

Poster Abstracts

DNA Damage and Repair

P1

The Influence of Nucleotide Pools on How Cells Choose to Repair Chromosome Breaks. Burkhalter MD, Ramsden DA. University of North Carolina, Chapel Hill, NC, United States.

DNA double strand breaks (DSBs) are highly dangerous lesions that can kill a cell and have the potential to induce carcinogenesis. Eukaryotic cells primarily use two different repair systems to deal with DSBs: nonhomologous end-joining (NHEJ) and homologous recombination (HR). Whereas the more error-prone NHEJ is active during the whole cell cycle, HR repairs DSBs during S and G2 phases and is considered to be mainly error-free. How does the cell choose between the two pathways? We explore the possibility of nucleotide pools being a determinant for this choice. Availability of nucleotides is a prerequisite for completion of HR, since this pathway involves extensive DNA synthesis. This need for nucleotides possibly could restrict HR to S and G2 phases, where dNTP pools are already elevated. No such restriction would apply for NHEJ, because this pathway does not include extensive DNA synthesis. To test this hypothesis we treated Chinese hamster ovary (CHO) cells that were synchronized in G1 with low levels of ionizing radiation (IR) to induce DSBs. Simultaneously, cells were treated with hydroxyurea (HU) for a limited time to inhibit ribonucleotide reductase, a key enzyme of *de novo* nucleotide synthesis. Applying colony formation assays we found that HU treatment does not affect the survival of HR-deficient cells. Contrastingly, HU sensitizes NHEJ-deficient cells to IR about five-fold. Cells that rely on HR for survival therefore are dependent on their ability to provide dNTPs for repair. To investigate whether HR is actually induced during G1 in NHEJ-deficient cells, we analyzed the formation of Rad51 foci as a measure for HR. Co-staining for cell-cycle markers allowed us to score cells at different stages in the cell cycle. NHEJ-deficient cells showed a higher number of Rad51 foci after irradiation than the corresponding controls. HR thus can be induced during G1 and serve as a backup pathway in case NHEJ fails. We also tested how HU affects the induction of HR in G1. Strikingly, Rad51 foci were reduced to background levels when NHEJ-deficient cells were pre-treated with HU. We conclude that initiation of HR is inhibited if cells are deprived of their ability to provide nucleotides *de novo*. Since the only known target of HU during G1 is P53R2, a damage-induced alternative subunit of ribonucleotide reductase, we currently investigate the role of this enzyme for initiation of HR and thus how it affects pathway choice.

P2

The Tp53 Gene is a Key Regulator of the Radioadaptive Response for DNA Damage *In Vivo*. Schmid TE, Marchetti F, Polyzos A, Bhatnagar S, Wyrobek AJ. Lawrence Berkeley National Laboratory, Berkeley, CA, United States.

The adaptive response (AR) is a phenomenon where a low dose exposure of ionizing radiation can provide significant cellular protection against a subsequent higher dose exposure, but the molecular mechanisms are poorly understood. The adaptive response has been widely observed in rodents *in vivo*, protecting animals against cellular and tissue toxicity and against a variety of genomic and cytogenetic damages. We investigated the AR for DNA damage across tissues in mice that received whole-body radiation, and found that both white blood cells and brain tissue show the AR. Mice were treated with 5 cGy of X-rays (priming dose) followed 6 hours later by a challenge dose of 2 Gy; fifteen minutes after the challenge dose, cells were analyzed by single-cell gel electrophoresis under alkaline conditions (alkaline Comet assay) to assess the relative magnitudes of residual DNA breaks and alkali-labile DNA damage. We further investigated the role of p53 function in the AR in adult male mice (C57BL6 wildtype and isogenic p53 null). Our findings confirmed the presence of the AR for DNA damage in the white blood cells of wildtype mice (37% tail DNA after challenge exposure versus 24% tail DNA after the split priming-challenge exposures, $p < 0.01$). However, preliminary analyses did not detect the AR in the p53 null mice (40% versus 41% Tail DNA, respectively). These findings indicate that wild-type male mice can manifest an AR for DNA damage across tissues, and that a normally functioning p53 is required for the AR for DNA damage *in vivo*. Supported by the Department of Energy Low Dose Program and the Lawrence Berkeley National Laboratory LDRD Program.

P3

Role of Single Stranded Break Repair and XRCC1 in Neurodegeneration. Kulkarni A, McNeill D, Wilson III D. National Institute of Aging, Baltimore, MD, United States.

