exposure of the cell to alkylating agents. Within human cells, mismatches such as G:T, or alkylated mispairs such as O^6meG:T, are bound by hMutS alpha (hMSH2+hMSH6). hMutL alpha (hMLH1+hPMS2) interacts with the DNA-bound hMutS alpha and is required for subsequent repair (G:T) or DNA damage signalling (O^6meG:T). Studies by us (and others) have shown that hMSH6 is phosphorylated at several serines within the N-terminus, both in vitro and *in vivo*. We are investigating the role of hMSH6 phosphorylation in the subsequent processing of a DNA mismatch as compared to DNA alkylation damage. We have found that hMSH6 is phosphorylated to a higher extent in the presence of a G:T mismatch rather than O^6meG:T. Conversely, gel shift-westerns demonstrate that a higher concentration of hMSH6 protein binds to O^6meG:T than to G:T mismatched DNA. We have also found that hMSH6 and hPMS2 are bound to chromosomal DNA for up to 48 hours after alkylation damage. However, if TPA is added several hours before harvesting for protein-bound chromosomal DNA, less hPMS2 is bound to the alkylation-damaged chromosomal DNA. This indicates that the phosphorylation status and the total amount of bound hMSH6 are both important for differentiation of signalling for mismatch repair (G:T) or for DNA damage pathway activation (O^6meG:T). This work was supported by NIH CA84412 (KJW) and NIH CA106575 (KJW).

P10

The Oxidative DNA Lesions, 8,5'-Cyclo-2'-Deoxyadenosine and 8-Oxo-7,8 Dihydro-2'-Deoxyadenosine, Differentially Alter the Binding of HSF1 and CREB Transcription Factors to Target DNA Sequences. Abraham J, Brooks P. National Institute on Alcohol Abuse and Alcoholism, Rockville, MD, United States.

Humans are constantly being exposed to environmental factors that can covalently modify DNA bases to form stable lesions. If not repaired, these lesions can lead to transcription/replication blocking or mutagenic bypass. Our previous work has focused on 8, 5'-cyclopurine deoxynucleosides, a unique class of oxidative DNA lesions that are specifically repaired by the NER pathway (see Brooks, *DNA Repair* 2008). Here we used EMSA to monitor the ability of two sequence-specific transcription factors, heat-shock factor 1 (HSF) and CREB, to bind to their target sequences when 8, 5'-cyclo-2'-deoxyadenosine (cyclo-dA) is present within their recognition sequences. For comparison we also tested the effect of 8-Oxo-2'-deoxyadenosine (8-Oxo-dA) in the same recognition sequences. Heat-shocked nuclear extract was used to evaluate the changes in binding activity of HSF. The cyclo-dA lesion in the target sequence essentially eliminated the binding activity of both HSF and CREB. In contrast, 8-Oxo-dA had no obvious effect on HSF-binding activity in comparison to lesion-free DNA. Notably, though, CREB binding increased when an 8-oxo-dA lesion was present in the CRE-target sequence. A competition EMSA showed about a 3-fold increased affinity for the 8-oxo-dA containing CREB target sequence compared to lesion-free DNA. Our results further emphasize the transcription inhibitory effects of the cyclo-dA lesion, and also identify a novel biological effect of 8-oxo-dA. We are currently addressing the mechanistic basis for differential effects of these lesions on transcription factor binding using molecular modeling.

P11

Role of Post-Translational Modifications in the Regulation of SV40 DNA Replication. Thompson EG, Henricksen LA, Dixon K. University of Arizona, Tucson, AZ, United States.

Proper control of DNA replication and repair is essential to prevent uncontrolled cell proliferation, cell death, or inherited genetic mutations. The regulation of core DNA processing proteins by post-translational modifications is one mechanism of functional control by the cell. One of these core proteins is replication protein A (RPA), a heterotrimeric protein (70, 34,14-kDa subunits) essential for DNA metabolism, including DNA replication, repair and recombination. RPA becomes phosphorylated on the N-terminus of RPAp34 in a cell-cycle dependent manner and in response to DNA damage *in vivo*. Previously it was observed that cellular extracts from UV-treated cells failed to support SV40 *in vitro* DNA replication, although replication activity was restored by titrating unmodified RPA back into the reaction. Thus, phosphorylation of RPAp34 in response to UV-induced DNA damage may directly influence the participation of RPA in different DNA pathways. In order to determine the mechanism of this inhibition, we are using phosphomimetic forms of RPAp34 to examine the importance of N-terminal phosphorylation sites to the ability of cellular extracts to support SV40 replication. Current data points to a cell-cycle dependent, switch-like response induced by RPAp34 hyperphosphorylation that inhibits replication in response to DNA damage. Studies also indicate that phosphorylation of RPA alters its structural conformation and protein-protein interactions. We are using the SV40 *in vitro* replication reaction with mutant RPAp34 proteins and DNA damaging agents to investigate the mechanism of this inhibition

P12

Susceptibility to Ionizing Radiation Induced Tumors and DNA Strand Break Repair in p53 Deficient and Wildtype Mouse Hematopoietic Stem Cells (HSC) *In Vivo* and *In Vitro*. French JE, Parron VI. NIEHS, NIH, Research Triangle Park, NC, United States.

Coordination of DNA strand break repair pathways is dependent on the phase of the cell cycle, DNA replication, and expression of the p53 tumor suppressor protein. The loss of heterozygosity (LOH) phenotype observed in ionizing radiation (IR) induced tumors is highly penetrant in the C57BL/6 (B6) mouse strain and is low in the DBA/2 (D2) strain. The magnitude of IR induced *Trp53* sequence loss is greatest in B6>B6C3F1>C3H>B6D2F1>D2. D2 alleles suppress both LOH and tumor prevalence. Using primary cultures of HSC from B6, C3, D2, and their F1 outcross progeny with B6 isogenic mice, we have observed significant strain or genetic differences in the abundance of DNA repair gene transcripts up to 3 h post-irradiation. Using flow cytometry, terminal deoxynucleotidyl transferase (TdT) or g-H2AX assays were used for quantification of DNA strand breaks. The magnitude and time required to resolve breaks is significantly different between haplotypes of HR and NHEJ genes. Misrepair of strand breaks and LOH may be a quantitative trait (polygenic) dependent upon the DNA damage repair capacity observed in each strain. Analysis of DSB repair gene haplotypes by similarity matrices indicates that haplotype diversity between isogenic strains is significant but some components of the DSB repair pathways are identical by descent. Determination of the allelic variants of mouse and human orthologs causally related to DNA damage and repair with altered function and susceptibility is critical in order to understand the differences in individual risk due from exposure to environmental mutagens.

P13

Radiation Quality Dependent Specific ATM-Mediated Phosphorylation. Whalen MK, Gurai SK, Zahed-Kargaran H, Pluth JM. Lawrence Berkeley National Lab, Berkeley, CA, United States.

To determine whether the physical differences between high and low LET radiation are reflected in the biological responses of exposed cells, we detailed phospho-protein profiles of three proteins (γH2AX, pATF2 $Ser^{490/498}$ and pSMC1 Ser^{957}) functional in radiation repair and signal transduction. Phosphorylated forms of these proteins have been documented to co-localize at sites of double strand breaks (DSBs) after low LET exposures, and two of these phosphorylations, pATF2 and pSMC1, are specifically ATM dependent. Flow cytometry-based methods were used to quantify total levels of each phospho-protein at various times after radiation. As expected, we observed a greater induction and persistence in γH2AX after Fe nuclei (high LET) exposure as compared to X-ray (low LET). In surprising contrast, pATF2 and pSMC1 showed markedly lower induction levels following Fe exposure as compared to equivalent doses of X-ray. Quantification of pATF2 and pSMC1 foci revealed fewer cells containing foci, and fewer foci per cell following Fe as compared to X-ray exposure. These findings suggest that responses to DSBs induced by high LET radiation are regulated differently from DSBs induced by low LET radiation. Further studies have been initiated to determine whether other ATM-mediated phosphorylations are also differentially regulated following high LET radiation, or whether these differences are unique to these pathways.

P14

Modeling the Expression Pathway Controls of the Radioadaptive Response Across Tissues in Mice. Bhattacharya S, Poylzos AA, Bhatnagar S, Chu SD, Lowe XR, Marchetti F, Wyrobek AJ. Lawrence Berkeley National Lab, Berkeley,CA, United States.

Tissues vary substantially in their sensitivity to radiation toxicity but little is known of tissue variation after low dose exposures. Exposure to low dose radiation can protect cells and tissues from subsequent DNA and chromosomal damage, a phenomenon known as the radioadaptive response. The goal of this research was to identify the molecular pathways associated with adaptive response protection in tissues. We investigated transcript profiles of six tissues (blood, bone marrow, cortex, cerebellum, and hippocampus) of C57BL/6J male mice isolated 4 h after a 200 cGy challenge dose, with and without a prior conditioning dose of 5 cGy. We applied bioinformatics analyses to identify and compare gene functions, gene interaction networks, and pathway enrichments among tissues of mice that received the conditioning plus challenge doses versus those that received the challenge dose only. Blood had the most differentially expressed genes associated with conditioning. Tp53- and Myc-associated gene networks and several pathways (IL-10, IL-6 and glucocorticoid receptor signaling) were enriched in blood, bone marrow and cortex of conditioned mice. Several gene functions (cell cycle and DNA repair, immune response and cell signaling) were more consistently modulated ($p<0.01$) across tissues of conditioned animals. Our findings provide insight into the molecular mechanisms and signaling pathways associated with the adaptive response in tissues after exposure to low dose ionizing radiation. Supported by the Department of Energy Low Dose Program and the Lawrence Berkeley National Laboratory LDRD Program.

P15

Towards Personalized Chemotherapeutics: Exploring Variations in DNA Damage/Repair in Cells and Tumors Treated with Carboplatin Using Accelerator Mass Spectrometry. Henderson PT[1,2], Li T[1], Zhang H[1], Malfatti M[2], Ma X[3], Turteltaub KW[2], de Vere White RW[2], Pan C-X[1]. [1]Biosciences and Biotechnology Division, Chemistry, Materials Earth and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, United States, [2]Department of Internal Medicine, Division of Hematology Oncology, School of Medicine, University of California, Davis, Davis, CA, United States, [3]Department of Biostatistics, School of Public Health, University of California, Los Angeles, CA, United States.

Accelerator mass spectrometry (AMS) is an ultrasensitive and precise method for detection rare isotopes that has found increasing use in DNA adduct detection over the last 15 years. We report progress towards biomedical applications of AMS for detecting cellular uptake and drug-DNA interactions. The mode of action for cancer drug carboplatin involves the formation of DNA adducts that leads to apoptosis in rapidly dividing cells. A variety of human cancer cell lines were exposed to a 100-fold concentration range of [^{14}C]carboplatin (1 μM and 100 μM). The carboplatin concentrations used represent a microdose (1/100th the therapeutics dose) and the therapeutic dose. Importantly, every cell line showed a linear dose-response with respect to DNA adduct formation as measured by AMS ($R>0.98$, $P < 0.001$). Nude mice with implanted tumors also showed a linear dose-response to [^{14}C]carboplatin administered via tail vein injection. Collectively, these data indicate microdosing as a promising strategy for development of predictive DNA adduct biomarkers of chemotherapy resistance. This work was performed in part under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Epigenetics

P16

Effects of Irradiation on DNA Methylation and Genomic Instability. Aypar U, Malkiel LN, Baulch JE. University of Maryland, School of Medicine, Baltimore, MD, United States.

Radiation is part of everyday life. In addition to natural background radiation, occupational, accidental and therapeutic exposures increase the radiation burden and potential for health risks. It has been shown that radiation via not only direct DNA damage, but also the initiation of genomic instability. The unexpectedly high frequency of transmission of genomic instability from irradiated cells to their progeny suggests the possibility that epigenetics, rather than conventional mutagenesis, play a role in radiation-induced genomic instability. One epigenetic mechanism in mammalian cells is DNA methylation at CpG dinucleotide sequences. Abnormal methylation patterns are usually found to have deleterious effects. In this study, we test the hypothesis that altered DNA methylation is an epigenetic effect of irradiation that has a mechanistic role in the induction and/or perpetuation of genomic instability. Normal human fibroblast cells (AG01522) and human colon carcinoma cells (RKO36) were exposed to x-rays, Fe ions and protons and evaluated for genomic instability and changes in methylation immediately following irradiation and at delayed time points using an arbitrarily primed methylation sensitive PCR screen. Preliminary data indicate differences in DNA methylation based on cell line and/or irradiation history. This method provides a global, unbiased measure of genomic DNA methylation, as well as a link between changes in methylation and radiation induced genomic instability. [This work is supported by NASA grants NNX07AT42G (JEB) and NNJ05HE73G (WFM).]

P17

Multiple Generational Exposure of *Drosophila melanogaster* to Ethanol Increased Toxic Effects of Chlorophenoxy Herbicides in Later Generations. van Gijssel HE, Gienger HM, Bata MA, Dobmeier AD, Blunck BM. Valley City State University, Valley City, ND, United States.

Chlorophenoxy herbicides have been used since the 1940's, meaning at least 3 generations of people have been exposed. Our lab has previously shown that the toxic effects of 2,4- Dichlorophenoxyacetic acid (2,4-D) and 2-Methyl-4-chlorophenoxyacetic acid (MCPA) increased over 4 generations of exposure using the *Drosophila* as a model organism. To exclude the effect of ethanol, which was used as a control, *Drosophila* were exposed to ethanol for 3 generations. Subsequently, embryos were collected using the 3rd generation parents and were added to vials containing food with either ethanol or 1 µM or 3 mM 2,4-D or MCPA. Vials were checked every 24 hrs and development was followed for 16 days. The experiment was repeated 3 times. Results showed that ethanol alone did not change development or survival of *Drosophila,* during 4 generations of exposure. However, development was significantly delayed in *Drosophila* exposed to 3 generation ethanol followed by 1 generation of chlorophenoxy herbicides. This delay was more pronounced than the delay found after exposure to 4 generations of chlorophenoxy herbicides or to 1 generation of chlorophenoxy herbicides without pre-exposure to ethanol. In addition survival was decreased in *Drosophila* pre-exposed to ethanol. Our data showed that mutigenerational exposure to ethanol alone does not affect development of *Drosophila* but increased the toxic effects of chlorophenoxy herbicides in later generations. The exact mechanism is not known, but epigenetic changes may be involved. We hope to use this model to study the role of alcohol consumption in susceptibility for disease.

P18

Spontaneous Mutagenesis Frequencies Correlate with APE1 Abundance in Murine Spermatogenic Cells. Perez M[1], Hildreth K[1], Herbert DC[1], McMahan CA[1], Izumi T[3], Mitra S[3], Walter CA[1,2]. [1]University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, [2]South Texas Veteran's Health Care System, San Antonio, TX, United States, [3]University of Texas Medical Branch at Galveston, Galveston, TX, United States.

Many dominant genetic disorders are associated with a paternal age effect due to an increased de novo mutant frequency (MF) in spermatogenic cells. The lacI transgenic mouse model also displays a paternal age effect and provides a system that can be manipulated to identify mechanisms that contribute to the paternal age effect. Base excision repair (BER) activity is high in murine spermatogenic cells but declines by 50% at old age. A decreased abundance of APE1, a BER protein, is associated with the decline in BER. In the present study, spontaneous MF was studied in spermatogenic cells obtained from 6-, 16-, and 26-month-old Ape1+/-, wildtype or human APE1 (hAPE1) transgenic mice to test the effects of altered APE1 abundance on germline mutagenesis. The prevalence of apoptotic cells was determined because cell death can potentially alter the MF. Spermatogenic cells obtained Ape1+/- mice displayed an elevated MF at 6- and 16-months-old compared to wildtype. At 26-months-old, MF was significantly and similarly further elevated in cells from wildtype and Ape1+/- mice. Spermatogenic cells from mice expressing hAPE1 displayed spontaneous MFs similar to wildtype mice at 6- and 16-months-old. However, cells obtained from hAPE1 mice at 26-months-old had a significantly lower MF than age matched controls. The reduced MF in spermatogenic cells from old hAPE1 mice was associated with a significantly greater prevalence of TUNEL positive cells. Combined, these results suggest that APE1 abundance plays a major role in regulating germline mutagenesis.

P19

Oxidative Stress, Antioxidant Defense System During Hepatocarcinogenesis Induced by Dietary Methyl Deficiency in Mice. Montgomery BA, Bagnyukova TV, Pogribny IP. National Center for Toxicological Research, Jefferson, AR, United States.

The methyl-deficient model of liver carcinogenesis in rodents is remarkably similar to the development of hepatocellular carcinoma (HCC) in humans and is one of the most extensively studied models of HCC. The pathological and molecular events leading to the HCC involves changes in transcription of many metabolism genes, apoptosis, increased hepatocellular proliferation, and substantial alterations of cellular epigenome, among others. Additionally, oxidative stress (OS) is believed to play a pivotal role in the pathogenesis of cancer, including liver cancer. In the present study, we examined the underlying mechanisms associated with the induction of OS in livers of mice fed a methyl-deficient diet (MDD), with special emphasis on the role of the antioxidant defense system. Here, we demonstrate that feeding C57BL/6J and DBA/2J mice MDD resulted in induction of OS state in the liver. The extent of OS was correlated, in a time-dependent manner, with the diminished capacity of the cellular antioxidant defense system, especially with the decreased expression and activity of the hydrogen-peroxide metabolizing enzymes, catalase and glutathione peroxidase. More importantly, these changes were more pronounced in the DBA/2J mouse strain susceptible to liver carcinogenesis. Together, these data clearly indicate the significance of antioxidant defense in the maintenance of a normal oxidant state in cells and strongly suggest the importance of OS in the promotion of liver carcinogenesis via induction of both genotoxic and epigenetic events.

P20

Mutagen-Exposed Female Germ Cells Mediate Delayed Mutagenesis in Early Stage Embryos. Gresham CS, Norris MB, Winn RN. ABEL University of Georgia, Athens, GA, United States.

Analyses of mutations in offspring transmitted by female germ cells have been hampered by numerous practical challenges. Further, genetic health risks based on analyses of male germ cells may not be directly applicable to female germ cells. To address the need for improved approaches to study mutagenesis mediated by female germ cells, we used a fish model that carries the *cII* mutation target gene. We detected mutations in the *cII* genes carried by offspring of ethylnitrosourea (ENU)-treated λ transgenic females and untreated, wildtype male medaka. Offspring that exhibited *cII* MF greater than 2-fold above controls were scored as mutants, and comprised up to 16% of the offspring, comparable to frequencies of mutant offspring derived from male germ cells. Mutant offspring exhibited a wide range of *cII* MFs, from ~2 to 500-fold (7.5 to 1585 $X10^{-5}$) induction compared to controls. Characterization of mutational spectra revealed both whole body and mosaic mutant offspring were produced, with mosaic mutants comprising the vast majority (93%) of mutant offspring. Whole body mutants were generated from mutations fixed either in gametes, or the one-cell stage, whereas mosaic mutants were generated from mutations fixed at or after the two-cell stage of development. These data show fixation of persistent DNA damage in mature oocytes was delayed until after fertilization. Consequently, as shown in male germ cells, post-fertilization processes contributed by the maternal genome, induce mutagenesis in early stage embryos. These data illustrate this fish model provides new insights into germ cell mediated mutagenesis.

P21

Susceptibility of Y Chromosome to DNA Damage. Xun L, Jia J, Wei F, Robbins w. UCLA, Los Angeles, CA, United States.

Introduction: Our group and others have shown that men exposed to borates in the workplace have a lower ratio of male to female offspring at birth. Subsequently, we showed elevated boron in biologic fluids predicted reduced ratio of Y to X chromosome bearing sperm (n=146 men, $p \leq 0.02$) not explained by conventional semen parameters, other exposures assayed simultaneously (Ca, Mg, Pb, Se, Cr, Cu, Sr, Zn), diet, alcohol, smoking, or known reproductive toxicants. The purpose of the present study was to determine if boron causes DNA damage preferentially in Y bearing sperm as a potential mechanism for reduced ratios of male:female offspring and Y:X sperm. Methods: Archived semen samples were randomly selected from three exposure groups (14 boron workers, 13 exposed community controls, 22 non-exposed controls). COMET assay was followed by *in situ* hybridization of COMETs with Y and X fluorescent probes; 200 cells were scored per sample. Results: Exposure group predicted Y:X ratio in COMETs (boron workers 0.92, community controls 0.95, non-exposed controls 1.00, $p<0.0001$)), as well as a trend in DNA damage (indexed by Tail length, Moment Arm, % DNA in tail), however, within each category of DNA damage, Y bearing sperm were over-represented in the heaviest damaged cells (deviation from expected 0.5, $p=<0.006$). Discussion: Finding heaviest DNA damage predominantly in Y bearing sperm is new. This was not modified by level of nicotine metabolite in blood, alcohol consumption, age, or boron exposure. It may suggest susceptibility of Y chromosome DNA to heavy damage and warrants further investigation.

P22

Tissue-Selective and Sex Differences in Brain of Mice Exposed to Ionizing Radiation. Koturbash I[1], Kutanzi K[1], Kolb B[2], Kovalchuk O[1]. [1]University of Lethbridge, Lethbridge, AB, Canada, [2]Canadian Centre for Behaviour Neuroscience, Lethbridge, AB, Canada.

For over 100 years ionizing radiation (IR) serves in medicine as a beneficial diagnostic and therapeutic instrument. However, there is a high risk that these benefits can be shadowed by the post-exposure complications that include cerebral necrosis, neurocognitive deficits and radiation-induced cancers. Despite the large number of studies and increasing interest from researchers, the exact mechanisms of post-exposure events in different brain regions remain enigmatic. With that in mind, we have evaluated early and delayed effects (6 and 96 hours after treatment) of ionizing radiation in selective brain tissues (cerebellum, frontal cortex, olfactory bulb and hippocampus) of male and female C57Bl6 mice. We have found the selective induction of DNA damage (measured by ROPS), global DNA methylation paralleled with the dysregulation of methylation machinery (DNMT3a, MeCP2, and MBD2), and an imbalance in proliferative and apoptotic proteins (PCNA, TRAX and p38) in irradiated brain tissues. Additionally, by analyzing the microRNA profile of the frontal lobe, hippocampal and cerebellar tissue of male and female mice, we have observed significant differences in the microRNA patterns in these brain regions, as well as gross perturbations of regulatory miRNAs in response to IR, that guide essential processes in the cell. Our findings constitute the first evidence of sex- and tissue-selective differences in the response of different brain regions to IR treatment.

P23

Epigenetic Regulation of Somatic and Transgenerational Response to Environmental Stress in Plants. Boyko A, Titov V, Yao Y, Kovalchuk I. University of Lethbridge, Lethbridge, AB, Canada.