Of the roughly one million molecular lesions that arise in DNA per mammalian genome per day, single strand breaks (SSBs) are one of the most frequent. XRCC1 is a non-enzymatic scaffold protein critical to the process of single strand break repair (SSBR). It recruits key enzymatic components of the pathway including APE1, Pol beta and DNA ligase III alpha via direct physical interactions. Some of the phenotypes of XRCC1 defective cells are enhanced sensitivity to DNA damaging agents, reduced SSBR and increased sister chromatid exchange. XRCC1 null mice die early during embryogenesis, underscoring the importance of this gene in genome stability. Recent studies have shown a direct link between SSBR and neurodegeneration. These studies have focused on the neurodegenerative disorders AOA1 (ataxia with oculomotor apraxia) and SCAN1 (spinocerebellar ataxia with axonal neuropathy) caused by mutations in the SSBR proteins Aprataxin and TDP1 (Tyrosyl phosphodiesterase), respectively. Both Aprataxin and TDP1 are targeted to SSBR processes via direct (Aprataxin) or indirect (TDP1) interactions with XRCC1, leading to speculation that XRCC1 plays a role in neurodegenerative disease. In this study we investigate the contribution of XRCC1 to dividing and non-dividing cellular states using XRCC1-deficient primary neuronal cells from mice and rat, and the human neuroblastoma cell line SY5Y. Our studies indicate that XRCC1 heterozygous mouse primary neuronal cells are highly sensitive to SSBs induced by the oxidizing agent Menadione. We observed a significant dose-dependent increase in both DNA damage and apoptosis in these cells relative to wild type controls. Notably, we did not detect a similar differential response to either Menadione or the alkylating agent MMS in XRCC1-downregulated SY5Y cells. Since SY5Y cells are dividing cells that can repair SSBs by alternative pathways, such as replication-mediated recombination, this outcome exemplifies the importance of XRCC1 and SSBR for genomic stability in non-dividing neuronal cells. We are currently attempting to confirm our findings via a comparative study in retinoic acid differentiated non-replicating SY5Y cells and in terminally differentiated rat neuronal cells.

P4

Effects Of MSH3 Gene Deletion on Targeted Homologous Recombination in ERCC1 Wild-Type or ERCC1 Knock-Out Cells. Adair GM, Rowley B, Robison T, Lowery M, Della Coletta L, Bolt A, Nairn RS. University of Texas, MD Anderson Cancer Center, Smithville, TX, United States.

In the yeast *Saccharomyces cerevisiae*, both MutS beta (Msh2-Msh3 heterodimer) and the Rad1-Rad10 nucleotide excision repair (NER) endonuclease are required (and epistatic) for processing and removal of long non-homologous tails from strand-invasion or single-strand-annealing recombination intermediates. Using *APRT* targeted recombination (gene correction) assays employing *ERCC1+* or *ERCC1* knock-out CHO cell lines, we have previously demonstrated that the Xpf-Ercc1 NER endonuclease is required for removal of long non-homologous tails from strand-invasion recombination intermediates in mammalian cells (ref. 1). To investigate the roles and possible epistasis of MutS beta in *ERCC1*-dependent or independent recombination pathways in mammalian cells, we generated isogenic wild-type (*MSH3+*, *ERCC1+*), *MSH3* gene deleted (*MSH3-*, *ERCC1+*), and compound mutant (*MSH3* gene deleted, *ERCC1-* knock-out) CHO cell lines. Each cell line carries the same hemizygous *APRT* target gene mutation (a 2-bp, exon 2, EcoRV site deletion) at the endogenous CHO *APRT* locus. We have used these three cell lines for recombination assays employing the same *APRT* targeting vectors used for our previous *ERCC1* studies. In one targeting vector (pAG6ins0.9), both arms of *APRT* targeting homology flanking a double-strand-break (DSB) are blocked by long non-homologous single-stranded tails that must be removed by Xpf-Ercc1 endonuclease for recovery of productive targeted integrations or vector (gap repair) correction events. In targeting experiments using this pAG6ins0.9 vector, *MSH3* gene deleted cells show a 2-fold reduction in overall targeted recombination frequency compared to wild-type (*MSH3+*, *ERCC1+*) cells. Compound mutant (*MSH3* del, *ERCC1* ko) cells show targeted recombination frequencies about 8-fold lower than wild-type, and 4-fold lower than those seen in the *MSH3* del cells. The compound mutant displays qualitatively different, and more severe recombination-deficient phenotypes than either a *ERCC1* ko or *MSH3* del cell line. These results suggest that Msh3 and Ercc1 are not epistatic for removal of long, 3'-ended, non-homologous tails from strand-invasion recombination intermediates in mammalian cells. Reference 1: EMBO J 19 5552 (2000).