Plants are capable of rapidly reprogramming patterns of gene expression, allowing fast acclimation and adaptation in response to specific environmental stress. We analyzed whether these conditions are associated with genome instability and whether the response is regulated epigenetically. For the experiments we used transgenic *Arabidopsis thaliana* plants carrying in the genome the substrate for the analysis of homologous recombination frequency (HRF). To analyze the mechanisms of epigenetic regulation we used Dicer-like mutants, *dcl2*, *dcl3*, *dcl4*. We found that various environmental stresses, such as UV, salinity, heavy metals and pathogens destabilize the genome of somatic cells. More over, we found that the immediate (G1) progeny of stressed plants inherited the changes in the genome stability. Next, by propagating this progeny with and without stress to the next generation (G2), we found the changes to persist only when plants were propagated in the presence of stress. The progeny of the stressed plants had hypermethylated genomes and showed increased tolerance to stress. While analyzing the response of *dcl2*, *dcl3 and dcl4* mutants to stress, we found these plants to differentially respond to all stresses and to be impaired in transgenerational response. It is thus possible that stress adaptation requires function of small RNAs and establishment of new pattern of genome methylation and chromatin structure. To summarize, here we report that stressed plants inherit the memory of stress and the maintenance of the response requires constant stress exposure.

P24

DMN Disturbs Sex Differentiation and Induces Transgenerational Damage From Affected Males. Ramos-Morales P, Vega CC. Lab Genetica y Toxicologia Ambiental, Fac Ciencias, Universidad Nacional Autonoma de Mexico, Distrito Federal, Mexico.

Transgenerational activity related to parental exposure to genotoxins had made evident that genetic screening must extend through several generations in order to improve our knowledge about their real *in vivo* effects. Some of the genetic damage which appears early in the lifespan of unexposed organisms, could happen as a consequence of the parental exposure. In a previous work we showed that the promutagen alkylant N-Nitrosodimethylamine (DMN) reduces the fertility, the progeny recovered from treated males and increases the somatic mutation events in the unexposed progeny of treated males. In this work we explore the reprotoxic and transgenerational effects of DMN on two *Drosophila* species: the well known *D melanogaster* and *D mülleri* which show yellowish testis that turn reddish in mature males. Third instar larvae fed until pupation standard food enriched with DMN. From each kind of fly, 40 males randomly chosen were individually mated with their respective untreated females. After the egg laid the number of gonads in each one *D mülleri* male was scored. As previously, DMN reduced the survival of exposed individuals, increased the male/female ratio and reduced the fertility and progeny/gonad recovered from treated males ($p<0.05$). In *D mülleri*, males with less than 2 gonads were recovered since the lowest concentration. The progeny/gonad was reduced according the number of gonads in parental male. 0 gonad males were quite sterile. The well known *D melanogaster* used together with *D mülleri* (colored testis) is a powerful tool to explore *in vivo* reprotoxic and transgenerational effects of genotoxins.

Mutagenesis and Carcinogenesis

P25

The Lowest Dose of Ionizing Radiation Detectable Using FISH Whole Chromosome Painting. Tucker JD, Luckinbill LS. Wayne State University, Detroit, MI, United States.

Chromosome translocations (TLs) are the hallmark of exposure to ionizing radiation and have a non-zero baseline frequency that increases with age and cigarette smoking. Sigurdson et al. (Mutat Res 652:112-121) recently published TL frequencies on 1,933 healthy, unexposed people. These data allow us to estimate the lowest radiation doses detectable using painting for TLs. To estimate these doses, we determined the number of TLs per 100 cell equivalents (CEs) in 5-year age intervals from 0 to 80+. The mean TL frequency per 100 CEs at ages 0, 20, 40, 60 and 80 are 0.04, 0.24, 0.67, 1.19 and 1.81, respectively. We then determined the number of TLs per 100 CEs needed in a putatively exposed individual that are in excess of these values in order to conclude that a significant increase had occurred. For these same ages, the numbers of excess TLs per 100 CEs are 0.15, 0.57, 1.01, 1.82, and 2.57. This dramatic rise indicates that the dose needed to cause a statistically significant elevation in TLs increases with age. We calculated this dose for these same five-year age intervals, using an established 137Cs dose response curve. The increases in TLs correspond to doses of 2.1, 4.9, 6.3, 8.6 and 10.0 cGy of 137Cs photons for these age intervals, respectively. This minimally detectable dose increased linearly with age at a rate of ~0.098 cGy/year. This approach is applicable to evaluating effects of other types of exposure, including cigarette smoke. It should also be possible to obtain separate estimates of the effects of radiation exposure in smokers and non-smokers.

P26

Maternal Dioxin Exposure Combined with a Diet High in Fat Increases Mammary Cancer Incidence Through Induction of Estrogen Metabolizing Genes CYP1B1 and COMT. La Merrill M[1,2], Harper R[1], Birnbaum L[3,1], Cardiff R[4], Threadgill D[1]. [1]University of North Carolina, Chapel Hill, NC, United States, [2]Mt Sinai School of Medicine, New York, NY, United States, [3]US EPA, Research Triangle Park, NC, United States, [4]University of California, Davis, CA, United States.

Epidemiological studies show that breast cancer risk correlates with total lifetime exposure to estrogens and that early life 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure can also increase cancer risk. Because both TCDD and adipocytes impact the estrogen pathway, we examined how TCDD and a high-fat diet (HFD) interact to alter breast cancer susceptibility. At 12.5 days post coitus, we exposed pregnant FVB/NJ female mice to 1 µg/kg of TCDD or vehicle and at parturition randomly assigned the nursing dams to a low-fat diet or HFD. Female offspring were maintained on the same diets after weaning and exposed to 7,12-dimethyl-benz[a]anthracene (DMBA) at post-natal days (PND) 35, 49 and 63 to initiate mammary tumors. A second cohort of females was treated identically until PND 35 or 49, when mammary gland morphology was examined, or at PND 50, when mammary gland mRNA and ERBB2 was analyzed. We found that maternal TCDD exposure doubles mammary tumor incidence among mice fed HFD. Among mice fed HFD, maternal TCDD exposure caused rapid mammary development and increased *Cyp1b1* expression and decreased *Comt* expression in mammary tissue. Mammary tumor *Cyp1b1* expression was also increased by maternal TCDD exposure. Our data suggest that HFD may increase sensitivity to maternal TCDD exposure, resulting in increased breast cancer incidence through changes in the timing of mammary differentiation and estrogen metabolism during puberty.

P27

Predicting the Genetic Toxicity of Kinase Inhibitors Based Upon Kinase Inhibitory Profiles. Olaharski AJ[1], Gonzaludo N[1], Goldstein D[1], Bitter H[1], Kirchner S[2], Uppal H[1], Kolaja K[1]. [1]Roche Palo Alto LLC, Palo Alto, CA, United States, [2]Hoffman-La Roche, Basel, Switzerland.

Inhibition of kinases involved with chromosome segregation during mitosis can cause aneuploidy and lead to a positive micronucleus assay (MNT) result. Because most small molecule kinase inhibitors (SMKIs) target the conserved ATP binding pocket, many SMKIs are not selective and are prone to non-pharmacologically related toxicities. Internal experience has demonstrated that a large proportion of Roche SMKIs test positive in an *in vitro* MNT, yet our understanding of the kinases mediating this toxicity is limited. To better understand this relationship, a statistical model was built correlating *in vitro* MNT results with inhibition profiles against 290 kinases, using a training set of 54 chemically diverse SMKIs. The model was developed in two steps: first, a pair of feature selection techniques were used to identify kinases most correlated with MNT results; and second, support vector machines, was used to generate a model based on the subset of selected kinases. Cross validation was used to estimate the accuracy of the model and to determine the optimal kinase set: The model's prediction accuracy for those 54 compounds was estimated to be 80% and 13 kinases comprised the optimal set of features, including CDK2, GSK3α & β, and PCTK1 & 2. Subsequently, the model has been applied to an independent 33 compound forward validation set where it properly predicted the MNT results for 25 kinase inhibitors (75%). Further validation of the model includes testing of an additional 30 SMKIs in the MNT assay, results which will be completed by September and incorporated into the dataset. These data have assisted medicinal chemists in designing SMKIs that avoid inhibiting these kinases reducing the likelihood that they will be genotoxic.

P28

Abstract withdrawn.

P29

Development of a New Array Comparative Genomic Hybridization Tool for High Resolution Analysis of Mouse Chromosome 11. Fuscoe JC[1], Han T[1], Wang J[2], Sawyer J[3], Mei N[4], Honma M[5], Chen T[4], Moore MM[4]. [1]Division of Systems Toxicology, NCTR, FDA, Jefferson, AR, United States, [2]Center for Food Safety and Applied Nutrition, FDA, College Park, MD, United States, [3]University of Arkansas for Medical Sciences, Little Rock, AR, United States, [4]Division of Genetic and Reproductive Toxicology, NCTR, FDA, Jefferson, AR, United States, [5]National Institue of Health Sciences, Tokyo, Japan.

The L5178Y mouse lymphoma thymidine kinase (Tk) assay is widely used to evaluate chemicals for their ability to induce mutation. Small colony mutants grow slowly and their induction is associated with clastogens. Large colony mutants grow at normal rates and their induction is associated with point mutagens. The reason(s) for the difference in growth rates is not clear but one hypothesis involves the deletion of a growth control gene located near Tk. We have developed an array comparative genomic hybridization (aCGH) chip that allows a high resolution view of DNA copy number variation along chromosome 11; the location of the Tk gene. The chip consists of 10,000 60-mer probes uniformly spaced along chromosome 11. An additional 1,000 probes that map to other chromosomes, whose copy number is not expected to change in Tk mutants, were included as normalization controls. We validated the approach with a bleomycin-induced mutant that has a G-banded karyotype showing a single chromosome 11 in addition to a pair of fused chromosomes 11. PCR showed loss of heterozygosity for the distal 25% of chromosome 11, including the Tk locus. Our aCGH analysis comparing this mutant to the parental cell population is consistent with 3 copies of chromosome 11 proximal to 11qB4 and two copies of chromosome 11 distal to 11qB4. This new tool will be valuable in evaluating the growth gene hypothesis for small colony mutants and will also provide high resolution definition of chromosome 11 damage.

P30

High Levels of Transcription Stimulate Base Substitution Mutations in Yeast. Lippert MJ, Alexander MP, Crall WC, Holmes MP. Saint Michael's College, Colchester, VT, United States.

High levels of gene transcription in the yeast, *Saccharomyces cerevisiae*, lead to elevated mutation rates, a phenomenon termed Transcription-Associated Mutation (TAM). In this study, we explored the role of transcription level on BS mutagenesis in yeast. Specifically, we created low- and high-transcription strains that possess a *lys2* nonsense allele (*lys2-C1696T*) regulated by the normal low-level *pLYS* promoter and the highly inducible *pGAL* promoter, respectively. We measured reversion rates and determined mutation spectra in low- and high-transcription strains. Results indicate a significant 13-fold elevated mutation rate in the high- versus the low- transcription strain, 14.8 x 10^{-9} and 1.1 x 10^{-9}, respectively. Moreover, under high transcription conditions G-to-T transversions predominated (15 of 23 or 65%) and G-to-C transversions comprised the majority (5 of 23 or 23%) of remaining BS. In contrast, under low-transcription conditions G-to-T and G-to-C transversions comprised 18% (4 of 22) and 14% (3 of 22) of total BS. Thus high levels of transcription stimulate BS mutagenesis specifically at G:C bps with a predominant induction of G:C-to-T:A transversions. We are currently using a genetic approach to test the hypotheses that TAM occurs at sites of oxidative DNA damage, specifically 8-oxoguanine lesions, or that the translesion synthesis DNA polymerase zeta (Pol ζ) participates in TAM. (M.J.L. was supported by the Vermont Genetics Network through Grant Number P20 RR16462 from the INBRE Program of the NCRR, a component of the NIH, and by NIH grant 1R15GM079778.)

P31

Riddelliine Induced Rat Liver Mutagenicity and Gene Expression Profile. Mei N[1], Guo L[2], Fuscoe JC[2], Chen T[1]. [1]Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR, United States, [2]Division of Systems Toxicology, National Center for Toxicological Research, Jefferson, AR, United States.

Riddelliine is isolated from plants grown in the western United States and is a prototype of genotoxic pyrrolizidine alkaloids (PAs), which are probably the most common plant constituents that poison livestock, wildlife, and humans worldwide. In this study, we investigated riddelliine-induced genotoxic effects and gene expression changes in the target tissue. The female Big Blue transgenic rats were gavaged with riddelliine 5 days a week for 12 weeks with 0.1-1 mg/kg body weight, the doses were demonstrated to induce liver tumour in a previous carcinogenesis bioassay. A significant dose-dependent increase in mutant frequency (MF) was found, and the MF in the high-dose group was 3-fold higher than that in the control group. Molecular analysis of the mutants indicated that there was a significant difference between the mutational spectra from riddelliine-treated and control rats. We also performed rat whole genome gene expression microarray to determine riddelliine-induced gene expression profiles in livers. By analysis with the Ingenuity Pathway Analysis software, we found that the differentially expressed genes were mainly involved in cancer, cell death, tissue development, cellular movement, tissue morphology, cell-to-cell signalling and interaction, and cellular growth and proliferation. We further analyzed the genes involved in metabolism, injury of endothelial cells, liver abnormalities, and cancer development in detail. These results provided further insight into the mechanisms involved in genotoxicity and carcinogenesis after exposure to riddelliine.

P32

DGGE-Based Detection of Mitochondrial tRNA Gene and Flanking Region Mutations in Umbilical Cord Tissue from HIV-1 Uninfected Infants Receiving Perinatal AZT-Based Therapies. Torres SM[1,2], Walker DM[3], Seilkop SK[4], Copeland WC[5], Walker VE[1,3]. [1]Lovelace Respiratory Research Inst., Albuquerque, NM, United States, [2]Univ. of New Mexico, Albuquerque, NM, United States, [3]BioMosaics Inc., Burlington, VT, United States, [4]SKS Consulting Srvs, Silver City, NC, United States, [5]NIEHS, NIH, Research Triangle Park, NC, United States.

A study was conducted to determine if mitochondrial DNA (mtDNA) polymorphisms and/or mutations were detectable in healthy newborns and if prepartum AZT-based HIV-1 prophylaxis was associated with significant increases in mtDNA mutations in uninfected infants born to HIV-1 infected mothers. Using a denaturing gradient gel electrophoresis (DGGE) method, mtDNA from umbilical cord tissue was screened for sequence variants in the 22 tRNA genes and flanking regions of the human mitochondrial genome. A total of 24 mtDNA sequence variants were identified in 19/52 (37%, 0.46 changes/subject) treated newborns compared to only 8 variants detected in 7/55 (13%, 0.15 changes/subject) unexposed newborns, resulting in a significant 3-fold elevation in AZT-exposed infants compared with unexposed controls ($P<0.001$). Six of the distinct sequence variants found in unexposed control infants represented previously reported polymorphisms that were predominately synonymous and homoplasmic. Uninfected Infants exposed to AZT/3TC and 'maternal HIV-1 infection' had an increase in non-synonymous heteroplasmic sequence variants at polymorphic sites (10 distinct variants) and novel sites (4 distinct variants) resulting in a significant shift in the spectrum of mutations ($P = 0.04$). This study is the first of its kind to provide evidence suggesting that AZT-based prophylaxis or treatment is significantly associated with mtDNA mutagenesis; albeit additional research is needed to determine the contribution of fetal responses to maternal HIV-1 infection in the absence of antiretroviral treatment to prenatal mtDNA mutagenesis.

P33

Mechanism of Inhibition of Arsenite Co-Carcinogenesis by Supplemental Selenium (Se). Rossman TG, Uddin AN, Burns FJ, Vega K. New York University School of Medicine, Tuxedo, NY, United States.

Arsenic (As) in drinking water is associated with skin, bladder, and lung cancers. Previously, we showed that arsenite in drinking water enhances solar UV-induced skin cancer in mice. Furthermore, dietary vitamin E (α-tocopheryl acetate) and pXSC [1,4-phenylene *bis*(methyleneselenocyanate)] blocked the cocarcinogenic effect completely (Uddin et al., 2005). Immunostaining for 8-oxo-dG revealed that both vitamin E and pXSC significantly reduced its level in the skin of irradiated mice. Antioxidants and Se compounds block spontaneous and arsenite-induced delayed mutagenesis (Rossman & Uddin, 2004). Taken together, this data suggested an antioxidant mechanism for Se. However, pXSC failed to block UV-alone carcinogenesis, whereas the antioxidant Vitamin E blocked it 30%, suggesting an alternative mechanism for pXSC. X-ray fluorescence of mouse skin showed diffusely elevated levels of As in the hair follicles and epidermis of mice given UV + As. However, As was entirely absent when mice were also given pXSC. In skin, As was diffusely distributed, whereas Se was strongly associated with cell and nuclear membranes. X-ray absorption near-edge spectra were consistent with the presence of the seleno-bis(*S*-glutathionyl) arsinium ion in the liver. Our results show that Se was uncommonly effective at completely preventing any As in skin even after 196 days of continuous exposure to 5 mg/l As in drinking water. Traces of the seleno-bis(*S*-glutathionyl) arsinium ion in the liver suggests that this compound sequestered As in the liver, from where it is excreted in bile, thus preventing its appearance in skin.

P34

Comparison of MicroRNA Expression, Genomic Gene Expression, DNA Adducts, Mutation Induction, and Tumor Incidence for Carcinogenesis of Aristolochic Acid in Rat Kidney. Chen T, Pearce M, Mei N, Guo L. National Center for Toxicological Research, FDA, Jefferson, AR, United States.

Aristolochic acid (AA) is a potent human carcinogen and has been associated with the development of urothelial cancer in humans, and kidney and forestomach tumors in rodents. To investigate the molecular mechanisms responsible for the tumorigenicity of AA, and to compare the different biomarker endpoints, we determined the microRNA and gene expression profiles, biomarker signature genes, DNA adduct formation and mutation induction in the target tissue kidney of Big Blue rats treated with a similar protocol that resulted in tumors. AA treatment that eventually resulted in kidney tumors in rats also resulted in significant dose-response increases in DNA adduct formation, mutation induction and biomarker gene signature scores in the rat kidneys. Microarray analysis of the microRNA and gene expression profiles indicated that AA significantly altered the expression of microRNAs and genomic genes that were associated with cancer development. Our results suggested that AA is a genotoxic carcinogen through a genotoxic mode of action in kidney. Also, the microRNA and gene expression profiles might be used as indicatives for carcinogens and offer explanations for the mode of action.

P35

The Potential Carcinogenic Effects of Some Tryptophan Metabolites. Chung K-T, Gadupudi GS. The University of Memphis, Memphis, TN, United States.

Tryptophan is an essential amino acid and not carcinogenic, but many tryptophan metabolites such as kynurenine, 3-hydroxykynurine, anthranilic acid, 3-hydroxyanthranilic acid, indole, skatole, indole-acetic acid, 5-hydroxytryptamine, etc. were reported to be involved in the carcinogenetic process. The urinary excretion of large amounts of some of these metabolites in patients with bladder cancer suggests the involvement of tryptophan metabolites in cancer. These metabolites could play a vital role in carcinogenesis when coupled with metal ion cofactors and nitrates, or deficiency of vitamin B6. The urinary excretion of abnormal levels of tryptophan metabolites in cancer patients is statistically significant, but the molecular mechanisms involved in the induction of cancer are not well explained. The proposed mechanisms involved in this process are auto-oxidation of the metabolites to form free radical intermediates that later form adducts with DNA. Some of these metabolites are detoxified in the liver by glucouronidation or sulphate ester formation. These detoxified glucouronides and sulphate esters could be regenerated in the bladder at low pH or by the action of the glucouronidases and sulphurylases. These metabolites could be activated metabolically and generate free radicals formation and production of intermediate endogenous carcinogenic compounds that ultimately form adducts with DNA and lead to mutagenesis. Though the free radicals and formation of adducts seems to be a convincing theory, their actual mechanisms involved in carcinogenesis are yet to be demonstrated.

P36

Gene Expression of Phase I and Phase II Metabolizing Enzymes and PAH-DNA Adduct Formation in Human Prostate as Risk Factors in Prostate Cancer Etiology. John K[1], Singh PB[2], Pratt MM[1], Ragavan N[2], Cole KC[3], Matanhelia SS[3], Phillips DH[3], Martin FL[2]. [1]National Cancer Institute, NIH, Bethesda, MD, United States, [2]Lancaster University, Lancaster, United Kingdom, [3]Institute of Cancer Research, Surrey, United Kingdom.

Two zones of the prostate, the peripheral zone (PZ) and the transition zone (TZ), which differ in cancer susceptibility, were examined for differences in the expression of Phase I/Phase II genes and PAH-DNA adduct formation. Clinically, there is a higher proportion of prostate tumors arising in the PZ, with fewer tumors in the TZ. Matched paraffin-embedded tissue sets (n=28) obtained from radical retropubic prostatectomies, were examined for the expression of cytochrome (*CYP*) P450s, *1A1*, *1B1* and *1A2*, *N*-acetyl transferase 1 and 2 (*NAT1* and *NAT2*) and catechol-*O*-methyl transferase (*COMT*), using quantitative real-time reverse transcriptase (RT)-PCR. Levels of *CYP1B1* and *NAT1* were significantly higher in the PZ compared to the TZ ($p<0.001$ and <0.01, respectively). Staining of PZ and TZ sections samples with antiserum elicited against r7, t8-dihydroxy-t-9, 10-oxy-7, 8, 9, 10-tetrahydro-benzo[*a*]pyrene (BPDE) DNA was followed by quantitation of nuclear staining using the Automated Cellular Imaging System (ACIS). PAH-DNA adducts were concentrated in the prostate glandular epithelial cells and levels were very high, compared to those seen for human cervix and placenta using the same method. However, there was no significant difference in PAH-DNA adduct levels for the PZ, and TZ samples, matched from 23 individuals. Therefore, though expression levels of CYP1B1 and NAT1 were higher in the PZ than in the TZ, PAH-DNA adduct levels were similar in both zones suggesting that additional factors (*e.g.*, local hormonal drivers) may contribute to tumor formation in the PZ.

P37

Human Toxicogenomic Analysis of Bromoacetic Acid: A Regulated Drinking Water Disinfection By-Product. Wagner ED[1], Muellner MG[2], Hudson ME[1], Attene-Ramos MS[1], Plewa MJ[1]. [1]University of Illinois, Urbana, IL, United States, [2]Nalco Company, Naperville, IL, United States.

We linked genomic DNA damage induced by the regulated drinking water disinfection by-product bromoacetic acid with toxicogenomic analysis using a PCR array for 84 genes related to human DNA damage and repair. We used a non-transformed, normal, fetal human small intestinal epithelial cell line (FHs 74 Int) to measure the modulation of gene expression after exposure to bromoacetic acid. Cells were exposed to a non-cytotoxic concentration of bromoacetic acid for 30 min or 4 h. Eleven genes showed altered gene expression after 30 min; 3 were down regulated. After 4 h exposure 13 genes exhibited altered gene expression; 8 were down-regulated. One gene displayed changed expression at both time points. Functions for the modified genes included: DNA double strand break repair (9 genes), cell cycle arrest (7), mismatch repair (2), base excision repair (2), nucleotide excision repair (1), apoptosis (1), and other (1). One suggested mechanism for bromoacetic acid-induced DNA damage is the generation of double strand breaks. These data are supported by DNA repair kinetic experiments using mammalian cells. Supported by AwwaRF Grant 4132 and WaterCAMP*W*S NSF Center Grant CTS-0120978.