P5

Screening *Mycobacterium Tuberculosis* Genome for Genes Involved in Cellular Response to Nitrosative Stress. Park S-H, Hart L, Cao W. Department of Genetics and Biochemistry South Carolina Experiment Station, Clemson University, Clemson, SC, United States.

The production of reactive nitrogen species (RNS) such as nitric oxide by macrophages helps to control infection by *Mycobacterium tuberculosis* (*Mtb*). However, the protection is imperfect and infection persists. To identify genes that *Mtb* requires to resist RNS, an expression plasmid library for *Mtb* genome was transformed into an *E. coli* mutant strain, which is hypersensitive to nitrous acid and other reactive nitrogen species. Those colonies that contained genes active in the cellular response to nitrosative stress were selected by screening the transformed cells in the presence of 0 mM, 20 mM, 30 mM, 40 mM, and 50 mM NaNO₂ in 100 mM NaAc (pH4.6). Those colonies selected from initial screening were confirmed by their survival under nitrosative stress. The confirmed colonies were sequenced to determine the genes involved. Genes previously known or unknown to be resistant to nitrosative stress are being identified. These genes are the candidates for further study as to how this bacterium resists nitrosative stress.

P6

Xanthine DNA Glycosylase Activity from Mismatch-Specific Uracil DNA Glycosylase (MUG). Lee H-W, Wright C, Cao W. Department of Genetics and Biochemistry South Carolina Experiment Station Clemson University, Central, SC, United States.

Deamination of DNA bases carrying an exocyclic amino group such as adenine (A), guanine (G), cytosine (C) under nitrosative stress leads to formation of hypoxanthine, xanthine (X) and oxanine (O), and uracil (U). Deaminated base damage can cause mutagenesis in the genome, potentially giving rise to a number of disorders, including cancer. Base Excision Repair (BER) is a major pathway involved in repair of modified DNA bases. The mismatch-specific uracil DNA glycosylase (MUG) was previously identified in the *E. coli* genome with activity against uracil in the order of U:G>U:C>>U:T. Examination of cell extracts led us to detect previously unknown xanthine DNA glycosylase (XDG) activity in *E. coli*. DNA glycosylase assays with purified enzymes indicated the novel XDG activity is attributable to MUG. Here, we report biochemical characterization of xanthine DNA glycosylase activity from MUG. Unlike the uracil DNA glycosylase (UDG) activity, the wild type MUG is active against all xanthine-containing DNA (C/X, T/X, G/X, A/X and single-stranded X). Interestingly, the XDG activity from MUG appears to be more robust than the UDG activity. Site-directed mutagenesis was performed on conserved motifs that are involved in base recognition. Amino acid residues important for XDG and UDG activities are being identified. The surprising finding of XDG activity indicates that the active site of MUG can not only accommodate pyrimidine deaminated base like uracil but also some purine deaminated base like xanthine.

P7

Rad51d-Defective CHO Cells Show Radiosensitivity and Radiation-Induced Chromosomal Aberrations in S and G2 Phases of the Cell Cycle. Hinz JM, Wilson PF, Urbin SS, Nham PB, Thompson LH. Lawrence Livermore National Laboratory, Livermore, CA, United States.

Introduction: Homologous recombination repair (HRR) uses an homologous template for repair of double-strand breaks (DSBs), such as those caused by ionizing radiation (IR), and requires the activity of BRCA2, Rad51, the five Rad51 paralogs, and other proteins. HRR occurs in the S and G2 phases at broken replication forks and between replicated chromatids. We wished to determine whether HRR helps mitigate DNA damage occurring in G1, which may persist into S. In addition, we wished to determine the role of HRR in repairing double-strand breaks that can produce chromosomal aberrations. Methods: We irradiated the HRR-proficient parental AA8 cells, Rad51D-defective 51D1 (*rad51d*) cells, and the gene complemented 51D1.3 with g-rays at doses of 150 or 300 cGy, and then separated cells into discrete, highly synchronous fractions throughout the cell cycle using centrifugal elutriation. Degree of synchrony and cell cycle position were monitored by flow cytometry. Cells from each elutriation fraction were plated for colony formation efficiency, and metaphase spreads were collected for the first, and every third, fraction for corresponding chromosomal aberration analysis. Results: We found that the *rad51d* cells lack classical S-phase-associated radiation resistance compared to the parental and gene-complemented control cells. In addition, we found for all cell lines increasing levels of chromatid-type aberrations (associated with unrepaired breaks after replication) as the cells progressed through the cycle, but the magnitude was much higher in the *rad51d* cells. Consistent with indistinguishable radiosensitivity of mutant and control cells in the G1 fractions, there was no difference between their induction of chromosome-type aberrations (associated with unrepaired breaks occurring in G1). Discussion: We conclude that HRR plays a key role in the repair of IR-induced damage during the S and G2 phases of the cell cycle, primarily through HRR acting on frank DSBs between sister chromatids, and possibly also in repairing broken replication forks. The similar sensitivity of parental and *rad51d* mutant cells in early G1 suggests that IR-induced DNA damage at this stage does not persist into S phase and result in broken replication forks, which require HRR for restoration.