P38

Genotoxicity of Titanium Dioxide (TiO_2) in Human Bronchial Epithelial Cells *In Vitro*. Falck GC-M[1], Lindberg HK[1], Suhonen S[1], Vippola M[2], Vanhala E[1], Savolainen K[1], Norppa H[1]. [1]Finnish Institute of Occupational Health, Helsinki, Finland, [2]Tampere University of Technology, Tampere, Finland.

TiO_2 nanoparticles cause oxidative stress, inflammation, and cytotoxicity in mammalian systems but information on the genotoxicity of TiO_2 is still limited. We aimed at evaluating the genotoxicity of two types of commercial TiO_2 on bronchial epithelial cells (BEAS 2B). Nanosized titanium(IV) oxide rutile (>95%; 10 x 40 nm; <5% SiO_2 coating) and anatase (99.7%, <25 nm) were assayed. Fine titanium(IV) oxide rutile (99.9%; <5 µm, average size 1 µm) was studied for comparison. The cells were cultured in the presence of several doses of each TiO_2 for 24, 48, and 72 h. The alkaline Comet assay was used to detect DNA strand breaks and the cytokinesis-block micronucleus (MN) assay chromosomal damage. Nanosized anatase and fine rutile induced DNA damage at several doses with all treatment times, with dose-dependent effects after 48-h and 72-h with nanosized anatase and after 24-h and (in one out of two experiments), 48-h and 72-h with fine rutile. Nanosized rutile increased DNA damage only at 80 µg/cm² after the 24-h treatment and at 100 µg/cm² after the 72-h treatment, the longer treatment showing a dose-dependent effect. In the MN assay, none of the TiO_2 samples induced micronuclei, except for anatase at 10 and 60 µg/cm² after the 72-h treatment. In conclusion, both nanosized anatase and (SiO_2-coated) rutile and fine rutile induced DNA damage in BEAS 2B cells, the nanosized rutile being less effective than the other two forms of TiO_2. Only nanosized anatase could increase the frequency of MN. [Funded by NANOSH, NMP4-CT-2006-032777.]

P39

Gene Selection and Gene Identification in Microarray Data Analysis. Chen JJ, Zou W, Chang C-W, Morris SM. National Center for Tox Res, Jefferson, AR, United States.

A common goal of microarray studies is to select a list of genes that express differently among different experimental conditions of interest. However, in comparative bacterial genomics studies the main goal is to identify which genes are present or absent. We present comparisons for the differences between the two goals with respect to the probe design, experimental design, and data analysis methods. Common studies use commercially available arrays having a large number of genes and a limited number of experimental conditions, such as control-versus-treatment or dose-response studies. Standard procedures developed for gene identification use the significance testing approach, and often require a sufficient number of biological replicates in order to achieve a desired statistical power. In comparative genome analysis, the arrays are custom-designed with probes specific to segments of a sequenced reference genome for target samples. The number of genes is generally small and the number of experimental conditions, such as bacterial samples, is large. The primary data analysis is to determine an optimal cut-off in which genes with intensity above the cut-off are classified as present and below the cut-off are classified as absent. Two microarray experiments are analyzed to illustrate approaches to gene selection and gene identification. The first experiment is a study of gene expression profiles of liver tissue from p53+/+ (Wild-Type), p53+/− (Heterozygous) and p53−/− (Knock-out) transgenic mice. The second experiment is a study of detection of antimicrobial resistance genes in 34 Salmonella isolates from a turkey production facility.

P40

Proteomic Profiling of Urinary Bladders From Mice Exposed to Sodium Arsenite. Winnik WM, Chilakapati J, Wallace K, Kitchin KT, Ortiz PA. U.S. Environmental Protection Agency, Research Triangle Park, NC, United States.

Arsenic, an environmental contaminant, has been linked with cancer of the bladder in humans. To study the mode of action of arsenic, female CH3 mice were exposed to 85 ppm sodium arsenite in their drinking water for 30 days. Following the exposure a comparative proteomic analysis of the mice bladders was performed by 2D difference gel electrophoresis (DIGE). Differentially expressed proteins were identified by nanospray-liquid chromatography-tandem mass spectrometry (LC-MS/MS) and database searching. Forty-five protein spots that showed changes in expression have been identified; some of the pathways affected by these proteins are valine, leucine and isoleucine degradation, glycolysis, actin cytoskeleton signaling, and the metabolism of fatty acids, glutathione, and pyruvate. This study follows our *in vitro* proteomic investigation of arsenic-induced protein expression in human bladder UROtsa cells. Protein expression changes resulting from the arsenic exposure may provide essential information for the elucidation of its mode of action. This abstract does not necessarily reflect EPA policy.

P41

Validation of Transgenic Rodent Gene Mutation Assays Using DNA Sequence Data. Douglas GR[1], Soper LM[1], Singer TM[2]. [1]Environmental Health Science & Research Bureau, Health Canada, Ottawa, ON, Canada, [2]New Substances Assessment and Control Bureau, Health Canada, Ottawa, ON, Canada.

The predictivity of genotoxicity tests for carcinogenicity is an important consideration in the acceptance and interpretation of such tests for regulatory use since mutagenicity is a primary event in the etiology of most cancers. Despite the close association between these two endpoints, it is an imperfect association. There is a small, but distinct, proportion of non-carcinogens that are genotoxic, presumably because mutagenicity per se was insufficient for the development of tumors in such cases. Furthermore, there are carcinogens that are non-genotoxic, due to mechanisms that do not involve genotoxicity as a primary event. Carcinogenicity, therefore, may not be the most appropriate endpoint against which to validate a new assay such as the transgenic rodent (TGR) gene mutation (GM) assay, since there is an expectation of non-concordance among test results. The most biologically relevant endpoint for use in validation of the predictivity TGR assays would be another, well-established in vivo gene mutation assay that is not limited to a single tissue. Since such an assay does not exist, we have used sequence data from the DNA isolated from mutant phenotypes (i.e. plaques or colonies) to estimate the Positive Predictive Value (PPV) of these presumptive mutant genotypes. The PPV is the proportion of mutant phenotypes that are confirmed as mutant genotypes. We have reviewed the data from over 140 studies in which a total of 32,751 mutant phenotypes were sequenced yielding 31,659 mutant genotypes and a PPV of 0.967, validating the effectiveness of TGR assays for detecting gene mutations.

P42

Abundant Expression of *CYP1A1* is Positively, While *CYP1B1* and *NQO1* Are Negatively, Associated With Benzo(a)pyrene (BP)-DNA Adduct Formation in Normal Human Mammary Epithelial Cells (NHMECs). Einem TL[1], Divi RL[1], Shockley ME[1], Keshava C[2], Weston A[3], Poirier MC[1]. [1]National Cancer Institute, NIH, Bethesda, MD, United States, [2]U.S. EPA, Research Triangle Park, NC, United States, [3]NIOSH, CDC, Morgantown, WV, United States.

Polycyclic aromatic hydrocarbons (PAHs), including BP, are activated to DNA binding species by Phase I enzymes and detoxified by Phase II enzymes. Identifying the enzymes involved in BP-DNA adduct formation may be useful for cancer prevention strategies. Our previous studies in NHMECs indicated that BP up-regulates *CYP1A1, CYP1B1* and *NQO1*. To understand how expression levels of these genes affect BP-DNA adduct formation, 16 NHMEC strains were exposed for 12 hr to BP (4μM), and BP-DNA adducts were measured by chemiluminescence immunoassay. The BP-DNA adduct levels ranged from 0.2–15.8 adducts/10^8 nucleotides. Gene expression levels (transcripts/ng RNA or tpn) of *CYP1A1, CYP1B1* and *NQO1*, measured by quantitative RT-PCR, showed large variations in unexposed and BP-exposed cells. In the 16 cell strains, basal tpn ranged from 56-836 for *CYP1A1*, 336-5587 for *CYP1B1* and 5943-40112 for *NQO1*. BP-induced tpn values were 251-13234 for *CYP1A1*, 4133-61627 for *CYP1B1* and 4456-55887 for *NQO1*. BP-induced *CYP1A1* was positively associated with BP-DNA adduct levels (r=0.737, *p=0.0003*). *CYP1B1* and *NQO1* tpn levels in unexposed cells were negatively associated with BP-DNA adduct levels (r=-0.495, *p=0.03; and* r=-0.423, *p=0.07*, respectively). These associations were further confirmed in 3 NHMEC strains by Western-blot for all the three proteins, ethoxyresorufin deethylase assay for CYP1A1/1B1, and dicumerol-sensitive NQO1 activity. These data indicate that, in NHMECs, BP-induced CYP1A1 is necessary for BP-DNA adduct formation, and high basal levels of CYP1B1 and NQO1 appear to protect against BP-DNA adduct formation.

P43

Mutant T-cells in Melanoma Patients: Probes for Immunological Responses. Albertini MR[1,2], Macklin MD[2], Zuleger CL[2], Newton MA[2], Albertini RJ[3]. [1]William S. Middleton Veterans Hospital, Madison, WI, United States, [2]University of Wisconsin Paul P. Carbone Comprehensive Cancer Center, Madison, WI, United States, [3]University of Vermont, Burlington, VT, United States.

We investigated *in vivo* mutant T-cells from patients with advanced melanoma for their utility as diagnostic and mechanistic probes to detect *in vivo* T-cell proliferation and to study the T-cell response to human melanoma. Mutant hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) deficient T-cells (MT) were obtained by 6-thioguanine (TG) selection from melanoma patient peripheral blood as clonal isolates or as mass cultures, and from tumor-bearing regional lymph nodes or distant melanoma sites as mass cultures. Wild Type T-cells (WT) were obtained from all sites by analogous means without TG selection. cDNA sequences of the T-cell receptor (TCR) beta chains were used as unambiguous biomarkers of *in vivo* clonality. *In vivo* T-cell clonal amplifications, identified by identical TCR-beta V-region, complementarity determining region 3 (CDR3), and J-region gene sequences (TCR usage), were significantly enriched among MT compared with WT. Common TCR usage by some MT was identified within and between melanoma patients. Study findings demonstrate MT trafficking between blood and tumor and suggest potentially shared immunogenic epitopes in melanoma patients. Preliminary flow cytometry analysis of tumor infiltrating lymphocytes from one patient stained with HLA-A2 pentamers containing peptides derived from melanoma associated antigens suggest enrichment of melanoma-reactive T-cells in MT compared to WT from that patient. We conclude that MT from melanoma patients are enriched for proliferating T-cells and are candidate probes to investigate *in vivo* T-cell responses to melanoma.

P44

Exploration of the Composition of Chromatin Buds in the *In Vitro* Human Lymphocyte Micronucleus Test. Araujo AT, Serrano L, Camacho R, Montero RD. Instituto de Investigaciones Biomedicas, UNAM, Mexico, DF, Mexico.

Gene amplification implies multiplication of specific sequences of the genome. It has been observed in cancer cells with numerous copies of oncogenes like c-myc and in cultured cell lines that resist the action of antineoplastic drugs like Methotrexate (MTX). It was recently proposed that gene amplification could be the origin of structures like the Chromatin buds (CHB) observed in the human lymphocyte Micronucleus test (ly-MN). CHB are formed when lymphocytes are treated *in vitro* and had been observed in *in vivo* studies of people exposed to genotoxic agents. Shimizu et al. (2000) described similar structures in tumoral cells that extruded double minutes (amplifications) from their nuclei and it has been interpreted that CHB observed in the ly-MN, either *in vitro* or *in vivo*, could be due to gene amplifications. To test this hypothesis whole blood cultures were set-up and treated with MTX at five concentrations from 0.01 to 2.5 mM. MN and CHB were evaluated according to the method of bromodeoxyuridine (BrdU) incorporation; an increase with the dose was found for both parameters. Simultaneous cultures without BrdU were used to look for the amplification of the gene dihydrofolate reductase (*DHFR*); FISH and immunodot blot were used to assess whether this gene was amplified using a probe of 231 bp corresponding to intron 5 and exon 5, obtained by PCR and labeled with digoxigenin by random priming. No evidence of amplification of the *DHFR* gene was observed under the conditions used in these experiments, which are the standard conditions for the *in vitro* MN test experiments. Ref. Mut Res 448:81-90,2000.

P45

Bystander Responses Induced by Mitomycin C, Phleomycin and Ionizing Radiation in Normal Human Lymphoblastoid Cells. Asur R, Thomas RA, Tucker JD. Wayne State University, Detroit, MI, United States.

Many studies have evaluated radiation-induced bystander effects, but few have evaluated the ability of chemicals to produce similar responses. Work by our laboratory has shown that phleomycin (PHL) and mitomycin C (MMC) both cause cells to release media-soluble factors that induce a bystander effect in a normal human B lymphoblastoid cell line. We evaluated the ability of these chemicals to induce a bystander effect through media transfer in another normal human B lymphoblastoid cell line. Micronuclei induction in cytokinesis-blocked binucleated cells was used as the endpoint. These 2 cell lines were exposed to different concentrations of PHL (50, 100, 200 and 300 µg/ml) or to MMC (50, 100, 200 and 300 ng/ml) for 1 hour. Unexposed cells were used as controls. After exposure, the cells were washed thoroughly to remove the residual chemical, incubated in fresh media, and grown for an additional 4 hours at 37°C. This "conditioned" media was then transferred to unexposed cells. Cytochalasin B (6 µg/ml) was added, and the cells were grown for another 28 hours. The cells were then evaluated for micronuclei induction. The data suggest that the direct and the conditioned media exposure of normal human lymphoblastoid cells to the chemicals resulted in micronuclei induction at the concentrations tested. A similar experiment was performed using acute doses of ionizing radiation (IR). Both cell lines exhibited radiation dose-responsive increases in bystander-induced micronuclei. The bystander effect appears to be part of a more general stress response and is not restricted to IR, as previously thought.

P46

Cell Proliferation of Pancreatic β-cells is Impaired by Arsenite Treatment. Sordo M[1], Burns AL[1,2], Salazar A[1], Ostrosky-Wegman P[1], Diaz-Villaseñor A[1]. [1]Instituto de Investigaciones. UNAM, México DF, Mexico, [2]Facultad de Medicina. UNAM, México DF, Mexico.

Exposure of rat pancreatic β-cells to arsenite impairs insulin transcription and secretion. Insulin is known to stimulate cell proliferation while arsenic has been shown to diminished cell proliferation both *in vivo* and *in vitro*. Thus, the present study was performed to characterize the effect of arsenite on the cell cycle and proliferation in pancreatic β-cells. RINm5F cells were exposed to 0, 0.5, 1, 2 and 5 µM arsenite for 72 h. The cell cycle was analyzed by flux cytofluorometry. In addition, mitotic index (MI) and replication index (RI) were evaluated. Both indexes declined in a dose-dependent manner. Arsenite produced an arrest in G2/M starting at 2µM whereas higher doses (5µM) produced morphological changes in the cells. The changes in cell proliferation could result from the reduction in insulin secretion or viceversa and should be further studied.

P47

GPI-deficient Rat Lymphocytes Have Mutations in the *Pig-A* Gene. Miura D[1], Mittelstaedt RA[2], Shaddock JG[2], Dobrovolsky VN[2], Heflich RH[2]. [1]Teijin Pharma Limited, Tokyo, Japan, [2]USFDA/National Center for Toxicolgocal Research, Jefferson, AR, United States.

We previously reported that measuring cells deficient in glycosylphosphatidylinositol (GPI) anchor synthesis by flow cytometry could form the basis of a rapid *in vivo* mutation assay. We also demonstrated that proaerolysin-resistant (ProAER^r) rat spleen T-cells are GPI deficient and presumed mutants for the phosphatidylinositol glycan complementation group A gene (*Pig-A*) (Miura et al., EMM 48:591). In order to confirm that *Pig-A* mutation is the cause of the GPI-deficient phenotype, male F344 rats were treated with 3 doses of 40 mg/kg *N*-ethyl-*N*-nitrosourea (ENU). Four weeks later, spleen cells were isolated from the rats and grown at limiting dilution in 96-well plates using a medium containing ionomycin, phorbol-12-myristate-13-acetate, and 2 nM ProAER. The frequency of ProAER^r spleen cells from control rats ranged from 1.3 to 4.8 x 10^{-6}, while treatment with ENU increased the frequency of resistant cells 100-fold. RNA was isolated from ENU-induced ProAER^r clones, and *Pig-A* cDNA was synthesized, amplified by nested PCR, and sequenced. Each of the ProAER^r clones that produced RT-PCR products contained a mutation that altered the coding properties of the *Pig-A* gene. We identified 19 independent mutations, with the largest percentage involving basepair substitution at A:T. The pattern of mutations was similar to what we previously reported for *Hprt* lymphocyte mutation in ENU-treated rats (Mittelstaedt et al., EMM 26:261-269). These results are consistent with our hypothesis that GPI-deficient splenocytes are *Pig-A* mutants and support the use of GPI deficiency for measuring *in vivo* mutation.

P48

Depressed Antioxidant Status in Pregnant Women on Iron Supplements: Mutagenic Implications. John A, Fidelia A, Grace T, Abiodun A, Fasola FA. University of Ibadan, Ibadan, Oyo, Nigeria.

Iron (Fe) is a commonly prescribed supplement in pregnancy, but its possible mutagenic potential is hardly considered. We determined the antioxidant status in pregnant women on Fe supplements and controls in Nigeria. Serum Fe level was significantly higher in the supplement than the non-supplement group ($P < 0.001$). Levels of the antioxidants, ascorbate, copper (Cu), zinc (Zn), and bilirubin were all significantly decreased ($p<0.05$, $p<0.001$, $P<0.05$ and $p<0.05$) respectively.The antioxidants; ascorbate, bilirubin, Cu, urate and Zn, were negatively correlated with serum Fe level (r -0.299, $P<0.05$, r-0.278, $P<0.05$, r-0.383, $P<0.05$ and r-0.0369, $P<0.05$) respectively except vitamin E. Serum Fe level increased steadily in the 3 trimesters in the supplement group, associated with inverse antioxidant status. In the non-supplement group, Fe levels were stable in the first 2 trimesters and rose in the last, associated with relative stability of all antioxidants except urate. The significantly higher Fe level in the second trimester was sustained in the third though to a lesser degree ($P<0.05$) and associated with significant decreases in the following antioxidants in the supplement group, ascorbate, bilirubin, Cu, and Zn ($P<0.02$, $P<0.02$, $P<0.02$ and $P<0.001$) respectively. These data imply depressed antioxidant status in the supplement pregnant group with attendant oxidative stress, probably prooxidant Fe-induced. This may lead to mutation or carcinogenesis in the presence of impaired innate repair system as suggested by the consistent reduction in some key members of the antioxidant system, particularly zinc.

P49

The Effect of Reduced Levels of Human DNA Polymerase β on Microsatellite Mutagenesis in Human Cells. Jacob KD[1], Sobol RW[2], Eckert KA[1]. [1]The Pennsylvania State University College of Medicine, Hershey, PA, United States, [2]The University of Pittsburgh, Pittsburgh, PA, United States.

Microsatellite mutations are sources of genetic variation and proposed to occur by DNA slippage. Polymerase utilization of slipped DNA intermediates is required in this mechanism, but the cellular polymerase responsible is unknown. We examined the role of human DNA polymerase beta (pol β) in mutagenesis. Overexpression of pol β increases spontaneous mutagenesis in mammalian cells for both microsatellite and non-microsatellite containing targets. We knocked down pol β in a non-tumorgenic human lymphoblastoid cell line and determined the effect on spontaneous mutagenesis. We used the oriP-tk shuttle vector system to examine mutation rates at a $[G/C]_{10}$ microsatellite relative to the HSV-tk coding region. In wildtype cells, the mutation rate of the HSV-tk control vector was $3.8x10^{-6}$. The $[G/C]_{10}$ repeat vector was less genetically stable with a median mutation rate of $5.5x10^{-5}$. Clonal cells containing the $[G/C]_{10}$ vector were stably transduced with a lentiviral vector containing a control shRNA or one of 3 different shRNAs targeted to the *pol* β gene. Pol β protein levels and mutation rates were analyzed for 10-20 clones isolated from each shRNA construct. Median mutation rates ranged from $2.4\text{-}3.5x10^{-5}$ in the knockdown cells. Mutational spectra were generated from randomly selected clones. In the absence of pol β, 72% of the mutations occurred at the repeat allele, compared to 27% for the parental cells. Our results indicate that the absence of pol β alters the specificity of spontaneous mutations. This result may reflect the activity of an alternate polymerase that substitutes for pol β.

P50

Thymidylate Synthase: Tumor Suppressor and Oncogene in Sporadic Breast Cancer? Barclay B[1], Murray D[2]. [1]Planet Biotechnologies Inc, St Albert, AB, Canada, [2]Cross Cancer Institute, Edmonton, AB, Canada.

We suggest here a role for thymidylate synthase (TYMS) in the etiology of sporadic breast cancer (SBC). The essence of the hypothesis is that thymine nucleotide stress (TNS), caused by interactions between polymorphisms in genes involved in one-carbon metabolism (TYMS, MTHF), nutritional deficiencies (folate, B_{12}) and exposure to environmental toxins (malathion, ethanol) results in the accumulation of gross chromosomal rearrangements (GCRs) throughout the genome of mitotic breast stem cells. GCRs at chromosome band 18p11.32, that include the TYMS gene, create chromosomal (deletions, 18p loss) and extrachromosomal (episomes, large palindromes) cytogenic drivers that alter TYMS activity, stabilizing an endogenous TNS phenotype. TYMS acts as a tumor suppressor in excess by repressing the p53 gene and the network of numerous genes under p53 control. Altered TYMS activity (both limitation and excess) increases rates of mitotic recombination and mutation for nuclear and mitochondrial genes, giving rise to a heterogeneous and genetically unstable tumor cell population. Ongoing TNS causes GCRs and mutations at numerous other tumor suppressor and oncogene loci that produces the genomic variation upon which selection forces act to channel SBC tumor cells into histopathological subtypes and driving later events in disease progression. Our interest here is in SBC but we note that our model may also apply to other cancers.

P51

Evaluation of *cII* Mutations in Big Blue Mice Fed Methylphenidate Hydrochloride for Up To 24 Weeks. Shelton SD[1], Manjanatha MG[1], Mattison DR[2], Morris SM[1]. [1]National Center for Toxicological Research/FDA/DGRT, Jefferson, AR, United States, [2]National Institute of Child Health and Human Development, Bethesda, MD, United States.

Methylphenidate hydrochloride (MPH), a widely prescribed pediatric drug for attention deficit hyperactivity disorder (ADHD), induces liver adenocarcinomas in B6C3F1 mice. We previously reported that MPH was metabolized to ritalinic acid in B6C3F1 mice. Further, the drug was not mutagenic or clastogenic as tested by the *Hprt* mutation assay in surrogate lymphocytes and the micronucleated-reticulocyte assay in the erythrocytes, respectively. In order to determine if the tumor induction was through a mutagenic mode of action in target tissue, groups of male Big Blue mice (B6C3F1 background) were fed with diets containing 50-4000 ppm of MPH for up to 24 weeks and *cII* mutant frequency (MF) and types of mutations were determined in the liver. Food consumption and body weight gain/loss were recorded weekly for each animal. Although the mice fed higher doses of MPH lost body weights initially, there was no significant difference in the total food consumption or body weight loss/gain at 24 weeks except for 2000 and 4000 ppm groups. The liver *cII* MFs in control animals ranged from 19-24 X 10^{-6} where as in MPH-treated animals, the *cII* MFs ranged from 18-27 X 10^{-6}. None of the MPH doses tested at 4, 12 or 24 weeks of treatment induced *cII* MFs that were significantly higher than the control MFs. Further, the molecular analysis of *cII* mutations showed that the majority of the MPH-induced mutations were GC->AT transitions consistent with the spontaneous mutations. There was no significant difference in the *cII* mutational spectra between mice fed the control and MPH diet. These results suggest that MPH is not mutagenic in mice and the induction of tumors in the liver is probably through a non-genotoxic mechanism.

P52

Arrest of Mitochondrial RNA Polymerase by the Malondialdehyde Adduct, M_1dG. Cline SD[1], Lodeiro MF[2], Marnett LJ[3], Cameron CE[2], Arnold JJ[2]. [1]Mercer University School of Medicine, Macon, GA, United States, [2]Penn State University, State College, PA, United States, [3]Vanderbilt University School of Medicine, Nashville, TN, United States.

M_1dG, the major endogenous DNA adduct produced by malondialdehyde, is found in both the nuclear and mitochondrial genomes. The exocyclic guanine adduct assumes an open-ring structure when paired with C, but remains closed opposite T or in single stranded DNA. PdG is a stable, closed-ring analog of M_1dG. Nucleotide excision repair removes M_1dG from nuclear DNA, but NER is absent in mitochondria, where these adducts may readily form. We hypothesize that persistent M_1dG adducts in the mitochondrial chromosome interfere with the expression of genes needed for oxidative phosphorylation resulting in diminished cellular energy production. To investigate M_1dG effects on mitochondrial gene expression, we utilized an *in vitro* transcription system with purified human mitochondrial RNA polymerase (mtRNAP) and transcription factors, mtTFA and mtTFB2. The transcription templates contained either the heavy strand or light strand promoter (HSP1 or LSP, respectively) with a downstream M_1dG or PdG adduct in either the transcribed or nontranscribed strand opposite C or T. As previously observed with nuclear RNA polymerase II, PdG was a strong block to mtRNAP elongation when located in the transcribed strand, while the arrest at M_1dG in the transcribed strand was less severe and greatest when M_1dG is opposite T. The extent of transcriptional arrest was similar after initiation at HSP1 and LSP. These findings suggest that M_1dG may inhibit gene expression from both strands of the mitochondrial chromosome.

P53

Cytotoxicity and Mitochondrial Membrane Damage Effects of Di-ethylhexyl and Mono-ethylhexyl Phthalates on Human TK-6 Lymphocyte Cells. Rosado C, Velez C, Zayas B. Metropolitan University, San Juan, Puerto Rico.

The main objective of this study is to determine the toxicity and mitochondrial interaction of the phthalates Di-ethylhexyl (DEHP) and its principal metabolite, Mono-ethylhexyl (MEHP) on a human lymphocyte cell line, TK-6. Phthalates are a family of compounds used widely in the manufacturing industry and in the production of plastics. Human can be exposed to phthalates through health care and beauty products. The effects of phthalates as estrogen disrupters and on the reproductive and respiratory systems have been reported by the scientific community. Studies on the identification of biomarkers of exposure to phthalates however are limited. In this study TK-6 lymphocytes cells were cultured on 25cm flasks on modified culture media with 10% FBS, and kept at 37°C and 5% of CO_2. TK-6 cells were exposed to DEHP or MEHP at doses ranging from 0.5uM, to 50uM. For determination of the IC50 (inhibition concentration) TK-6 cultures were exposed to BQs for 48 and 72 hours and cytotoxicity assessed by Trypan Blue exclusion. Apoptosis induction through mitochondrial membrane permeability was also determined. For apoptosis related analysis cells were treated with the respective IC50 concentrations for 72 hours. Included positive controls were Valinomycin, (permeate mitochondrial membrane) and Staurosporine an apoptotic agent. Membrane permeability were determined by fluorescent analysis with MITO PT. Preliminary cytotoxity analysis with DEHP and MEHP indicate IC50s of 32uM 26uM respectively. Mitochondrial permeability analysis is in the process of completion.

P54

Gene Polymorphisms and the Risk of Head and Neck Squamous Cell Carcinoma in Brazil. Garcia SMN[1], Curioni OA[2], Brasilino M[2], Kohler P[1], Gattás GJF[1]. [1]FMUSP, São Paulo, Brazil, [2]Heliopolis Hospital, Sâo Paulo, Brazil.

The Head and Neck Squamous Cell Carcinoma (HNSCC) is the fifth cancer most incident between male population and the seventh between female in Brazil. Chronic alcohol consumption is an important risk factor for the development of various types of cancers, specially the HNSCC. A genetic predisposition may influence cancer risk because the rate of alcohol metabolism is genetically determined. Two principal groups of enzymes are involved in the alcohol metabolism, Alcohol dehydrogenase (ADH) and Aldehyde dehydrogenase (ALDH). For several of these enzymes more than one genetic variant exists, it can interfere in the different rates of the alcohol metabolism, increasing the toxic metabolite acetaldehyde in the body. A hospital-based case-control study was conducted in Sao Paulo, Brazil, to evaluate genetic risks associated to the polymorphism of alcohol metabolism genes (*ADH1CIle350Val and ADH1BArg48His*). We here analyzed, by PCR-RFLP, 246 patients (228 male and 18 female) with confirmed HSNCC and 216 cancer free controls (191 male and 25 female) admitted as in-patients in the same hospital. No significant differences were detected in *ADH1CIle350Val* when the two populations were compared (OR, 1.189; 95% CI, 0.6511- 2.170), by the other side, *ADH1BArg48His* mutate allele *(ADH1BArg48His* and *ADH1BHis48His*) was more frequent in the control group (11.78%) compare to HNSCC patients (6.48%) apparently conferring disease protection for this group (OR, 0.5186; 95% CI, 0.26- 1.01). We are increasing our patients and control sample and alcohol metabolism genes polymorphisms to confirm these data.

P55

Randon Mitochondrial Mutations in Human Age-Dependent Pathologies. Vermulst M[1], Bielas J[1], Wanagat J[1], Kujoth G[2], Prolla T[2], Rabinovitch P[1], Loeb L[1]. [1]Dept. of Pathology, University of Washington, Seattle, WA, United States, [2]Dept. of Medical Genetics, University of Wisconsin, Madison, WI, United States.

Mitochondrial DNA (mtDNA) mutations have been reported to contribute to the pathology of a number of age-related diseases, including Parkinson disease, Alzheimer's dementia. sarcopenia, and metastatic cancers. We have developed assays that can track mitochondrial mutations over time in a variety of tissues and experimental settings. This methodology, termed the Random Mutation Capture assay is highly sensitive; it utilizes single molecule amplification to detect rare mtDNA mutations among millions of WT bases. We measured the spontaneous mutation frequency of mitochondrial DNA at a single base pair level in mice, and reported a 10-fold increase in mtDNA point mutations as a function of age. Surprisingly, we found that mitochondrial mutator mice, which carry a proofreading deficient copy of DNA polymerase gamma in a single allele, can sustain a >100 fold increase in single-base substitutions without exhibiting an aging phenotype. This suggests that mitochondrial point mutations alone do not limit the natural lifespan of WT mice. The tolerance of mitochondrial mutator mice to random mitochondrial mutagenesis could result from the multiplicity of mitochondrial genomes per cell, or accelerated degradation of mutated mtDNA. In contrast to point mutations, the frequency of non-clonal deletion mutations in normal and mutator mice correlates with the aging phenotype. Clonal mtDNA mutations also accumulate in many age associated diseases with and may serve as a marker for disease detection and stratification.

P56

Nuclear and Mitochondrial Mutations in Cancer. Bielas JH[1], Vermulst M[1], Fox EJ[2], Ericson NG[1], Loeb KR[3], Rubin BP[4], O'Sullivan JN[2], True LD[1], Loeb LA[1]. [1]Dept. of Pathology, University of Washington, WA, United States, [2]Centre for Colorectal Disease, Dept. of Gastroenterology, St. Vincent's University Hospital, Dept. of Medicine, University College Dublin, Dublin, Ireland, [3]Div. of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [4]Dept. of Anatomic Pathology and Dept. of Molecular Genetics, Taussig Cancer Center and the Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States.

Cancers contain numerous clonal mutations and chromosomal aberrations. We have hypothesized that the large numbers of somatic mutations observed in human cancers cannot be explained by the extremely low rate of mutation in normal cells. Rather, cells must acquire a mutator phenotype during tumor evolution. We investigated this hypothesis by using a recently developed assay able to assess the spontaneous frequency of nuclear and mitochondrial random point mutations in human tissues. In normal tissues, the frequency of spontaneous nuclear somatic mutations was exceedingly low (less than 10^{-8} per base pair), whereas tumors on average exhibited greater than 210-fold increase in the incidence of mutation. The difference between the frequencies of point mutations in normal and neoplastic tissues is statistically different (P=0.009). Thus, the genomes of human cancer cells display greatly elevated frequencies of random nucleotide point mutations, or point mutation instability (PIN). Using the same assay we have determined that the frequency of mitochondrial mutations in normal tissues - and are currently investigating whether neoplastic tissues also display PIN within their mitochondrial genomes. At present, our data clearly document tumor heterogeneity at the single nucleotide level, provide strong support for the hypothesis that cancer cells express a mutator phenotype at the single-nucleotide level, indicate that increased mutagenesis persist during tumor progression, and suggests that PIN might serve as a novel prognostic indicator for tumor progression.

Environmental Genotoxins and Risk Assessment

P57

Induction of DNA Repair *Ogt*-alquil Enzymes by *Roheo Discolor*, Antimutagenic Ethanolic Extract. Arriaga-Alba M[1], Gonzales-Avila M[2], Ruiz-Perez NJ[1], Sanchez-Navarrete J[1]. [1]Hospital Juarez DE MEXICO, Mexico DF, Mexico, [2]Universidad Politecnica de Pachuca, Pachuca-Cd Sahagún, Zempoala Hidalgo, Mexico.

Introduction: *Roheo discolor*, a mexican plant employed on cancer, mycosis and infections, is not mutagenic on Ames test, neither genotoxic on the Unscheduled DNA Synthesis. Ethanolic extract is good antioxidant similar to α-tocopherol, both it have yet known mechanisms of action. Objective: The aim of this work is to know its antimutagenesis mechanisms. Material and Methods: Antimutagenic properties were evaluated against mutations induced by (2AA) or (2AF) on TA98 and UTH8413, or (ENG) and (MNNG) on TA100 and UTH8414 or by mytomicine-C on TA102. Employing S. typhimurium YG7100 (ada-/ogt+), YG7104 (ada+/ogt-) and YGT108 (ada-/ogt-), its antimutagenicity to alkylating agents was studied. Results: *R. discolor* did not reduced framshift mutations. It neither improved DNA excision repair on strain UTH8414 (hisG46 uvrB+). It reduced base pair substitution mutations by MNNG or ENG when added simultaneously or before alkylating agents. This antimutagenic effect is observed on TA100 ada+/ogt+ or YG7100 ada-/ogt+. On *S. typhimurium* deficient in Ogt DNA repair protein; YG7108 ada-/ogt- or YG7104 ada+/ogt-, any antimutagenic effect was observed. Conclusions: Antimutagenicity of *R. discolor*, against mytomicine C is reliable with its antioxidant properties; antimutagenic against MNNG or ENG only when *Ogt* demetilase DNA repair enzyme is present. The fact that antimutagenicity was not observed in *ogt-* strains suggests that *R. discolor*, induces the DNA *Ogt* enzyme necessary to repair alkylated DNA with ethyl or methyl groups. *R. discolor* may be suitable to prevent cancer induced by alkylating agents.

P58

Antimutagenic Properties of Group B Vitamins. Arriaga-Alba M, Ruiz-Perez N-J, Sanchez-Navarrete J, Lopez Del Angel B, Flores-Lozada J. Hospital Juarez De Mexico, Mexico DF, Mexico.

Introduction. Antimutagens are antioxidants, metabolism inhibitors, or induce DNA repair enzymes. Group B vitamins, cofactors on biochemical synthesis, might be antimutagens. Objectives. The aim of this work is to known the antimutagenitic properties of vitamins B against several types of mutagens and its possible mechanism of action. Material and Methods. Mutagenic and antimutagenic assays were performed with the Ames test. Vitamins B_1, B_6 or B_{12} were evaluated alone or in the presence of (MNNG) or (ENNG) on strain TA100, (2AF) or (2AA) on strain TA98 and Norfloxacin (NOR) or Nalidixic acid (NLX) on strain TA102. Antimutagenic results were analyzed by Dunnett´s Multiple Comparison test, using Graph Pad Prism software 2.01. Results. Vitamins B_1, B_6 and B_{12}, inhibit NOR or NLX mutagenesis ($P<0.001$). None of them were antimutagenic against framshift mutagens: 2-AF or 2-AA. B_1 reduced mutations induced by the alkylating agents MNNG or ENNG ($P<0.001$). They did not reduce quinolones effect on 24 uropathogenic E. coli strains. Conclusions. B_1, B_6 and B_{12} are antioxidants who inhibit quinolones induced ROS. They dif not inhibit, *in vitro* metabolic activation of 2AA and 2AF. B_1, a cofactor on DNA synthesis, induced the DNA alkyl transferases, reducing mutations by MNNG or ENNG. These vitamins, which are important components on human diet, may be a useful to reduce genotoxic risk of alkylating and ROS induced mutations.

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Genotoxic Exposure Assessment in Depleted Uranium (DU) Exposed Gulf War I Veterans: Sixteen Years of Follow Up. McDiarmid MA[1,2], Gucer P[1,2], Oliver M[1,2], Engelhardt SM[2], Squibb KS[1,2], Dorsey C[1,2], Vacek PM[3], Ardell SK[3], Albertini RJ[3]. [1]University of Maryland, Baltimore, MD, United States, [2]Dept. Veterans Affairs Medical Center, Baltimore, MD, United States, [3]University of Vermont, Burlington, VT, United States.

Exposure to depleted uranium, an alpha-emitting heavy metal, has prompted the inclusion of markers of genotoxicity in long-term surveillance of a dynamic cohort (N=77) of DU exposed Gulf War veterans followed since 1994. Using urine Uranium (U) concentration as the measure of U body burden the cohort was stratified into a low U (<0.10 µg U/g creat) and high U group (≥0.10 µg U/g creat). Outcomes include markers of both mutagenicity and clastogenicity: frequencies of sister chromatid exchange (SCE), chromosomal aberrations using classical G-banding (CA) and FISH techniques which have shown mixed and generally non-significant differences between U groups. Mean hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutant frequencies (MF) however have shown an almost 50% increase between the low vs high U group (MF=20.9±.8-vs-28.8±9.5; p=0.42) in 2007. In regression models, the soldiers with the highest U body burden over four testing rounds, do show a statistical difference in adjusted MF X 10^{-6} (57.11 vs.14.37) for those with urine U≥10.0 compared to those with urine U<0.10 ($p<0.001$), suggesting a possible genetic threshold effect. Mutational spectra determined for mutant isolates recovered from samples obtained in 2003 revealed 94 unique mutations in the high U group of which 25.5% were gene deletions (2.1% total dels; 23.4% partial dels) compared to 148 unique mutations in the low U group of which 16.2% were gene deletions (3.4% total dels; 12.8% partial dels). The between group difference in partial deletion frequencies is significant (p=0.03).

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The Role of Dietary Micro-Nutrient Supplementation to Maintain Genomic Stability. Vaglenov A[1,2], Shinn B[1], Edelbrock M[3], Schwaner T[3], Simonian A[4]. [1]School of Pharmacy, University of Findlay, Findlay, OH, United States, [2]National Center of Radiobiology and Radiation Protection, Sofia, Bulgaria, [3]College of Sciences, University of Findlay, Findlay, OH, United States, [4]College of Engineering, Auburn University, Auburn, AL, United States.

The objectives of this study were to determine each of the following both prior to and four months after daily intake of two Centrum tablets: 1) baseline DNA damage, 2) anti-genotoxic and therapeutic effects, and 3) susceptibility to induced radioresistance. The group examined for baseline curves determination consisted of 194 healthy donors, made up of both men and women and smokers and non-smokers. DNA damage was determined by cytokinesis-block assay of peripheral blood lymphocytes. For the determination of susceptibility, blood samples from the same persons have been "*in vitro*" irradiated with 1 Gy gamma-rays of 137 Cs before and after the termination of treatment. The baseline MN regression lines showed strong age, gender and smoking dependencies. After treatment with Centrum® an antigenotoxic effect was evident for smokers and non-smokers. Our results demonstrate that, at three months after the end of prophylaxis, both radiated and non-radiated MN levels are still lower than baseline values determined prior to trial initiation. Thus, Centrum® may be used for anti-mutagenic and anti-carcinogenic protection of persons exposed to tobacco and alcohol.

P61

Polymorphisms (SNPs) in DNA Repair Genes and Bladder Cancer Risk. Michiels S[1], Laplanche A[1], Boulet T[1], Dessen P[3], Guillonneau B[5], Méjean A[6], Desgrandchamps F[7], Lathrop M[4], Sarasin A[3], Benhamou S[2,3]. [1]Institut Gustave Roussy, Villejuif, France, [2]INSERM U794, Paris, France, [3]CNRS FRE2939, Villejuif, France, [4]Centre National de Génotypage, Evry, France, [5]Institut Mutualiste Montsouris, Paris, France, [6]Hôpital Necker, Paris, France, [7]Hôpital Saint-Louis, Paris, France.

Cigarette smoking is an established risk factor for bladder cancer. Tobacco exposure results in DNA damage, which, if left unrepaired can lead to the process of carcinogenesis. SNPs in DNA repair genes may therefore modify susceptibility to bladder cancer through gene-gene and gene-smoking interactions. We investigated a panel of 652 SNPs from 85 genes categorized in 6 major pathways in 201 bladder cancer cases and 326 controls matched for age, sex and hospital. We evaluated effects of haplotypes within genes, of global genetic variation in each pathway, as well as gene-gene and gene-smoking interactions by a regression tree. To account for multiple testing, we estimated for the p-value cut-off an expected proportion of false discoveries. Haplotype-analysis suggested potential associations ($p<0.05$) for 4 genes but came with an associated false discovery proportion of 0.24 (*POLB* and *FANCA* genes) and 0.62 (*OGG1* and *POLG* genes). DNA damage signaling and double-strand break repair pathways showed a higher number of SNPs with differential allele frequencies between cases and controls as compared to what would be expected by chance ($p<0.02$ and $p=0.05$). The first split on the regression tree is smoking status indicating smoking is the main risk factor. Distinct patterns of risk were then suggested in never- and ever-smokers. In never smokers, no genetic variants were found, whereas in ever smokers, a potential interaction between SNPs in *XRCC5* and *LIG1* genes was found. Bladder cancer is a complex disease and large-scale studies are needed to better identify gene-gene and gene-environment interactions.

P62

The Mutagenicity and Dioxin-Like Activity of Biodiesel Emissions. Gagnon ML, White PA. Health Canada, Ottawa, ON, Canada.

Alternative fuels are currently being assessed to determine their ability to reduce the risks of adverse health effects associated with exposure to diesel exhaust. The aim of this study was to assess the toxicological properties of (bio)diesel emissions using two *in vitro* bioassays. Mutagenic activity was assessed using the Salmonella mutagenicity assay and dioxin-like activity was assessed using the DR-CALUX assay. (Bio)Diesel particulates, collected on Teflon-coated filters via a constant volume dilution tunnel, were extracted using pressurized fluid extraction and the adsorbed organics were separated on open silica into polar aromatics and non-polar neutral compounds. Results indicate that organic extracts of (bio)diesel particles contain direct- and indirect-acting polar aromatic mutagens as well as polar and non-polar Ah-receptor agonists. A reduction in the mutagenic activity of direct-acting compounds was observed for the polar aromatic fraction with increasing biodiesel content in the fuel (e.g., 48% reduction for biodiesel blend B20 compared to ULSD on *Salmonella* TA98 without metabolic activation. Conversely, an increase in dioxin-like activity with increasing biodiesel fuel content was observed for both the polar and non-polar fractions (e.g., 144% increase for the non-polar fraction and 111% increase for the polar aromatic fraction of biodiesel blend B20 compared to ULSD). These results will provide a framework for evaluating the toxicological hazards of biodiesel emissions, and eventually identify fuel choice and engine design scenarios that minimize the risks of adverse health effects.

P63

Flow Cytometric Determination of the Micronucleus Frequency in Juvenile Rhesus Monkeys Chronically Exposed to Methylphenidate Hydrochloride. Bishop ME[1], Hotchkiss CE[3], Lin C-J[2], Chen JJ[2], Mattison DR[4], Morris SM[1]. [1]DGRT/NCTR/FDA, Jefferson, AR, United States, [2]DPMN/NCTR/FDA, Jefferson, AR, United States, [3]WANPRC/UWashington, Seattle, WA, United States, [4]OPP/NICHD/NIH, Bethesda, MD, United States.

Our laboratory has participated in a multi-center effort to evaluate the genotoxicity of methylphenidate hydrochloride (MPH). In our study, juvenile, male rhesus monkeys were chronically exposed to oral doses of MPH diluted in Prang. Initial doses were (1) Control, Prang only, 10 animals (2) Low, 0.15mg/kg of MPH twice per day, 10 animals, and (3) High, 1.5mg/kg of MPH twice per day, 10 animals. These doses were increased to approximate the serum levels of pediatric patients (2.5mg/kg, twice/day, Low) and a five-fold increase (12.5mg/kg, twice/day, High) over clinical levels. The baseline frequency of micronucleated erythrocytes (MN-RETs) was determined at the start of dosing and at monthly intervals for 18 months. The Litron Monkey MicroFlow® kit (customized) was used to evaluate 18,000 $CD71^{pos}$ cells for the presence of micronuclei by flow cytometry. The baseline frequency of MN-RETs in all 30 animals was 0.21± 0.03% (SE). The data were divided into two groups for statistical analyses: (1) the initial doses (5 months of exposure) and (2) the increased doses (12 months of exposure). An ANOVA mixed model with the dose as a fixed effect was used to determine if there were any significant effects of dose for either dosing regimen. The F-test indicated that there were no significant effects on dose ($P = 0.436$, initial doses and $P = 0.181$, increased doses). These data may be interpreted that, within the limits of sensitivity of detection of these methods, exposure to MPH does not result in an increase in the frequency of micronucleated reticulocytes in juvenile rhesus monkeys.

P64

Antioxidant and Anticlastogenic Capacity of Prickly Pear Juice. Hernández-Ceruelos A, Madrigal-Santillán E, Valadez-Vega C. Instituto de Ciencias de la Salud, Area Académica de Medicina, Universidad Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, Mexico.

Prickly pear (PP) is the fruit of cactus (Opuntia ficus-indica (L) Miller), there are many varieties of the fruit with different colors, all rich in antioxidant compounds like vitamin C, betalaines and polyphenols, know for their health benefits, making PP a good candidate as a chemoprotective agent. The aims of the study were to compare the antioxidant capacity of the green, yellow and red prickly pear juice (PPR) by DPPH colorimetric method, and to select the best variety to determine its anticlastogenic capacity against methyl methanesulfonate (MMS). Results showed that red variety had the best antioxidant capaicty, therefhore, it was selected to perform the *in vivo* micronucleus test. Groups of 5 animals were treated daily by oral route for two weeks with water as negative control, and 0.75, 0.5 and 0.25 ml of PPR juice. Two samples of blood were taken in the first and second week to determine the possible genotoxicity of the juice. After this period, groups treated with juice were administered with MMS (40 mg/Kg) by I.P. route. Blood samples were obtained at 0, 24, 48, 72 and 96 h after the mutagen injection and smears were stained with Giemsa, micronucleus (MN) frequency and polichromatic and normochromic (PCE/ENC) rate were determine. Results showed that after two weeks of treatment PPR is not genotoxic or citotoxic by itself and a significative decrease on the MN frequency was observed when compared with the positive control in all the groups treated with juice, proving an strong anticlastogenic capacity, suggesting that other test must be done to explore all its advantages.

P65

Functional Food Potential of Fermented Red Wine Pomace. Yoshikawa K. Kinki University, Nara City, Japan.

While consumptions of wine increases, an abundant by-product is the grape pomace produced by compression of unrefined red wine and seeds during the middle of the main fermentation. Pomace are used for raw materials of brandy such as grappa and the marl, but most are used compost. Putrefactive bacteria from pomace could also possibly polluted the winery. Therefore, there is a need to re-utilize by-products from pomace. I examined its functional foods. I divided the grape pomace into rinds and seeds, freeze dried and transformed them into powder. Samples were extracted from the powder using acetone, diethyl ether etc.. The functional food potential was examined by checking for the presence of antimutagenicity through Ames test performed using *Salmonella typhimurium* TA98, 100.In addition, I examined antioxidation activity by the DPPH radical elimination method. The freeze-dried samples extracted organic solvents, showed antimutagenicity. I was able to identify the existence of antioxidation material as in the extract of rinds and the seeds using the same samples. In addition, antimutagenicity was also detected after high-pressure steam sterilization of freeze-dried powder, indicating a potential for application to heated processed food and development of supplements.

P66

Genotoxicity of Methylphenidate Hydrochloride in the Rhesus Monkey. Dobrovolsky VN[1], Shaddock JG[1], Manjanatha MG[1], Miura D[3,1], Mattison DR[2], Morris SM[1]. [1]NCTR, Jefferson, AR, United States, [2]NICHD, Bethesda, MD, United States, [3]Teijin, Tokyo, Japan.

We have investigated the mutagenicity of methylphenidate hydrochloride (MPH) in cells of peripheral blood derived from male juvenile rhesus monkeys chronically exposed to the drug. 10 animals received orally a daily dose of 0.30 mg/kg (later increased to 5 mg/kg); 10 animals received a daily dose of 3.0 mg/kg (later increased to 25 mg/kg); and 10 animals received vehicle only. One additional animal received two i.p. injections of 77 mg/kg ENU – one at the beginning of the study and another 16 months later. All animals were monitored for the presence of *HPRT* mutant lymphocytes in the blood before the MPH treatment and every 3 months over a period of 19 months. In addition, all animals were analyzed for the presence of *PIG-A* mutant erythrocytes at 24 months. The *HPRT* mutant frequency (MF) in animals receiving vehicle ranged from 0 to 8×10^{-6}, and from 0 to 4×10^{-6} in the MPH-treated monkeys. The ENU-treated animal exhibited an increase in the *HPRT* MF (up to 30×10^{-6}) that remained high (though gradually decreasing) over the monitoring period. Similar observations were made using the *PIG-A* gene target. The controls had a low *PIG-A* MF ($8\pm4\times10^{-6}$), the ENU-treated animal had a MF more than 5 fold higher (46.5×10^{-6}), and the MPH-treated animals had a *PIG-A* MF consistent with the control values ($11.7\pm5.7\times10^{-6}$ for the low dose and $9.3\pm4.1\times10^{-6}$ for the high dose, $p>0.1$). A single animal treated with low MPH dose had an elevated *PIG-A* MF of 22.6×10^{-6} (a potential outlier). Our results suggest that, within the limit of the detection sensitivity in our model, MPH is not a gene mutagen in rhesus monkey.

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Frequency of Chromosomal Aberrations in Prague's Mothers and Their Newborns. Sram RJ[1], Rossnerova A[1], Balascak I[2]. [1]Institute of Experimental Medicine AS CR, v.v.i., Prague, Czech Republic, [2]Faculty of Medicine, Charles University, Prague, Czech Republic.

The capital city of Prague is one of the most polluted areas of the Czech Republic. The impact of air pollution on the level of chromosomal aberrations is systematically studied. Analyses were performed using fluorescence *in situ* hybridization (FISH) with whole chromosome painting for chromosome #1 and #4. The studied groups were 42 mothers and their newborns, living in Prague. The average age of mothers was 29 years (20-40). The blood samples were collected during fall 2007 and winter 2008. Average levels of c-PAHs and B[a]P from stationary monitoring were 19.3 ± 12.8 ng/m^3 and 2.6 ± 1.8 ng/m^3, respectively. The levels of stable (one-way and two way translocations) and unstable (acentric fragments) chromosomal aberrations in both groups were analyzed. The mean genomic frequency of translocations by FISH (F_G/100) was 0.09 ± 0.013 vs. 0.80 ± 0.79 ($p<0.001$) for newborns vs. mothers. The level of stable aberrations significantly increased with age. The frequency of unstable aberrations did not differ between those two groups. We observed 64% of unstable aberrations vs. 36% of stable aberrations in newborns and 20% vs. 80% in mothers. The level of aberrations in newborns showed the significant increase in the group of children born to older mothers. We did not find significant differences in levels of aberrations between fall 2007 and winter 2008. Our present results indicate: the frequencies of aberrations in newborns are very low and represented mainly by acentric fragments. Our concentrations of c-PAHs did not affect genomic frequency of translocations in newborns. Grants by Min. of Education CR 2B06088 and AS CR AVOZ50390512.

P68

Enhancement of Bleomycin Genotoxicity in Yeast by Conventional and Unconventional Intercalating Agents. Hoffmann GR, Laterza AM, Sylvia KE, Tartaglione JP. College of the Holy Cross, Worcester, MA, United States.

Interactions between bleomycin (BLM) and intercalating agents were studied in an assay for mitotic gene conversion and point mutations in yeast. BLM is a glycopeptide antibiotic and cancer chemotherapy drug whose properties as a radiomimetic chemical have led to extensive study in genetic toxicology assays. Its mechanism of action includes abstraction of a hydrogen from the 4' position of deoxyribose, followed by processing of the resultant free radical to form single- and double-strand breaks in DNA. Various chemicals modulate the genetic activity of BLM; the interactions include antimutagenesis and enhancement of genotoxicity. The chemicals studied here include both classical intercalating agents with three fused heterocyclic rings and agents whose structures do not immediately reveal the likelihood of intercalation but that are suggested by computer modeling to intercalate into DNA. Both the conventional and unconventional intercalating agents, whether they are genotoxic themselves or not, were found to enhance the induction by BLM of mitotic gene conversion at the *trp5* locus and reversion of the *ilv1-92* allele in *Saccharomyces cerevisiae* strain D7. The data are consistent with the interpretation, based on micronucleus assays in mammalian cells, that BLM enhancement can serve as an indicator of intercalating activity (R. D. Snyder et al., Environ. Mol. Mutagen. 44:163, 2004; R.D. Snyder, Mutat. Res. 623:72, 2007). The agents studied in D7 include nonreactive acridines, acridine mustards, nitroacridines, chlorpromazine, chloroquine, mefloquine, tamoxifen, diphenhydramine, and benzophenone.

P69

Development of an Automated *In Vitro* Micronucleus Analysis Method in CHL/IU Cells. Muto S, Sugiura K, Kurabe M, Aruga C, Yamamura E, Uno Y. Mitsubishi Tanabe Pharma Corporation, Kisarazu, Chiba, Japan.

An automated *in vitro* micronucleus analysis method in CHL/IU cells was developed and evaluated for its availability as a rapid screening of genotoxicity of chemicals. The CHL/IU cells were plated on collagen I-coated 96-well microplates and incubated for 24 h. Cells were treated with clastogens, aneugens and pharmacetceutical candidates in Mitsubishi Tanabe Pharma Co. (MTPC) for 6 h with or without S9-mix followed by a 18 h recovery period or 24h without S9-mix. After the cells were fixed at the end of the incubation period, the nuclear DNA and cytoplasmic RNA were stained with Hoechst 33342 and SYTO RNA select, respectively. The images of cells were collected with a high contents screening system (IN Cell analyzer 1000, GE healthcare) and analyzed by an image analysis software (Developer toolbox, GE healthcare) which analytical protocol was optimized for the assay with CHL/IU cells. The same images were separately examined by manual scoring to compare the test results. There was a good correlation (R = 0.95) in the incidence of micronucleated cells between automated and manual analyses. The analysis of mean micronucleus size showed that aneugens caused micronuclei which was larger than that from clastgen-treated cells, indicating that the aneugens might be identified with this simple method. The automated *in vitro* micronucleus analysis would be acceptable as a rapid screening of genotoxicity instead of a conventional microscopic analysis on micronucleus induction.

P70

Flow Cytometric Evaluation of Micronucleated Polychromatic Erythrocytes in Bone Marrow and Micronucleated Reticulocytes in Peripheral Blood Following Acute and Repeat Dosing Regimen of Chemicals. Shi J[1], Krsmanovic B[1], Torous D[2]. [1]BioReliance Corporation, Rockville, MD, United States, [2]Litron Laboratories, Rochester, NY, United States.

The flow cytometric (FCM) method for evaluating micronuclei (MN) in erythrocytes has been developed and proven to be more sensitive, reliable and reproducible compared with traditional microscopic enumeration. To validate the FCM method in-house at BioReliance using *In Vivo* MicroFlow®, we conducted a systematic evaluation of bone marrow and peripheral blood of mice treated with well-characterized MN inducers such as cyclophosphamide, methyl methanesulfonate and vinblastine, as well as a nongenotoxic chemical, S-adenosylmethionine chloride. Bone marrow and peripheral blood samples were harvested at 24 and 48 hours following a single dose, 24 hours following the 5th repeat dose and on Days 1, 8, 15, 22 and 29 in a 4-week repeat dosing study. The frequencies of micronucleated polychromatic erythrocytes/reticulocytes (% MN-PCE/MN-RET) were determined by microscopy (BioReliance), and flow cytometry at both BioReliance and Litron. The results were comparable; moreover, the FCM method enhanced assay sensitivity throughout the studies and the FCM data obtained from BioReliance and Litron showed a high-degree correlation. In addition, sampling once a week in a 4-week repeat dosing study provided valuable insight into the generation, turnover and relationship between MN-PCEs in bone marrow and MN-RETs in peripheral blood. In conclusion, in agreement with other comparative studies, MN frequency evaluated by MicroFlow is validated at BioReliance and the presented data support it's use for the evaluation of MN frequencies in bone marrow and peripheral blood of mice following chemical exposure. Future validation will be conducted in more species including rat, canine and human.

P71

Coupling Cytotoxic Biomarkers With *In Vitro* Comet Assay. Shi J, Springer S, Bruce S, Sly J, Scherer M, Cecil M, Pant K, Escobar P. BioReliance Corporation, Rockville, MD, United States.

The Alkaline Comet assay detects single-strand breaks, double-strand breaks and alkali-labile sites in DNA. It is among a number of widely used genotoxicity tests for product safety assessment. Concurrent cytotoxicity measurement is recommended to be conducted with *in vitro* Comet assay to avoid possible false positive results due to excessive cytotoxicity. Conventional cytotoxicity assessment methods, including trypan blue exclusion assay and mitotic index, often underestimate cytotoxicity, revealing the need for developing reliable cytotoxic biomarkers to provide high sensitivity and indications of cytotoxic mechanisms. Here, we introduced the ATP determination assay and the Caspase-3/7 activation assay in combination with *in vitro* Comet assay to test a number of cytotoxins, genotoxins and apoptosis inducers in TK6 human lymphoblast cell line. The ATP determination assay quantifies the ATP amount in metabolically active (live) cells, thus correlates well with cell number and viability. The Caspase-3/7 activation assay measures the activation of these two proteases, which indicates the onset of apoptosis. Our data showed that these biomarkers provide rapid, robust and accurate measurement for cytotoxicity compared with traditional methods, which could potentially be applied in determination of top dose in the Comet assay as well as other *in vitro* genotoxicity tests. Moreover, the observation that induction of apoptosis accompanies with many chemical-induced cytotoxicity indicated the mode of action (MOA) of the test chemicals and suggested that apoptosis plays an important role in this process.

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Cross-Validation of Miniature Ames and *In Vitro* Micronucleus Assays to Facilitate Earlier Genotoxicity Screening During Lead Optimization. Sawant SG[1], Pant K[2], Szkudlinska A[2]. [1]Amgen Inc., Thousand Oaks, CA, United States, [2]BioReliance Corporation, Rockville, MD, United States.

Genotoxicity is an essential part of preclinical safety evaluation. Many pharmaceutical companies conduct screening studies early on in drug discovery to minimize genotoxicity-related compound attrition during the GLP-development phase. At present, screening Ames (using 100 mm plates) and HPBL micronucleus assays (using 5 mL cell cultures) require a total of 500 mg test article. Compound for screening studies are generally obtained from a batch prepared for the *in vivo* toxicology studies. However, a positive finding in these studies delays/stops further development of the molecule. Identifying the genotoxic potential of drug candidates before *in vivo* tox batch preparation could further minimize loss of time/resources and help with the selection of drug candidates free from genotoxicity liabilities. This would require assays that use minimum compound, as only milligram quantities of material are usually made in the earlier research batches. Therefore we validated miniaturized versions of Ames (using 24 well plates) and HPBL micronucleus assays (using 1 mL cell cultures) that could be conducted with only 40 mg of compound. Ten compounds were selected for the validation studies. For both validation studies the regular and miniaturized formats were performed in parallel. Comparison of the studies demonstrates a high concordance for both mutagenicity and genotoxicity results between the miniaturized and the standard screening formats. These results show that the miniaturized assay formats provide an opportunity to conduct screening genotoxicity studies early on in drug discovery.

P73

Evaluation of Micronucleus Frequencies and DNA Damage in Male Rats Administered Methylphenidate Hydrochloride (Ritalin) for 28 Days. Witt KL[1], Recio L[2], Shepard K[2], Green A[2], Baldetti C[2], Winters J[2], Davis J[3], Caspary W[1], Hobbs CA[2]. [1]National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States, [2]Genetic Toxicology Division, ILS, Inc., Research Triangle Park, NC, United States, [3]Integrative Toxicology Division, ILS, Inc., Research Triangle Park, NC, United States.

A variety of studies have been conducted recently to clarify the risk for induced genetic damage following exposure to methylphenidate (MPH), a frequently prescribed drug for management of symptoms of attention deficit/hyperactivity disorder. Although most studies reported no changes in genetic damage endpoints associated with MPH exposure, one recent study [Andreazza et al. 2007, Prog Neuropsychopharmacol Biol Psychiatry 31(6):1282-8] reported an increase in DNA damage detected by the comet assay in blood and brain of Wistar rats exposed to 2 or 10 mg/kg MPH; no increases in micronucleated lymphocyte frequencies were observed. To clarify this report of MPH-induced DNA damage, we treated male Wistar-Han rats with 0, 2, 10 and 25 mg/kg MPH by gavage once daily for 28 consecutive days and assessed micronucleated reticulocyte (MN-RET) frequencies in blood, and DNA damage in blood, brain, and liver, 4 hr after the last dosing. Flow cytometric evaluation of blood samples revealed no significant increases in MN-RET, and the %RET among total erythrocytes, a measure of bone marrow toxicity, was unaltered at any dose. Furthermore, comet assay evaluations of blood leukocytes and liver tissue, as well as striatum, hippocampus, and frontal cortex tissues of the brain showed no increases in primary DNA damage in MPH-treated rats in any of the three treatment groups, and no MPH-induced histopathological changes were observed in brain tissue. Thus, the reported observations of MPH-induced DNA damage in blood and brain tissue of rats exposed for 28 days were not confirmed in this study.

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Miniaturization and Further Automation of the *In Vitro* Micronucleus Assay. Bryce S, Phonethepswath S, Avlasevich S, Bemis J, Dertinger S. Litron Laboratories, Rochester, NY, United States.

This laboratory has described a flow cytometric method for scoring *in vitro* micronuclei (MN) [Environ. Molec. Mutagen. (2006) 47:56-66]. This *In Vitro* MicroFlow™ method labels necrotic and mid-/late-stage apoptotic cells with ethidium monoazide. Cells are then washed, stripped of their cytoplasmic membranes, and incubated with RNase plus a pan-nucleic acid dye. Here, we report modifications to the method whereby all procedures are accomplished in the same 96 well plate. For these experiments, TK6 cells were treated continuously for 24 - 30 hrs with 6 diverse genotoxicants. Additionally, 6 non-genotoxicants were evaluated up to cytotoxic concentrations. With the assistance of liquid handlers, treatments, staining, and flow cytometric analyses occurred automatically. Flow cytometry-based MN frequencies were calculated based on the analysis of 10K nuclei per well. Since a consistent number of latex particles were added to each specimen, nuclei to bead ratios were collected concurrently with MN frequencies, and these data were used to derive relative survival (RS) measurements. Dose-dependent MN induction was observed for each of the genotoxic agents studied. Regarding non-genotoxicants, negative results were obtained when top concentration was limited to 50% RS. Collectively, these data suggest that *in vitro* MN screening can be accomplished in a miniaturized and highly automated format. Further work is needed to characterize assay sensitivity and specificity with a greater number of chemicals, and also to assess the transferability of the method through inter-laboratory trials.

P75

Cytotoxicity and Mutagenicity of Stereoisomers of 3-Epoxybutane-1, 2-diol at Low Concentrations in TK6 Cells. Meng Q[1], Hackfeld L[2], Hodge R[2]. [1]Battelle Toxicology Northwest, Richland, WA, United States, [2]University of Texas Medical Branch at Galveston, Galveston, TX, United States.

3-Epoxybutane-1, 2-diol (EBD), a reactive metabolite of 1, 3-butadiene (BD), potentially plays a significant role in the mutagenic and carcinogenic effects of BD. Earlier studies showed that (2R, 3S)-EBD induced significant mutagenic response at 40 μM and was significantly more cytotoxic and mutagenic than other stereoisomers of EBDs in TK6 Cells. To evaluate further the cytotoxicity and mutagenicity of EBDs at lower concentrations, TK6 cells, a human lymphoblastoid cell line, were exposed to 0, 5, 10, 20, 30, 40 μM of each form of EBD for 24 hour, and the cytotoxicity was evaluated immediately after exposure by determining relative cell survival. (2R, 3S)-EBD was cytotoxic at 20, 30, and 40 μM. The other three stereoisomers of EBD did not cause cytotoxic response at any exposure concentrations. TK and HPRT mutant frequencies (M*f*s) were measured at exposure concentrations of 0, 5, 10, or 20 μM using a cell cloning assay. (2R, 3S)-EBD caused statistically increased HPRT and TK M*f*s at 20 μM, but not at lower concentrations. HPRT and TK M*f*s were not increased in cells exposed to other three stereoisomers of EBD. These results indicate that stereostructure of EBD has an important impact on the cytotoxicity and mutagenicity of EBD, and these differences of mutagenic potency associated with stereostructure may contribute significantly to the species differences in mutagenic and carcinogenic effects of BD in rodents. Supported in part by the Health Effects Institute agreement 01-5 and grant number ES-06676 from the National Institute of Environmental Health Science.

P76

A Mini Version of the Mouse Lymphoma Cell Thymidine Kinase Locus Assay. Hou S, Bjurström M, Bolcsfoldi G. Safety Assessment, AstraZeneca R&D Södertälje, Södertälje, Sweden.

We have developed a mini version of the mouse lymphoma assay (MLA) that employs the standard MLA protocol, but high cell-density incubation (5 x 10^5 cells in 2 mL treatment medium) in 50-mL tubes to reduce amount of test compound without increasing test variability. The Mini assay was compared with the standard assay (5 or 10 x 10^6 cells in 10 or 20 mL treatment medium, in 50-mL tubes) in concurrent tests using 3-hour exposure to negative and positive reference compounds. The two assays produced similar levels of solvent control mutant frequencies (MF) both in the presence and in the absence of S9. An excellent reproducibility between replicate cultures, treated or untreated, in both relative total growth and MF, was achieved in the Mini assay. Both cytotoxicity and mutagenicity were similar in the two assays for 9-aminoacridine (-S9), hycanthone (-S9), hydrogen peroxide (-S9), cyclophosphamide (+S9) and 7,12-dimethyl-1,2-benzanthracene (+S9). Lower cytotoxicity was seen in the Mini assay than in the standard assay for formaldehyde (-S9) and 4-nitroquinoline-N-oxide (-S9), but the magnitude of mutant induction was the same at comparable cytotoxicity levels. Negative results were obtained for 2-aminopurine (-S9), Triton X-100 (-S9) and phenol (-S9). It is concluded that the Mini MLA detects mutagenic activity reliably and can be used as an alternative to the standard MLA for genotoxicity evaluation of early discovery compounds. The assay can be run as a combined range-finder and mutagenicity test up to 1-2 mM that allows considerably less compound usage (10-20 mg) and higher throughput.

P77

Cytotoxic and Genotoxic Potential of Surface and Waste Waters Using the Allium and Comet Tests. Radic S[1], Stipanicev D[2], Cvjetko P[1], Širac S[2], Marijanovic Rajcic M[2], Pevalek-Kozlina B[1], Pavlica M[1]. [1]University of Zagreb, Zagreb, Croatia, [2]Hrvatske vode-Legal Entity for Water Management, Zagreb, Croatia.

Analysis of genotoxicity is of a great importance due to the pollution of the environment by genotoxicants including risk assessment evaluation. In this study the response of the *Allium cepa* and *Lemna minor* L. genetic material to the presence of potential cytotoxic and genotoxic substances in surface and wasterwater samples was monitored. Also the suitability of the Allium test and alkaline comet assay as systems for environmental monitoring of surface and wastewaters were investigated. The surface waters were collected at three sampling sites along the river Sava and its confluents while wastewater samples were collected from sewage and industrial effluents near Zagreb, Croatia. Morphological modifications in the A. *cepa* roots, inhibition of root growth and cell division, aberrant cells in metaphase and anaphase as parameters of Allium test were observed. Tail extent moment as a measure of DNA strand breaks was evaluated by using comet assay on *Lemna minor* plants. The most polluted water samples caused the inhibition of root growth over 50% (even up to 65%), decrease of mitotic index over 40%, increase of aberrant cells for more than 10 times and increase of tail extent moment for more than 35 times in comparison to control. Obtained data demonstrate the mutagenic activity in some samples of tested waters and suitability of both tests for complex assessment of surface as well as waste waters.

P78

Mouse Mutation Assay Based on the *Pig-a* Gene. Phonethepswath S, Bryce S, Bemis J, Dertinger S. Litron Laboratories, Rochester, NY, United States.

This laboratory has described a *Pig-a* gene mutation assay based on blood from Sprague Dawley rats [Bryce *et al.,* Environ. Molec. Mutagen. 49 (2008) 256-264]. The underlying premise is that lack of GPI anchored proteins on the surface of RBCs should represent a reliable phenotypic marker of *Pig-a* mutation. Here, we extend this work to include CD-1 mice. After RBC enrichment, anti-CD24-PE was used to differentiate wt versus GPI anchor-deficient cells (presumptive mutants). Cells were then incubated with SYTO 13 to discriminate mature erythrocytes from reticulocytes (RETs). Flow cytometry was used to score mutants in two erythrocyte populations: total RBCs and RETs. Mutagen treatments (ENU, 40 mg/kg/day, 3 days; or DMBA, 75 mg/kg/day, 3 days) increased the frequency of GPI anchor-deficient RBCs and RETs. Whereas induction of mutant RETs was evident by one week post-exposure, two weeks were needed in the case of RBCs. The magnitudes of these responses were maintained until termination of the experiment (5 weeks post-exposure). Collectively, these data suggest mutations that generate GPI anchor-deficient erythrocytes occur in cells with considerable self-renewing capacity, and that these mutational events are neutral. These are highly desirable characteristics of an *in vivo* mutation assay, and support further development of this system. In the near-term, further work is needed to more firmly establish the equivalence of the GPI-anchor deficient phenotype with *Pig-a* mutation, evaluate additional genotoxic and non-genotoxic chemicals, and identify the most appropriate treatment/harvest schedules.

P79

Identification of Promutagens Using 'S9', Hepatocytes And HepaRG Cells, With The GADD45aGFP Genotoxicity Assay. Walmsley RM[1,2], Tate M[2], Jagger C[2], Rabinowitz A[2], Hughes C[2], Cahill PA[2], Knight AW[2], Billinton N[2]. [1]University of Mancheser, Manchester, United Kingdom, [2]Gentronix Ltd, Manchester, United Kingdom.

Introduction: Recent developments in the supply of fresh hepatocytes, and the availability of more metabolically competent hepatic cell lines, have allowed a reassessment of the choice of exogenous metabolic activation (MA) in genotoxicity assessment. The most commonly used MA system is the S9 extract of chemically induced rodent livers. These extracts have a skewed representation of phase 1 and phase 2 activities, and show strong species differences. Methods: Data will be presented from 4 new metabolism studies using the TK6 hosted GADD45a-GFP reporter assay. The first and largest study using Aroclor-induced rat S9 provided a comparative baseline. The second study demonstrates that human S9 can also be used. The third study used co-culture of reporter cells with stabilized primary rat hepatocytes from Abcellute (with and without enzyme induction). The fourth study used co-culture with the HepaRG cell line from BioPredic. Results: 1. The studies establish compatibility of the reporter assay with the various MA sources. 2. All systems produced predictable results. Discussion: Follow up studies for compounds that give a positive result with MA are directed towards understanding relevance to human. This study suggests that valuable data can be derived quickly and easily using human, and by implication other S9 sources in the GADD45a-GFP assay. The hepatocyte and HepaRG studies demonstrate that it may now be possible to get a more representative view of *in vivo* metabolism, though progress will require a well characterized set of test compounds where *in vivo* metabolic fate has been well established.

P80

Impaired Mitochondrial Function May Cause Liver Toxicity in Nevirapine-Treated B6C3F1 Mice. Desai VG, Moland CL, Lee T, Branham WS, Beland FA, VonTungeln LS, Fuscoe JC. NCTR, Jefferson, AR, United States.

A non-nucleoside reverse transcriptase inhibitor, nevirapine (NVP), is effective in suppressing HIV-1 load in adults and in reducing mother-to-child transmission of the virus. However, NVP use has been associated with severe liver toxicity within the first few weeks of treatment. Liver toxicity has also been reported in patients administered nucleoside reverse transcriptase inhibitors and this has been related to mitochondrial dysfunction. The role of mitochondria in NVP-induced liver toxicity, however, is still unknown. We, therefore, examined the transcriptional level of 542 mitochondria-related genes, using a MitoChip in the liver of B6C3F1 female mice treated with NVP. Mice were treated orally by gavage with 100 and 375 mg/kg body weight/day for 28 days from postnatal day 28 through 55 and were humanely sacrificed 24 hours after the last dose. At the 375 mg/kg dose, microarray analysis showed a significant down-regulation of both mitochondrial and nuclear genes involved in oxidative phosphorylation compared to controls, suggesting altered respiratory function. In addition, genes associated with mitochondrial genome maintenance, fatty acid oxidation, the Krebs cycle, apoptosis, and the mitochondrial membrane transport system had decreased expression levels. Altogether, the results suggest that NVP-induced impaired mitochondrial function leading to oxidative stress may play a role in drug-related liver toxicity.

P81

Cyclophosphamide and Etoposide Canine Studies Demonstrate the Cross-Species Potential of the Peripheral Blood Micronucleated Reticulocyte Endpoint. Torous D[1], McKeon M[2], Schmuck G[3], Xu Y[2], Burgess S[2], Avlasevich S[1], Dertinger S[1], Kirkland D[4]. [1]Litron Laboratories, Rochester, NY, United States, [2]Covance, Vienna, VA, United States, [3]Bayer Healthcare, Wuppertal, Germany, [4]Covance, Harrogate, United Kingdom.

Accumulating data suggests that peripheral blood micronucleated reticulocytes (MN-RETs) represent an endpoint of cytogenetic damage, even in species with efficient splenic filtration function. Studies performed evaluated both dose response and time-course of micronucleus (MN) induction in the bone marrow and blood of beagle dogs after dosing with cyclophosphamide (CP) or etoposide (Eto). CP was administered daily via intravenous injection for 5 days at 0, 6.25, 12.5 or 25 mg/m^2/day. Eto was administered via iv injection for 2 days at 0, 1.56, 6.25, and 12.5 mg/m^2/day. Blood specimens were collected for analysis before dosing as well as at several intervals during treatment, and bone marrow was prepared at necropsy. Blood was prepared using the *In Vivo* MicroFlow® method and analyzed at Litron, while bone marrow was analyzed at Covance via microscopy (May-Grunwald and also acridine orange staining). Robust MN-RET induction was observed in the blood of all CP-treated dogs by Day 4, with dose-related increases evident by Day 3. Comparable dose-related increases were observed in the bone marrow with microscopy-based scoring. While significant MN induction was not observed in the blood or bone marrow of dogs treated with Eto at 1.563 mg/m^2/day, marked dose-related increases were noted in both compartments for the 6.25 and 12.5 mg/m^2/day groups. Collectively, these results demonstrate the utility and sensitivity of blood-based automated MN-RET measurements in canines. These data have important implications in regard to the reduction and refinement of animal usage in genetic toxicology investigations.

P82

Dietary Polyphenols as Topoisomerase II Poisons: B Ring and C Ring Substituents Determine the Mechanism Of Enzyme-Mediated DNA Cleavage Enhancement. Bandele OJ, Clawson SJ, Osheroff N. Vanderbilt University School of Medicine, Nashville, TN, United States.

Dietary polyphenols are diverse compounds that are linked to human health. Many of their effects have been attributed to the ability to poison (enhance DNA cleavage by) topoisomerase II. Some polyphenols are traditional, redox-independent topoisomerase II poisons, and interact noncovalently with the enzyme. Others enhance DNA cleavage in a redox-dependent manner that requires covalent adduction to topoisomerase II. To define the elements that dictate the mechanism by which polyphenols poison topoisomerase II, the activities of two classes of polyphenols against human topoisomerase IIalpha were examined. The first included catechins: (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), and (–)-epicatechin (EC). The second included flavonols: myricetin, quercetin, and kaempferol. EGCG and EGC were redox-dependent topoisomerase II poisons, kaempferol and quercetin were traditional poisons, myricetin utilized both mechanisms, and ECG and EC displayed no significant activity. Rules are proposed that predict the mechanism of bioflavonoid action against topoisomerase II. The first rule centers on the B ring. While the C4'-OH is critical for the compound to act as a traditional poison, the addition of –OH groups at C3' and C5' allows it to act as a redox-dependent poison. The second rule centers on the C ring. The structure of the C ring in the flavonols is aromatic and planar. Disruption of these attributes abrogates enzyme binding and precludes the ability to act as a traditional topoisomerase II poison. Supported by NIH grants GM33944, CA09582, and GM78744.

P83

The Mutagenic Activity of High-Energy Explosives, Contaminants of Concern at Military Training Sites. McAllister JE[1,2], Gingerich JD[1], White PA[1]. [1]Health Canada, Ottawa, ON, Canada, [2]University of Ottawa, Ottawa, ON, Canada.

The genotoxicity of energetic compounds that commonly occur in contaminated soils at military training sites has not been rigorously tested. The Salmonella and Muta™Mouse *in vitro* mutagenicity assays were employed to examine the mutagenic activity of selected energetic compounds including TNT, tetryl, RDX, and HMX, as well as explosives-contaminated soil samples. Salmonella analyses employed strains TA98 (frameshift), TA100 (base-pair substitution), and the metabolically enhanced YG1041, with and without exogenous metabolic activation (S9). Results indicate that TNT is a direct-acting mutagen, eliciting significant responses without S9. Strains TA98, TA100, and YG1041 yielded mutagenic potencies of 0.87±0.03, 1.72±0.08, and 1.32±0.06 revertants/µg TNT, respectively. In contrast, tetryl elicited significant responses both with and without S9, exhibiting mutagenic activity in all strains. Potencies ranged from 1.27±0.13 to 14.98±1.67 revertants/µg tetryl. Testing of soil samples yielded significant responses in strains TA98 and YG1041, with and without S9. Mutagenic potencies ranged from 3.48±0.13 to 16.20±0.97 revertants/mg soil equivalent. Responses obtained using the Muta™Mouse assay in FE1 cells indicate that TNT can induce lacZ mutations, with and without S9. In contrast, there is little evidence to support the mutagenic activity of tetryl or RDX. HMX appears to be toxic to FE1 cells. Testing of soil samples in the Muta™Mouse assay is currently underway. Analysis of other energetic compounds and contaminated soil samples is warranted in order to reliably estimate mutagenic hazard.

P84

Genotoxicity of Carbon Nanomaterials *In Vitro*. Lindberg HK[1], Falck GC-M[1], Suhonen S[1], Järventaus H[1], Catalán J[1,2], Vippola M[1,3], Vanhala E[1], Savolainen K[1], Norppa H[1]. [1]Finnish Institute of Occupational Health, Helsinki, Finland, [2]University of Zaragoza, Zaragoza, Spain, [3]Tampere University of Technology, Tampere, Finland.

Data on the possible genotoxicity of nanoparticles are still scanty. We have examined *in vitro* the potential genotoxicity of two commercially available carbon nanomaterials: single-wall (SWCNT; SES Research) and multi-wall (MWCNT; SES Research) carbon nanotubes. Genotoxicity was assessed by the analysis of DNA damage in human bronchial epithelial cells (BEAS 2B) and mesothelial cells (MeT-5A) and micronuclei (MN) in BEAS 2B cells. The cells were cultured with various doses (5-80 µg/cm²) of SWCNTs and MWCNTs. The single cell gel electrophoresis (comet) assay was applied to study DNA damage after 24-h and 48-h treatments. The induction of MN was examined by the cytokinesis-block method after 48-h and 72-h exposures. Our preliminary results indicate that the 24-h and 48-h treatments with SWCNTs induce DNA damage in BEAS 2B cells, with a dose-dependent effect after the 48-h treatment. With MWCNTs, an increase in DNA damage was observed in BEAS 2B cells only after the 24-h treatment, but no dose-dependency was seen. In MeT-5A cells, both 24-h and 48-h treatments with SWCNTs increased DNA damage, with a significant dependence on dose in the 48-h treatment. MWCNTs produced DNA damage in MeT-5A cells both after the 24-h and 48-h treatments in a dose-dependent manner. The analysis of the micronucleus data is presently in progress. In conclusion, our preliminary results suggest that both single and multiwall carbon nanotubes have genotoxic potential in human epithelial and mesothelial cells *in vitro*, with a more pronounced effect in mesothelial cells. [Supported by NMP4-CT-2006-032777.]

P85

The Effect of Heat Shock on DNA Integrity in Leaves of *Nicotiana tabacum* L. Cvjetko P, Balen B, Peharec P, Pavlica M. University of Zagreb, Zagreb, Croatia.

Plants are sessile organisms incapable to escape from the harmful environment. Therefore, plants have evolved a variety of responses to protect and preserve cellular homeostasis. Moreover, many different forms of abiotic stress, temperature as well, are known to disrupt metabolic balance inducing oxidative damage in the cells which respond by triggering the antioxidant defense, including peroxidases. Heat-induced DNA damage and DNA damage response are two interrelated mechanisms involved in maintaining integrity of the genome, but in plants are poorly understood. Therefore, to determine the possible genotoxic effect of short-term heat shock treatment at 42°C the alkaline comet assay has been applied to leaf cells of *Nicotiana tabaccum* 1- and 3-month old. Peroxidase activity was also evaluated as biomarker of temperature stress. Increased level of DNA damage and induction of peroxidase activity were noticed in response to heat shock treatment at 42°C. A new peroxidase isoform appeared only in 3-month old treated leaves. Following 24- hour recovery period, a further increase in DNA damage and tendency of DNA repair was observed in 1-month and 3-month old plants, respectively. Obtained results suggest genotoxic effect of high temperature but also that interplay between DNA damage and repair depends on the stage of leaf development.

P86

Validation of a Multi-Endpoint Assay in Rats: Bone Marrow Micronucleus, Comet and Flow Cytometric Peripheral Blood Micronucleus. Bowen D[1], Henderson D[1], Kidd D[1], McGarrey S[1], Pearce G[1], Torous D[2], Whitwell J[1], Williams L[1], Kirkland D[1]. [1]Covance Laboratories, Harrogate, United Kingdom, [2]Litron Laboratories, New York, NY, United States.

With the publication of revised draft ICH guidelines, there is scope and potential to establish a combined multi-end point *in vivo* assay to alleviate the need for multiple *in vivo* assays, thereby reducing time, cost and animal usage. We present here the results of a validation trial in which we have combined bone marrow micronucleus (measuring potential chromosome breakage and loss in developing erythrocytes), with comet (DNA strand breakage) in stomach and liver. This allows a variety of potential target tissues (site of contact, site of metabolism and peripheral distribution) to be assessed. In addition, we have incorporated the assessment of peripheral blood micronuclei (in young reticulocytes) via the use of the validated MicroFlow flow cytometric method to further complete a broad assessment of potential DNA damage in a multitude of tissues. A series of genotoxic mutagens were tested which are known to act via a variety of different modes of action (direct and indirect acting clastogens, gene mutation, cross-linking and aneugenic compounds). This combination approach has been performed with minimal changes to the standard and regulatory recommended sampling times for the standard assay designs.

P87

Time Course of Chemical-Induced *In Vivo* Genotoxicity Evaluated Using a Combined Protocol for Micronucleus and Comet Analyses. Hobbs CA[1], Recio L[1], Shepard K[1], Winters J[1], Green A[1], Baldetti C[1], Streicker M[1], Davis J[1], Caspary W[2,3], Witt KL[2,3]. [1]Integrated Laboratory Systems, Research Triangle Park, NC, United States, [2]Toxicology Branch, NIEHS, Research Triangle Park, United States, [3]National Toxicology Program (NTP), Research Triangle Park, United States.

To minimize the use of animals and maximize information gained from *in vivo* genotoxicity assays, the NTP is examining protocols to simultaneously measure micronucleated reticulocyte (MN-RET) frequencies by flow cytometry, and primary DNA damage by the Comet assay. To evaluate the feasibility of a combined protocol, male B6C3F1 mice were administered ethyl methanesulfonate (EMS) by gavage once daily for 3 days and blood was collected for MN-RET analysis 4 and 28 hr after the final dose. In this repeat dosing experiment, the frequency of MN-RET peaked at the first sampling (28 hr after the second dose) and remained high through the second sampling (28 hr after the third dose). Another set of mice received a single EMS treatment and blood was collected 24, 48, and 72 hr later. Following the single treatment, MN-RET frequency increased at 24 hr and peaked at ~48 hr. A third experiment was conducted to evaluate optimal sample time for the Comet assay. Mice were administered EMS once daily for 4 days; blood was sampled 3, 4, 6, and 8 hr after the final dose. Similar levels of EMS-induced DNA damage were detected at all 4 sample times. The peak genotoxicity observed on Day 4 supports use of a combined 4-day protocol for parallel evaluation of MN-RET and DNA damage in the same set of experimental animals. Additional experiments are underway to measure DNA damage in mice after a single treatment with EMS, compared with the levels observed after repeated dosing. Results from this study may help to clarify whether induced DNA repair processes influence the amount of damage detected after multiple treatments.

P88

Dose-Response Relationship, Kinetics of Formation and Persistence of s-[2-(N7-guanyl) ethyl]glutathione DNA Adduct in Livers of Channel Catfish (*Ictalurus punctatus*) Exposed *In Vivo* to 1, 2-Dichloroethane. Means JC[1], Jemal A[2]. [1]Southern Illinois University, Carbondale, IL, United States, [2]American Cancer Society, Atlanta, GA, United States.

Formation of DNA adducts by reactive chemicals or their metabolites are often a precursor of tumorigenesis. The dose-response, kinetics of formation and the persistence of S-[2-(N^7-guanyl)ethyl]glutathione (GEG) hepatic DNA adducts following *in vivo* aqueous exposure to 1,2-Dichloroethane (EDC) were studied in juvenile channel catfish (*Ictalurus punctatus*). For dose-response, Fish were exposed for 4 h to concentrations of: 0, 50, 100, 200, and 600 mg/liter of EDC in dechlorinated (DCI) water, respectively. For kinetics and persistence, fish were exposed to 200 mg/liter of EDC in 50 liter of DCI water for 4 h, while control fish were maintained in DCI water. Treatment group fish were rinsed and placed in clean water and sampled at 2, 4, 8, 24, 48, 96, 360 and 504 h total post-exposure holding time and controls at 2 and 504 h. Fish were then euthanized and livers prepared for DNA adduct analysis. DNA GEG adducts were quantified by isotope dilution using LC/Electrospray ionization/MS/MS. GEG adducts formed rapidly in liver and ranged from non-detectable (<10 fmol GEG/mg DNA) in controls to 350 pmol/mg DNA in the high dose group and dose-response was linear up to 200 mg/liter. EDC adducts were detectable three weeks after end of exposure at levels well above the detection. The results from this study provide evidence that channel catfish may have potential to serve as sentinel organisms for dichloroethane contamination and genetic damage occurring in aquatic environments.

P89

Study of Genotoxicity and Oxidative Stress Biomarkers in Rats Exposed to Toluene, Chloroform, Methylene Chloride and a Mixture of Them. Belmont JA[1], Serrano L[1], Fanjul ML[2], Prieto J[2], Montero RD[1]. [1]Instituto de Investigaciones Biomedicas, U.N.A.M., Mexico, DF, Mexico, [2]Facultad de Ciencias, U.N.A.M., Mexico, DF, Mexico.

The presence of chloroform, methylene chloride and toluene has been described in two Mexican rivers (Montero2006). Increased genotoxic damage was found in residents living at the banks of these rivers and in residents who posses GSTT1 null (Montero 2006) and CYP2E1 RsaI c1/c1 polymorphisms, suggesting that the damage could be due to oxidative stress. The objective of this research is to study the oxidative stress response and the genotoxicity through time due to the chronic exposure to a mixture of chloroform, methylene chloride and toluene in a rat model. In an explorative experiment intended at the standardization of methods and to the evaluation of sensitive biomarkers, 5 week old male Wistar rats were injected i.p. with each contaminant and with a mixture of them. 1/10th of the LD50 of each chemical were administered daily during 3 days. Animals were sacrificed after completion of treatments; liver, kidney and brain were recovered for homogenization and bone marrow was used for the MN assay. Preliminary results: no changes were found in hepatic glutathione levels; this means that either there was no ROS production or that antioxidant systems were working properly. We will explore the activity of the enzymes CYP2E1 and GSTT1, both inducible in liver, in order to assess if there was an induction under this regime of exposure. On the other hand, increased micronucleus frequencies were found in the bone marrow of rats treated with methylene chloride and the mixture, even though only the mixture was close to significance (p=0.08). Reference: Montero et al. 2006. Mutagenesis 21(5):335-342.

P90

Evaluation of the Butter Flavoring Diacetyl and the Fluorochemical Paper Additive Lodyne P-208® For Mutagenicity. Whittaker P[2], Begley TH[2], Clarke JJ[1], San RH[3], Dunkel VC[4]. [1]BioReliance, Rockville, MD, United States, [2]US FDA, College Park, MD, United States, [3]Consultant, Gaithersburg, MD, United States, [4]Consultant, Bethesda, MD, United States.

Diacetyl (2,3-butanedione) is a yellowish liquid that is usually mixed with other ingredients to produce butter flavor or other flavors in a variety of food products. Inhalation of butter flavoring vapors was first associated with clinical bronchiolitis obliterans among workers in microwave popcorn production. Recent findings have shown irreversible obstructive lung disease among workers not only in the microwave popcorn industry, but also in flavoring manufacture, and in chemical synthesis of diacetyl. It has been reported that perfluorochemicals utilized in food packaging are migrating into foods and may be sources of oral exposure. Because of recent concerns about the presence of perfluorochemicals such as those found on microwave popcorn bags (e.g. Lodyne P-208E®) and diacetyl in foods, we evaluated both compounds for mutagenicity using the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells. Lodyne P-208E® was less toxic than diacetyl and did not induce a mutagenic response. Diacetyl induced a highly mutagenic response in the L5178Y mouse lymphoma mutation assay in the presence of human liver S9 for activation. There was an increase in the frequency of small colonies in the assay indicating that diacetyl causes damage to multiple loci on chromosome 11 in addition to functional loss of the thymidine kinase locus.

P91

Carcinogen Exposure, Mutant DNA Biomarkers, and Human Cancer Risk. Sampliner DS, Elespuru RK. Food and Drug Administration, Silver Spring, MD, United States.

Tests for safety assessment of FDA-regulated products such as medical device materials, drugs and food additives depend on genotoxicity testing and long term cancer bioassays. Even with these tests, assessing the risk to humans is difficult, especially in regard to particular exposures. We have undertaken an analysis of several major classes of carcinogen for which information exists on human exposure, tumor development, and mutant DNA biomarkers (p53 or K-ras) recovered from tumors. The carcinogens include aflatoxins, polycyclic hydrocarbons, ultraviolet radiation, aromatic amines, and nitrosamines. Our analysis of the available literature includes data on exposure (intake concentrations, urinary metabolites, adduct formation) that may be used to generate estimates of human exposure. This is compared with rates of tumor formation and recovery of DNA mutant biomarkers. The data are used to generate the following information: 1) What are the exposures to known carcinogens required for human cancer generation 2) Is the range of exposures between different classes of carcinogen large or small, i.e. is the required exposure very different for different classes of carcinogen 3) To what extent are mutant DNA biomarkers recovered 4). This data may be useful in establishing levels of concern or thresholds, or otherwise aid in estimating the risk to humans of known exposure to products testing positive in pre-clinical assays.

P92

A Novel Mutagenic Potency Ratio Method to Assess the Excess Lifetime Cancer Risk of Complex PAH Mixtures in Contaminated Soils. Lemieux CL[1], Long A[1], Lundstedt S[2], Tysklind M[2], Lambert IB[3], White PA[1]. [1]Mechanistic Studies Division, Safe Environments Programme, HECSB, Health Canada, Ottawa, ON, Canada, [2]Department of Chemistry, University of Umeå, Umeå, Sweden, [3]Department of Biology, Carleton University, Ottawa, ON, Canada.

Traditional cancer risk assessments for complex mixtures, such as contaminated soils, are based on either surrogate mixtures, or the concentrations and relative potencies of known carcinogens in the mixture. For the latter, risk is estimated as the sum of the risks attributable to each known carcinogen in the mixture. We have used a mutagenic potency ratio (MPR) approach to determine the excess lifetime cancer risk posed by ten PAH-contaminated soils, obtained from contaminated gasworks sites, wood preservation facilities and one coke oven site. The mutagenicity of non-polar and semi-polar aromatic fractions from these soils was assessed in the Salmonella mutagenicity assay and the Muta™Mouse FE1 *lacZ* transgene mutation assay. The MPR method used mutagenic potencies derived from these assays to infer the magnitude of exposure to a surrogate with known carcinogenic (oral) potency, namely benzo[*a*]pyrene. This value was used to estimate excess lifetime cancer risk for non-dietary ingestion by a construction worker. When mutagenicity data from the *lacZ* transgene mutation assay were employed, the MPR method underestimated risk as compared to the traditional approach, suggesting that current risk assessment practices for complex PAH mixtures are conservative. However, when *Salmonella* mutagenicity data were used, the MPR approach overestimated risk for 9 of the 10 soils examined. This discrepancy may be attributable to the oversensitivity of the Salmonella mutagenicity assay. The MPR approach is attractive because it does not require a priori knowledge of mixture components, and does not assume dose additivity. However, it should only be used for mixtures containing chemicals with the same mode of action.

P93

Mutagenicity Study of Jet Fuels and Their Additives Using the Ames Salmonella Assay. Blanco-Yu FB. Seton Hill University, Greensburg, PA, United States.

A study of the mutagenic activity of jet fuels and their additives was undertaken using the Ames Test. JP-8, JP-8+100, and a synthetic liquid made from natural gas using the Fischer-Tropsch process were tested for mutagenic activity with concentrations ranging from 0, 25, 50, 75 and 100 % vol/vol, respectively, with high-grade acetone. Initial results of the Ames Test without metabolic activation using *Salmonella typhimurium* TA 1535 and TA 97 gave negative mutagenic results. A number of nonhydrocarbon performance additives to JP-8 include an anti-oxidant, methyl tert-butyl ether (MTBE); an ice inhibitor, di(ethylene glycol) monomethyl ether (diEGME); and a proprietary formulation for fuel stabilization, Turboline FS 100, were tested in a similar fashion as the jet fuels. The additives MTBE and diEGME gave negative results versus the tester strains TA 1535 and TA 97 in the Ames assay. However, MTBE and diEGME had some form of genotoxic effect at the highest doses, 75% vol/vol and 100% vol/vol, as evidenced by the thinning of the background lawn. Turboline FS100 was not only found to be a potential mutagen but it was also genotoxic to *S. typhimurium* TA 1535 and TA 97. Turboline FS100 is a complex mixture that includes naphthalene, Naphtha solvent (found to be non-mutagenic in TA 1535 and TA 97), 1,2,4-trimethylbenzene, and two trade secret ingredients. The Ames Salmonella Assay with metabolic activation also confirmed the potential mutagenicity effect of Turboline FS100 in tester strains TA 1535 and TA 97.

P94

Mutagenic Effects of Acrylamide and Glycidamide in the Testes of Big Blue Mice. Azuma M, McDaniel L, Manjanatha M, Shelton S, Mei N. Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR, United States.

Acrylamide (AA) exposure can cause degeneration in testicular tissue. Also, AA is a rodent genotoxic carcinogen, and induces mutation and tumors in tissues such as the lung, skin and liver. Therefore, it is possible that AA is mutagenic in the testes. In this study, we investigated whether AA and its metabolite glycidamide (GA) possessed mutagenic toxicity in germ cells. Male Big Blue transgenic mice were administered 100 or 500 mg/liter of AA and equimolar doses of GA in the drinking water for 4 weeks. Testicular *cII* mutation frequency (MF) was determined 21 days after the last treatment, and the types of the mutation in *cII* gene were analyzed by DNA sequencing. The spontaneous MF in testicular tissue from untreated mice was obviously low, compared with that reported for liver tissue. AA treatment significantly increased the MF in the testes at either the low or high dose, and the MF in the high dose group was significantly higher than that in the low dose AA group. The MF in GA-treated mice was also significantly increased, and the difference in the MF between the two doses of GA was statistically significant, implying a dose-dependent mutagenic effect of GA, an effect similar to that of AA. Although no significant difference was found between the mutagenicities of AA and GA at any of the doses used, the highest MF was observed in the high dose GA group. The results for the mutation spectra were also demonstrated. These results suggest that AA and GA may possess not only non-genotoxicity but also genotoxic effects on testes, consequently resulting in genetic events critical to next generations.

P95

Automated Analysis of Micronuclei in Binucleate Human Lymphocytes. Norppa H, Järventaus H, Lindberg H, Falck G. Finnish Institute of Occupational Health, Helsinki, Finland.

Until recently, the most successful application of image analysis to cytogenetics in genetic toxicology has been automated metaphase finding which has much shortened the time required for chromosome aberration (CA) analysis. The analysis is interactive, since CAs are identified by the operator from the metaphases found by the equipment. One of the well-known advantages of the micronucleus (MN) assay, in comparison with the CA assay, is the relatively easy analysis, suitable for automation. Automated MN analysis frees the operator for more interesting work. We have utilized a metaphase finder for automated analysis of MN in binucleate human lymphocytes, based on fluorescence detection and a commercially available computer program which has been adjusted for our purposes. The system identifies binucleate cells and micronuclei in them. We have used an *in vitro* treatment with mitomycin C, a well-known clastogen, for testing the feasibility of the automated MN analysis. Our results show that the automated assay is well applicable to MN analysis of binucleate human lymphocytes. Results adequately comparable to manual scoring are obtained, when the criteria for a micronucleus are set so that the system will first record somewhat more micronucleated cells than there are in reality. After the automated finding, the positive cells are checked by the operator. Although the interactive use of the program requires some operator time, the automated test is faster than the manual assay. As with any automated microscopic approach, uniform and high-quality slides are required for reliable results.

P96

Micronucleus Frequency in Hamsters Peripheral Blood. Salazar AM[1], López-Cuevas E[1], León S[2], Flisser A[2], Ostrosky-Wegman P[1]. [1]Instituto de Investigaciones Biomedicas. UNAM, México DF, Mexico, [2]Facultad de Medicina, Mexico DF, Mexico.

Hamsters are a successful rodent experimental model for the study of infections, vaccines and carcinogenesis, nevertheless they have not been used for genotoxic evaluations. Interestingly, hamsters although being mammals, show polychromatic erythrocytes in peripheral blood, allowing the evaluation of micronucleus (MN) in these cells. Only one paper reporting the frequency of MN in peripheral blood in these animals, together with other 35 different species was found (Zuñiga et al, 1996) Thus, the aim of the present study was to analyze the basal frequency of MN in ten male hamster erythrocytes (9 month old). Blood samples were collected from the orbital venous plexus and to assess MN frequency, methanol-fixed slides were stained with acridine orange. For each animal, 3000 polychromatic erythrocytes or reticulocytes (MN-PCEs) and 6000 normochromatic erythrocytes (MN-NCEs) were scored.. The frequencies of MN-PCEs and MN-NCEs were 0.237% and 0.230%, respectively. Values ranging between 0.1 - 0.5. Noteworthy to mention that MN were well defined and easy to score Our data although different from the ones reported by Zuñiga et al (0.063%) are similar to the ones reported in other rodents. The present results suggest that hamster might be a good model in the evaluation of genotoxicity.

P97

Revision of ICH S2 Guidance: J&JPRD Experience With Integrated Approaches for *In Vivo* Genotoxicity Assesment. van der Leede BM, De Boeck M, Van Goethem F, Van Gompel J. Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V., Beerse, Belgium.

In the revised ICH S2 guidance on genotoxicity testing for pharmaceuticals, integration of *in vivo* genotoxicity assessment into repeat-dose toxicity studies is one of the new features. This integration ties in with ICH's commitment to promote the 3Rs principle and allows use of toxicological findings and toxicokinetic aspects for the interpretation of genotoxicity data. In addition, assessment of genotoxicity in other target tissues (*e.g.* by means of the comet assay) could be relevant in case of low exposure in bone marrow. Overall, two battery options are possible: 1) bacterial gene mutation test, *in vitro* mammalian cell test and *in vivo* test for chromosomal damage; 2) bacterial gene mutation test and *in vivo* test for genotoxicity with two tissues (with recommendations for top dose selection). For the integrated design, there is concern about reduced sensitivity, since the top dose would typically be lower than in acute studies, particularly when testing under option 2; about feasibility (*i.e.* practical issues of integration) and applicability (*i.e.* in how many cases is testing according to option 2 possible). Furthermore, practical experience in integration of comet assay into repeat-dose studies is very limited among the pharma companies. Our in-house experience will be presented by means of compound cases for which the possibility to conduct the micronucleus test as a stand-alone acute assay or integrated into a repeat-dose toxicity study was evaluated by strictly following the acceptance criteria for dose selection. We furthermore present data on a combined micronucleus and comet assay design.

P98

Reduction of False Positives in *In Vitro* Genotoxicity Assays. Fowler P, Jeffrey L, Young J, Kirkland D. Covance Laboratories Ltd, Harrogate, UK, United Kingdom.

Current *in vitro* genetic toxicology assays have a high rate of reported positive results, when compared with rodent carcinogenicity data. This effect is compounded when several tests are combined. A recent analysis of published data highlighted the inaccuracy of current *in vitro* assays, the false positive rate with a combination of assays was found to be at least 80% (Kirkland *et al* 2005). As identified at a recent ECVAM workshop (Kirkland *et al*, 2007) this poor predictivity was expected to be worst in p53-deficient cell lines derived from rodent origin, particularly Chinese hamster cell lines. As part of a larger framework for improvement of *in vitro* genetic toxicology assays the performance of currently used cell lines is being investigated and compared with p53-competent cells. Comparisons have been made between Chinese hamster Lung (CHL), Chinese hamster Ovary (CHO), V79 and Human peripheral blood lymphocytes (HULY), with a selection of compounds that are accepted as producing false positive results in *in vitro* clastogenicity assays (Kirkland *et al*, 2008). Initial comparisons have been made between six such compounds using the *in vitro* micronucleus assay to compare clastogenic potential and highlight any differences in sensitivity between cell lines. Micronucleus assay data from these comparisons highlights differences in response between different cell lines, sensitivity also differs markedly particularly when comparing levels of toxicity and when compared with p53-competent cells. This work is funded by the European Cosmetic Industry Association COLIPA, ECVAM and NC3Rs.

P99

Syrian Hamster Embryo (SHE) Cell Transformation Assay (CTA) With Conditioned Medium Without Any X-Ray Irradiated Feeder Cells. Pant K[1], Bruce SW[1], Sly JE[1], San RHC[1], Scott A[2], Carmichael P[2]. [1]BioReliance Corporation, Rockville, MD, United States, [2]SEAC Unilever, Colworth Sharnbrook Bedford, United Kingdom.

The SHE CTA has traditionally been conducted with a feeder layer of x-ray irradiated cells to provide growth support to the target cells seeded in low numbers. We have tried seeding the target cells in conditioned media prepared from the stock culture flasks in lieu of plating them on a feeder layer. The need for an x-ray irradiated feeder cell layer necessitated the maintenance of an x-ray machine and the additional step to seed feeder cells prior to plating target cells. With freshly prepared conditioned medium there was essentially no difference in the number of target cell colonies in the conditioned medium and in the plates with the x-ray irradiated feeder cell layer. The plating efficiencies of the vehicle controls were within the historical range for the standard SHE CTA. In each experiment, the positive control benzo(a)pyrene [B(a)P] elicited a significant increase in morphological transformation (MT) frequency, with or without x-ray irradiated feeder cells. Two compounds, D(2-ethylhexyl)phthalate (DEHP), CAS # 117-81-7 and N-Nitroso-N-methylnitroguanidine (MNNG), CAS # 70-25-7 were tested in the SHE CTA with and without an x-ray irradiated feeder layer and using a 7-day exposure regimen. The results were comparable in experiments performed with standard SHE CTA with x-ray irradiated feeder cells and without x-ray irradiation. The results of this study demonstrate the feasibility of conducting the SHE CTA without the use of an x-ray irradiated feeder layer, thereby simplifying the test procedure and facilitating the scoring of MT colonies.

P100

Skin and Lung Comet Assays: Current Developmental Status. Williams L, McGarry S. Covance Laboratories Ltd, Harrogate, UK, United Kingdom.

The Single Cell Gel Electrophoresis Assay (Comet assay) is more commonly being used in Genetic Toxicology as a second *in-vivo* test and problem solving tool. The Comet Assay is a simple and sensitive method to determine single strand breaks (SSB), alkali labile sites (ALS), and DNA-DNA or DNA-protein crosslinking. One main advantage of this assay is the ability to assess DNA damage in any tissue from which single cells can be obtained. The effects of genotoxic agents can be assessed in target tissues and/or site of contact tissues. For dermal and inhalation or intratracheal dose routes the site of contact tissues are the skin and lung respectively. Therefore, it is valuable to be able to assess the skin and lungs for DNA damage in the Comet assay. For both tissues, there are several technical complexities in obtaining single cell suspensions of the target cells. When single cell preparations were prepared from lung tissue, it was observed that there was a high proportion of blood cells present. Therefore, a number of methods were tested in order to remove blood cells from the lung cell preparations. The methodology for obtaining keratinocytes reported in the literature and has been assessed in our laboratory and adapted to improve the quality of the single cell preparations. Combinations of mechanical dissociation and enzyme digestion methods were investigated in order to obtain single cell suspensions. Methodologies used to obtain skin and lung single cell suspensions will be discussed.

P101

"False" Positive Reduction in *In Vitro* Genotoxicity Assays, Estimation of Toxicity and Implications for Selection of Maximum Dose. Fowler P, Jeffrey L, Young J, Kirkland D. Covance Laboratories LTD, Harrogate, UK, United Kingdom.

Current *in vitro* genetic toxicology assays have a high rate of reported positive results, when compared with rodent carcinogenicity data. This effect is compounded when several tests are combined. A recent analysis of published data highlighted the inaccuracy of current *in vitro* assays, the false positive rate with a combination of assays was found to be at least 80% (Kirkland *et al* 2005). As part of a larger framework for improvement of *in vitro* genetic toxicology assays the performance of currently used cell lines is being investigated and compared with p53-competent cells. In this study, we have compared different measures of estimating toxicity after exposure to previously identified chemicals giving rise to "false" positive results in the in vitro micronucleus assay. Emphasis has been placed on chemicals that have a steep toxicity profile and a maximum testing concentration limited by toxicity. Cell counts were performed as well as mitotic index and replication index measurements. A battery of intracellular toxicity endpoints was also investigated including metabolic activity, membrane permeability, apoptosis, protein synthesis and neutral red uptake. Our results demonstrate that certain measures have potential to seriously over estimate toxicity, the implication of which is a higher maximum testing concentration which may contribute to the generation of "false" positive results with *in vitro* genotoxicity assays. This work is funded by the European Cosmetic Industry Association COLIPA, ECVAM and NC3Rs.

P102

Combined Protocol for Simultaneous Measurement of Micronucleated Erythrocyte Frequencies and DNA Damage in Rodents. Recio L[1], Hobbs-Riter C[1], Shepard K[1], Baldetti C[1], Streicker M[1], Winters J[1], Caspary W[2], Witt KL[2]. [1]ILS, Research Triangle Park, NC, United States, [2]NTP/NIH/NIEHS, Research Triangle Park, NC, United States.

Assessment of *in vivo* genotoxicity has historically focused on effects in the bone marrow using the rodent erythrocyte micronucleus (MN) assay. Although this assay has proven useful, limiting evaluation to a single tissue may miss effects that are more pronounced in other target tissues. The *in vivo* alkaline (pH>13) comet assay detects a spectrum of DNA damage and enables the assessment of genotoxicity in almost any tissue since cell division is not required. To gain a more comprehensive assessment of *in vivo* genotoxicity, we are evaluating a protocol that allows for simultaneous scoring of MN in peripheral blood reticulocytes (MN-RET) using flow cytometry and DNA damage in multiple tissues using the comet assay. Test agents were administered once daily for 4 days to male B6C3F1 mice or F344 rats, and animals were killed 4 hours after the last dosing. Initial studies used 4 model genotoxicants: acrylamide, ethylmethanesulfonate, cyclophosphamide, and vincristine. All 4 chemicals induced MN-RET and DNA damage in at least one tissue. We have now expanded our testing to include 11 structurally diverse chemicals. Among these, 3 were positive in both assays, 2 were negative in the MN assay but positive for DNA damage in blood leukocytes, and the rest were negative in both assays. None induced MN-RET without showing evidence of DNA damage in at least one tissue. Further studies with additional test compounds are ongoing in mice and Wistar-Han rats to more thoroughly evaluate the usefulness of data obtained through this combined testing protocol. This work is funded by NIEHS/NTP NO1-ES-35514.

P103

Biomarkers in the *In Vivo* Human Lymphocyte Micronucleus Test in Relation to Metabolic Polymorphisms of Enzymes *CYP1A1*, *CYP2E1*, *GSTT1*, *GSTM1* and *NQO1*. Suarez K, Davila VM, Serrano L, Montero RD. Instituto de Investigaciones Biomedicas, U.N.A.M., Mexico, DF, Mexico.

Metabolic polymorphisms have been shown to be related with individual susceptibility to the effects of toxic substances of which PAHs and VOCs are some of the most common contaminants found in urban as well as in industrial areas. Susceptible individuals living in environments polluted with mixtures of these compounds are expected to manifest increased levels of biomarkers like chromosomal aberrations or micronuclei. Frozen blood samples taken in a previous monitoring study of people living at the banks of rivers polluted with VOCs were used to determine metabolic polymorphisms: *CYP1A1*2C*, *CYP2E1 RsaI, GSTT1 null, GSTM1 null* and *NQO1*2*. Biomarkers evaluated in the peripheral lymphocyte micronucleus (MN) test at the time of the study were analyzed in relation to each polymorphism: micronucleated cells, cells with more than 1 MN, chromatin buds (CHB), cells with more than 1 CHB, nucleoplasmic bridges (NPB) and apoptotic figures and it was assumed that each event occurred independently. Cells with more than 1 MN were increased in individuals carrying genotypes *GSTT1 null* and c1/c1 for the *CYP2E1 RsaI* polymorphisms ($p<0.05$), whereas cells with NPB were increased in individuals carrying the *GSTT1 null* genotype. Other parameters like age, gender, smoking and body mass index did not correlate with these biomarkers. Conversely, cells with more than 1 CHB were increased in individuals carrying the *GSTM1 native* genotype ($p=0.05$). These results support the idea that each of these genotoxic events arise from different mechanisms involving both the exposure and the individuals' susceptibility.

P104

Validation of the *In Vitro* Micronucleus Assay and Fluorescent *In Situ* Hybridization (FISH) in Human Lymphocytes. Farabaugh CS, Roberts DJ, Roy SK, Middendorf CA, Stankowski, Jr LF. Covance Laboratories, Inc., Vienna, VA, United States.

We previously reported validation of the *in vitro* micronucleus (MN) assay in human peripheral blood lymphocytes, comparing whole blood and isolated lymphocytes. Treatments included the clastogens mitomycin C (MMC) and cyclophosphamide (CP), and the aneugens vinblastine (VIN) and colchicine (COL). Newer studies indicate that culture size may be substantially reduced, using only 1/10 the amount of test article previously required, without sacrificing sensitivity. In comparing 10-mL control cultures to 1-, 2-, 3-, 4- and 5-mL test cultures, significant increases in the frequency of micronucleated binucleate cells (%MN-BN) were observed for 9/10 cultures treated with 0.300 μg/mL MMC, as compared to the matched de-ionized water control of equal size ($p\leq 0.05$; an elevated MN frequency was observed in the remaining case, but it did not reach significant levels). Additional experiments also have been performed to evaluate the use of FISH to differentiate between micronuclei formed by clastogenic or aneugenic events. Treatment with 0.6 and 0.7 μg/mL MMC, 50 and 60 ng/mL VIN, 40 ng/mL COL, and 50 μg/mL CP again induced significant increases in %MN-BN. Subsequent FISH analyses using a human pan-centromeric DNA probe revealed 89-100% of VIN- and COL-induced micronuclei were centromere positive, while only 9-12% of those induced by MMC and CP were. These results confirm that: (i) test article usage can be minimized significantly by reducing culture size; and (ii) when combined with FISH, the *in vitro* MN assay is suitable for detecting, and discriminating between, clastogenic and aneugenic events.

P105

Defining Criteria for the Evaluation of the Ames II™ Mutagenicity Assay. Bruce SW, Sly JE, Cecil MW, Springer SD, Klug ML, Scherer MC, Pant K. BioReliance Corporation, Rockville, MD, United States.

In recent months the Ames II™ Mutagenicity Assay has gained popularity as an early screening tool to define lead drug candidates. The Ames II™ assay utilizes two *Salmonella typhimurium* strains: TA98 (for frameshift mutations) and TAMix (an equimolar mixture of TA7001 to 7006 for base-pair mutations) plated in triplicate 384-well plates. Wells are determined to be revertants if the indicator medium undergoes a change from purple to yellow or a colony is clearly visible in the well. The difficulty with the Ames II™ assay is evaluating what constitutes a positive result due to very low backgrounds in the negative controls. We at BioReliance have been using the following evaluation criteria. For each tester strain, statistical analyses are performed. The mean and standard deviation of the revertant wells, and the fold induction over the negative control are calculated. The mean number of revertants for the negative control must be 1.0 or greater. If the value is less than 1.0, 1.0 is substituted for that value. The mean number of revertants per test article dose level must be at least 2-fold over the negative control in two increasing dose levels. The mean number of revertant wells per test article dose level should be greater than the historical negative control range for each tester strain. In order to have a positive (mutagenic) evaluation of the test article, all of the criteria must be true. If all three criteria are not true, the test article is evaluated as negative (non-mutagenic). We have tested many compounds and found that these criteria give a great concordance with the standard Ames assay.

P106

Application of the Acellular Comet Assay to Genotoxicity Testing for Pharmaceuticals. Vasquez MZ. Helix3 Inc., Morrisville, NC, United States.

The use of different cell types ranging from bacteria to hamster to human and the cellular processes ranging from cytotoxicity to division to repair introduces variability in *in vitro* genotoxicity tests including the comet assay. This makes interpreting the results of *in vitro* tests and determining their biological relevance difficult at best. And with the development of new mechanistic-based pharmaceuticals, the complexity of determining genotoxic potential increases substantially. The acellular comet assay eliminates the need to account for the cell types and processes that can modulate the accurate detection of genotoxicity by directly exposing to a test compound nuclear DNA from lysed cells (acellular DNA) rather than live cells. Acellular DNA, stripped of its protective and functional proteins, is more susceptible to damage induced by compounds and/or metabolites that interact directly with DNA while it is also resistant to the induction of strand breaks associated with cell death. Therefore, acellular DNA may be exposed both in the absence and presence of metabolic activation to test compound concentrations ranging from very low concentrations to concentrations up to and exceeding the cytotoxic dose in live cells thus optimizing the sensitivity and selectivity of the assay. To demonstrate the ability of the acellular comet assay to detect and interpret the damage induced by various compounds and/or pharmaceuticals with unknown or complex genotoxicity profiles, data from multiple experiments are presented.

P107

Genotoxicity of Acrylamide and Glycidamide in Big Blue Rats. McDaniel LP[1], Dobrovolsky VN[1], Shaddock JG[1], Mei N[1], McGarrity LJ[1], Miura D[2], Doerge DR[1], Heflich RH[1]. [1]U.S. FDA/NCTR, Jefferson, AR, United States, [2]Teijin Pharma, Tokyo, Japan.

Acrylamide (AA), which is generated during the cooking of starchy foods, is carcinogenic by chronic exposure to F344 rats. While AA produces negative or very weak responses in standard genotoxicity assays, we previously demonstrated that AA and its epoxide metabolite, glycidamide (GA), induce liver *cII* and lymphocyte *Hprt* mutations in Big Blue mice (Manjanatha et al., EMM 47:6-15). The significance of these findings to cancer mode of action is unclear because there are no published studies on the chronic carcinogenicity of AA in mice, and our previous study used relatively high doses of AA. In the present study, we exposed groups of 8 male and female Big Blue rats to the equivalent of approx. 5 and 10 mg/kg bw/d of AA or GA via the drinking water, a treatment regimen comparable to those used for the cancer bioassays (<5 mg/kg bw/d AA). After two months of dosing, the rats were euthanized, and blood was taken for micronucleus assay; spleens for lymphocyte *Hprt* mutant assay; and liver, thyroid, and mammary gland (from females) for *cII* mutant assay. Neither AA nor GA increased the frequency of micronucleated reticulocytes. In contrast, both compounds produced small (approx. 2-3-fold background) but significant increases in lymphocyte *Hprt* mutant frequencies ($p<0.05$), with the increases having dose-related linear trends ($p=0.045$ to $p<0.001$). Preliminary analyses of *cII* mutant frequencies in mammary gland (tumor target tissue) and liver (nontarget tissue) are negative. The results from this study indicate that AA is a gene mutagen in the rat via metabolism to GA under conditions that produce tumors.

P108

Validation of Flow Cytometry to Assess Mitotic Index in the Human Lymphocyte Chromosome Aberration Assay. Roberts DJ, Middendorf CA, Stojhovic G, Roy SK, Stankowski Jr LF. Covance Laboratories, Vienna, VA, United States.

The chromosome aberration assay is commonly used in the ICH battery to identify genotoxic agents. Dose selection for this assay is determined by some measure of cytotoxicity. For human lymphocytes the de facto standard is mitotic index (MI). Historically, that has meant manual microscopic evaluation which is time consuming, labor intensive and exhibits scorer variability. However, an automated flow cytometric (FCM) method is possible using antibodies to phosphorylated histone H3 (ser10). This marker is expressed by eukaryotes only during mitosis, and has proved to be a reliable tool for assessing MI. During our validation studies, human whole blood was collected and the mononuclear cell layer was isolated and washed. Isolated lymphocyte cultures were established and treated 48 hr after PHA stimulation with cyclophosphamide (3 hours +S9), or with mitomycin C or colchicine (22 hours –S9). Colcemid® was added 2 hours prior to harvest (at t = 72), and cultures were divided and processed for simultaneous manual and FCM determination of MI. Comparisons of MI generated by FCM and two qualified technicians indicated FCM consistently yielded higher MIs than manual scoring. For 37 samples analyzed by each (74 individual FCM vs manual comparisons), r^2 was 0.9655. Also, the target doses selected for evaluation chromosome aberrations (i.e., that which induced an ~50% decrease in MI) would be equivalent or adjacent in all cases. These results confirm FCM is an acceptable alternative to microscopic MI evaluation. Additional benefits are the inherent objectivity of FCM scoring and automation of data collection.

P109

Interpreting Data From *In Vitro* Genotoxicity Tests Using the Acellular Comet Assay. Dewhurst NE, Vasquez MZ. Helix3, Inc., Morrisville, NC, United States.

The *in vitro* comet assay is increasingly used to determine the biological relevance of the results from other genotoxicity tests. However, to replicate the conditions used in other genotoxicity tests and/or to optimize the sensitivity of the comet assay, a time course experiment including multiple sample times is necessary. The acellular comet assay can offer a faster, cheaper and less complicated alternative, as it can be performed with only one sample time. In the acellular comet assay, once damage is expressed, it can not be repaired or progress to cell death. This can make assessing compounds with ambiguous migration patterns easier to interpret. For example, compounds such as Mitomycin C can induce crosslinks after only a half hour of exposure. These crosslinks can lead to chromosomal aberrations, which can lead to cell cycle arrest followed by cell death. In addition, because chromosomal aberrations may be induced by cell death or cell death may be induced by chromosomal aberrations, it is imperative that the initial crosslinking event is detected to have an accurate understanding of the genotoxicity of a compound. The acellular comet assay is more sensitive than the *in vitro* comet assay in live cells and once damage is expressed, it cannot be repaired or progress to cell death. Therefore, damage can be detected using a single three hour sample time. To demonstrate how the acellular comet assay can be a better alternative to the *in vitro* comet assay, data from time course experiments performed using the *in vitro* and acellular comet assays are presented.

P110

Target Organ Selection for the *In Vivo* Comet Assay: Genotoxicity Versus Tumorigenesis. Sivers CL, Vasquez MZ. Helix3 Inc., Morrisville, NC, United States.

Target organ selection in the *in vivo* comet assay is one of the most critical processes for assessing the genotoxicity of a compound. However, the site of tumorigenisis may not be the site of genotoxicity due to the mechanistic pathway of the compound. For example, 7, 12 dimethyl benz[a]anthracene (DMBA) is known to induce mammary tumors; however DNA damage has not been detected in the mammary tissue. DMBA induced mammary tumors may be a result of DNA damage induced in a component of the endocrine system, such as the pituitary gland. Using the comet assay to measure DNA damage induced in the endocrine system may provide insight into the mechanisms of tumorigenisis for diseases such as breast cancer and prostate cancer. To demonstrate that genotoxicity maybe detected at sites other than the site of tumorigenisis, data from multiple experiments is presented.

P111

Comparison of the Cytotoxicity and Mitochondrial Membrane Permeability of Benzazolo[3,2-a]quinolinium (BQs) on Tumor vs Normal Cell Lines. Molina D[1], Velez C[1], Carro S[3], Hernandez W[2], Arroyo LV[1], Cox O[2], Zayas B[1]. [1]Metropolitan Univ., San Juan, Puerto Rico, [2]Univ. of Puerto Rico, San Juan, Puerto Rico, [3]Univ. of North Carolina, Chapel Hill, NC, United States.

This study presents the toxicity and mitochondrial interactions of five benzazolo[3,2-*a*]quinolinium salts (BQs) on A431 tumor cells and a preliminary comparison with toxicity on normal TK-6 lymphocytes. Tested drugs were NBQ38, ABQ38, NBQ95, ABQ95 and BQ108. For determination of the IC50 (inhibition concentration) A431 and TK-6 cultures were exposed to BQs for 48 hours and cytotoxicity assessed by Trypan Blue exclusion. Apoptosis induction through mitochondrial membrane permeability was also determined. For apoptosis related analysis cells were treated with the respective IC50 concentrations for 24, 48 or 72 hours. Included positive controls were Ellipticine (structural analog), Valinomycin, (permeate mitochondrial membrane) and Staurosporine an apoptotic agent. Membrane permeability and Caspases activation on A431 were determined by fluorescent analysis with MITO PT, Magic Red and FLICA fluorescent dyes respectively. Extended cytotoxicity indicated that ABQ's are more potent cell inhibitors than NBQ's. Fluorescence microscopy analysis on A-431 showed that mitochondrial membrane was permeabilized as early as 24 hours for ABQ's and completely permeabilized at 72 hours for all BQ's. In contrast TK-6 preliminary results indicated higher tolerance to the tested NBQs and ABQs with an average IC50 of 55 uM in comparison to 30 uM on tumor cells. Mitochondrial permeability analysis with TK-6 is being performed at this stage. However results with A431 demonstrate predominant apoptotic route with permeabilization of the mitochondrial membrane and caspases activation.

P112

Detection of Weak Mutagens in Transgenic Rodent Mutation Assays: Challenging the International Workshops on Genotoxicity Testing Protocol Recommendations. Singer TM[1,2], Douglas GR[1], Gingerich JD[1], Williams A[3], Soper L[1], Lambert IB[2]. [1]Mutagenesis Section, Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada, [2]Department of Biology, Carleton University, Ottawa, ON, Canada, [3]Biostatistics Section, Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada.

The development of transgenic rodents containing multiple copies of chromosomally integrated bacterial reporter genes has provided an *in vivo* model that may fill existing genotoxicity testing gaps. This work aims to provide insight into the applicability of the International Workshops on Genotoxicity Testing (IWGT) expert panel recommendation that a treatment duration of 28 days and a sampling/manifestation time of 3 days (28+3) would be optimal for the detection of a broad spectrum of mutagens with varying potency. Male lacZ transgenic mice (5-7/group) were administered the weak mutagen ethyl carbamate daily by gavage during the treatment period at doses up to the MTD, as follows: (a) 7 day treatment period, 3 day sampling time; (b) 7 day treatment, 28 day sampling time; (c) 28 day treatment, 3 day sampling time; (d) 28 day treatment, 28 day sampling time; (e) 56 day treatment, 3 day sampling time. Mutant frequencies in the bone marrow and liver were determined. In the bone marrow, a significant increase in mutant frequency was observed under conditions (b) to (e). In contrast, mutant frequencies in the liver were significantly elevated over the control only after the more prolonged treatments of conditions (c), (d) and (e). For the selection of a single sampling time that would be sufficient to detect a mutagenic response induced by weak mutagens in tissues with varying rates of cell proliferation, our results suggest that the 28+3 IWGT experimental protocol is sufficiently robust. This protocol should become the initial basis for the development of an OECD Test Guideline.

P113

Monitoring of Environmental Polycyclic Aromatic Hydrocarbons (PAH) in Soil and Fish From the Cucharillas Marshland, Catano, Puerto Rico. Nieves P, Lopez WL, Zayas B. Metropolitan University, San Juan, PR, United States.

Polycyclic aromatic hydrocarbons (PAHs) are by-products from incomplete combustion of carbonaceous materials. PAHs have the capacity to move through the environment via the atmosphere, water and soil. Some of these PAHs are known to have carcinogenic effects in humans and animals. Soils are important reservoirs for the PAHs, and fish that inhabit IN contaminated areas can also bioaccumulate PAHs. Through food ingestion humans can be exposed to these environmental carcinogens. The fact that the Cucharillas marshland is used for recreational activities presents a potential source of human exposure to these carcinogens. The aim of this study is to evaluate the presence of PAHs such as benzo[*a*]pyrene, benz[*a*]anthracene, and benzo[*b*]fluoranthene from soil and Tilapia from the Cucharillas marshland. Sediment and Tilapia obtained from contaminated areas in the marshland. For extraction and identification of PAHs from fish tissues, a microwave extraction technique was optimized and applied. As controls, Tilapias from Plata Lake were collected and analyzed. Identification of the monitored PAHs was made via HPLC-MS analysis. For PAH extraction from soil, a sonication method is being applied to compare with the more traditional Soxhlet method. Recovery of BaP from sediment with the optimized method resulted in 90% recovery. Co-chromatography gave preliminary results that indicate the presence of PAHs in fish at the µM level. In sediments fewer PAHs have been identified. This study presents evidence of the presence of environmental carcinogens on the Cucharillas marshland.

P114

Effect of Carbon Nanotubes-DNA Interaction on the Cellular Genomic Stability. Barajas Lemus C, Camacho Carranza R, Espinoza Aguirre J, Hernandez Ojeda SL. Instituto de Investigaciones Biomedicas UNAM, México, Mexico.

The carbon nanotubes (CNT) are nanoparticles conformed by hexagonal structures of atoms of carbon, which bend to form tubes. The CNT possess extraordinary mechanical, thermal and electronic properties, which confer them multiple potential uses. The Institute of Occupational Medicine of Edinburgh, calculates in 20,000 the exposed workers to the CNT, and the National Science Foundation, foresees that in 2015 there will be 2 million workers. Taking account of this, we consider that is important to make genotoxicity tests to assure the integrity of the workers as well as that of the consumer. We have designed a series of tests to determine if the CNT affect the genomic stability using *Salmonella typhimurium*: the Ames test to determine the mutagenic capacity of the CNT, the measuring frequencies of duplication-segregations, chromosomal invertions, plasmidic stability and changes in the efficiency of restriction enzymes. Preliminary results suggest that the single walled CNT don't induce bacterial DNA breaks, but they interfere in the repair mechanisms when for another means recombinogenic sustrats are produced, in particular if the RecFOR pathway is suppressed. The results in the Ames test have been negative. However the bacterial tolerance to CNT toxicity strongly suggests that the nanoparticles are not entering efficiently into the cell; probably because of the cellular wall. Therefore we are using bacterial protoplasts and/or electroporation to introduce the CNT into the cell.

P115

Combined Use of Multiple Biomarkers to Evaluate the Genotoxic Activity of the Herbicide Glyphosate. Ramos-Morales P, Muñoz JA, Rivas H, Muñoz A, Hernandez BR, Herrera JJ, Muñoz LB. Lab Genetica y Toxicologia Ambiental, Fac Ciencias, Universidad Nacional Autonoma de Mexico, Distrito Federal, Mexico.

Glyphosate (Roundup®) is one of the most used herbicides for controlling weeds in agriculture, forestry and aquatic systems. It inhibits enzymatic activity leading to blocking of aromatic amino acid synthesis. Reports about its genotoxic activity are controversial. In *D. melanogaster* induces Sex Linked Recessive Lethal Mutations. In this work, seven *Drosophila* biomarkers were combined to value the effect of Glyphosate on: whole organisms, the reproductive efficiency of treated males and the induction of somatic mutation. Wild type flies (wt) and flies with biomarkers to determine somatic mutation (standard) were employed. Third instar wt and standard larvae fed until pupation with standard food enriched with Glyphosate (prepared for surfactant use was 100% and 15 successive dilutions). Adults recovered were counting and sexed. 30 wt males randomly chosen were individually mated with unexposed wt virgin females to produce progeny. Another side, the wings of standard flies were mounting and the number and size of spots on the wings of each one of 60 flies was scored. Six of seven biomarkers showed differences between control and experimental flies ($p<0.05$). Only higher concentrations were toxic. Middle concentrations affected the male/female ratio. Low and high concentrations modified the reproductive activity, increased the frequency of somatic mutation and discriminated the most susceptible organisms among the exposed flies. The combined use of biomarkers informing about different levels of impact on *in vivo* models allow us to discard the false negative responses inherent to individual biomarkers.

P116

Potential Role of Environmental Mutagens in the Development of Human Lymphoid Malignancies. DeVoney D, Sonawane B, Jinot J, Bateson T, Vandenberg J. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C., United States.

Carcinogenicity is generally considered a multistep process by which a cell attains key attributes resulting in transformation. Environmental mutagens which come into contact with immune tissue, thymus or bone marrow, may result in a range of lymphocyte-derived malignancies. It is well accepted that toxic action in the bone marrow may result in lymphohematopoietic malignancies, as stem cells and progenitor cells may be transformed or acquire mutations expressed later. Lymphocytes present unique vulnerabilities for carcinogenic transformation not only in the bone marrow but as mature and differentiated cells. Unlike other somatic cells, lymphocyte function includes: 1) trafficking between tissues, 2) clonal expansion in response to immune challenge, 3) somatic hypermutation, 4) apoptosis for selection of successful clones, 5) apoptosis to down-regulate immune response, and 6) long-lived memory cells. B-cell leukemias, lymphomas and multiple myelomas, with DNA rearrangement in the variable chain region are clones of post-germinal center B-cells. Additionally, translocations generating known oncogenes are collocated with DNA regions rearranged during normal B-cell response to antigen. Finally, lymphocytes populate the lymph tissue and epithelium of the respiratory tract and gut, key portals of entry for infectious agents and endogenous compounds. These vulnerabilities of the humoral immune system offer unique opportunities for the direct action of environmental mutagens. (Disclaimer: The views expressed in this abstract are those of the authors and do not represent U.S. EPA policy or endorsement.)

DNA Repair and Damage Responses

P117

Dynamic Compartmentalization of BER Proteins Into Nuclei and Mitochondria is Governed by Oxidative DNA Damage Levels and Sumoylation. Griffiths LM, Swartzlander D, Wilkinson KD, Corbett AH, Doetsch PW. Emory University, Atlanta, GA, United States.

Oxidative DNA damage is the most frequently occurring DNA damage and occurs in cells due to oxidative stress caused by environmental exposures and cellular metabolism. Base excision repair (BER) is the primary pathway for the repair of nuclear and mitochondrial oxidative DNA damage. It is unknown whether localization of DNA repair proteins in response to levels of oxidative DNA damage plays a role in BER regulation. Also, little is known about the effect of post-translational modification of BER proteins and whether these modifications can act as a regulatory mechanism to control localization to sites of oxidative stress. To address this issue, *S. cerevisiae* BER DNA glycosylase/ AP lyase proteins, Ntg1 and Ntg2, were evaluated in response to cellular and mitochondrial oxidative stress. It was determined that the localization of Ntg1 is responsive to nuclear and mitochondrial oxidative DNA damage induced by oxidative stress; while, Ntg2 localization is solely nuclear. Furthermore, post-translational modification of Ntg1 and Ntg2 by sumoylation (small ubiquitin-like modifier) was evaluated as Ntg1 and Ntg2 possess seven and one consensus sequences for SUMO binding, respectively. Our results demonstrate that Ntg1 and Ntg2 are sumoylated, and sumoylation is associated with localization of Ntg1 to nuclei in response to oxidative DNA damage. Collectively, these results indicate that the orchestration of BER during cellular oxidative stress is dictated by the relative DNA damage levels harbored within nuclei and mitochondria which is manifested in the localization of repair proteins to these organelles. This work is supported by NIH grant ES011163.

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Mapping the Localization of DNA Repair Machinery to Sites of Genomic DNA Damage and Chromosomal Instability in *Saccharomyces cerevisiae*. Morris LP, Degtyareva N, Doetsch PW. Emory University, Atlanta, GA, United States.

Cells are constantly exposed to reactive oxygen species (ROS) produced both endogenously and exogenously, resulting in many types of DNA damage. Left unrepaired, adverse outcomes result including increased mutation accumulation, transformation and cell death. Repair of oxidative DNA damage is primarily through the base excision repair (BER) pathway, but the nucleotide excision repair (NER) pathway processes some of the same lesions. Also, translesion synthesis (TLS) and recombination (REC) enable cells to tolerate DNA damage when BER and NER are compromised. Elevated levels of unrepaired oxidative damage arise following disruption of BER alone and of both BER and NER in *S.cerevisiae* strains, leading to large-scale genomic rearrangements. An oxidative damage-associated fragile site within a 30-kilobase region on *S. cerevisiae* chromosome II has been identified. We hypothesize that when BER is severely compromised, oxidative DNA damage levels increase and other pathways such as TLS and REC compensate, leading to genetic instability and then, large-scale genomic rearrangements. The relationship between compromised repair of oxidative damage and genetic instability will be explored by first determining the genomic regions preferentially repaired by BER within the *S. cerevisiae* genome using the chromatin immunoprecipitation (ChIP) assay with DNA microarray analysis (ChIP-on-chip). We will then determine the distribution of BER machinery when the ability to repair such damage is compromised. This study will provide insight into how the distribution of oxidative damage repair governs genomic stability. Funded by NIH grant ES011163.

P119

Identifying Novel Human Damage Response Proteins Based on Yeast Orthology. Svensson P, Rebecca Fry R, Samson LD. Biological Engineering Department and Center for Environmental Health Sciences, MIT, Cambridge, MA, United States.

Cells are continuously exposed to damage and to avoid detrimental effects for the organism, elaborate pathways have evolved to ameliorate the effects of that damage. Sensitivity to several DNA damaging agents has been extensively studied in *S. cerevisiae* deletion mutants (Begley et al, 2004). The path from damage to cellular outcome remains elusive and evidence has implicated many functional processes, e.g. vesicle transport, RNA processing, chromatin modification and protein degradation, in yeast. The yeast sensitivity results were projected onto human protein-protein interaction data to identify potential toxicity-modulating proteins in humans. The resulting network revealed high connectivity between proteins required for damage recovery and many of the cellular processes identified in yeast were also significantly enriched in the human network. In this project, genes from the core interaction network are being silenced by RNA interference. Stable cell lines lacking functional protein are studied after exposure to differently acting DNA damaging agents: simple alkylating MMS, bulky alkylating 4-NQO and oxidizing t-BuOOH. To date, efficient gene silencing has been achieved in the human embryonic kidney cell line 293T for a number of targets of different pathways. Studies of the cellular sensitivity to the damaging agents have identified several novel toxicity-modulating proteins in human cells.

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Analyzing Genetic Interactions to Elucidate DNA Damage Responsive Networks and Toxicity Mechanisms of Environmental Agents in Yeast. Quirós L, Svensson P, Wang E, Fry R, Samson LD. Biological Engineering Department and Center for Environmental Health Sciences. MIT, Cambridge, MA, United States.

A myriad of new chemicals have been introduced into our environment and exposure to these agents can act on cells through different mechanisms of toxicity, some of them having DNA as the major target. High-throughput genomic-based techniques have become important tools to establish and clarify toxicity-modulating pathways of potential environmental carcinogens. Analysis of global responses in yeast provides a description of systems level interactions to identify new pathways of damage recovery upon exposure to genotoxicants. Compounds are screened in liquid culture in a microtiter format and detailed growth measurements are determined to reveal effects on ~4,800 different haploid gene deletion strains and ~1,100 hemizygous diploid deletion strains of *S. cerevisiae*. The pattern of which yeast deletion strains are sensitive, resistant or unaffected is predicted to indicate the mechanisms by which the compounds induce toxicity. Previous results using a solid agar assay and the alkylating agent MMS (Begley et al, 2004) have been recapitulated using this high-throughput liquid assay. Also, new groups of sensitive strains have been identified. Computational approaches allow us to identify biological networks connecting diverse cellular processes implicated in the response to the toxicant. This research was supported by Unilever.