P8

Human DNA Polymerase ν (POLN): Residues Important for its Low Fidelity and Bypass Activity. Takata KT, Wood RD. University of Pittsburgh, Pittsburgh, PA, United States.

Introduction: DNA polymerase N (POLN) is the most recently discovered nuclear DNA polymerase in human cells (1). It is an A-family DNA polymerase related to *E. coli* pol I, *Taq* pol I, human POLQ and *Drosophila* Mus308. POLN is preferentially expressed in vertebrate testis but little is known about its function. POLN can catalyze accurate translesion synthesis past a 5S-thymine glycol (Tg), which strongly inhibits replication by B-family DNA polymerases. The strand displacement activity of POLN is also stronger than exonuclease-deficient Klenow fragment (2). POLN is a low-fidelity enzyme favoring incorporation T almost as often as C for template G (2), and can mediate frequent GC to AT transitions (3). Methods: To explore the basis for these features of POLN, we examined an alignment of the DNA polymerase domain with common A-family DNA polymerases. In the highly conserved Motif 4, a positively charged Lys is present at residue 679 of POLN, in a position that is an uncharged Ala in *E. coli* pol I or Thr in *Taq* pol I. This residue is essential for fidelity of the prokaryotic enzymes. K679A and K679T mutants of POLN were constructed, expressed in *E. coli*, purified, and tested for activity. A single aromatic residue in motif 4 of the A-family DNA polymerases, either Phe or Tyr, is highly conserved and critical for ddNTP selectivity. POLN has a Y682 at this position, and is inhibited by ddTTP on a poly(dA)-oligo(dT) template. We also prepared Y682F POLN and tested its activity. Results: The K679A or K679T POLN mutants are active but poorly incorporate T opposite template G nor bypass 5S-Tg. The Y682F POLN mutant is resistant to ddTTP. Furthermore, Y682F POLN barely incorporates T opposite template G or bypasses 5S-Tg. The positively charged K (or R) residue and the aromatic residue Y in Motif 4 are conserved in POLNs. Our data indicate that these residues are critical for the unusual fidelity and bypass properties of POLN. References: 1) Marini, F., Kim, N., Schuffert, A., and Wood, R. D. (2003) *J Biol Chem* 278(34), 32014-32019, 2) Takata, K. I., Shimizu, T., Iwai, S., and Wood, R. D. (2006) *J Biol Chem* 281, 23445-23455, and 3) Arana, M. E., Takata, K., Garcia-Diaz, M., Wood, R. D., and Kunkel, T. A. (2007) *DNA Repair (Amst)* 6(2), 213-223.

P9

Novel Xanthine DNA Glycosylase (XDG) Activities in Mammalian and Yeast Systems. Dong L¹, Glass RA¹, Meira LB², Samson LD², Cao W¹. ¹Clemson University, Clemson, SC, United States, ²Massachusetts Institute of Technology, Cambridge, MA, United States.

DNA that stores genetic information is frequently damaged in cells. The DNA bases carrying an exocyclic amino group [adenine (A), guanine (G), cytosine (C)] encounter deamination even under physiological conditions. Xanthine (X) is derived from deamination of guanine, it is potentially cytotoxic and mutagenic lesions. However, in eukaryotes, studies on the enzymatic repair of these lesions are limited. So far, Alkyladenine DNA glycosylase (AAG) is the only glycosylase that contains xanthine DNA glycosylase activity in mammalian systems. Here, we report our recent findings on addition xanthine DNA glycosylase activity in *Aag* knockout mouse tissues and other mammalian tissues. Data obtained from xanthine DNA cleavage assay using the purified human DNA glycosylases demonstrated that a uracil DNA glycosylase hSMUG1 contained weak but detectable XDG activities. Analysis of the partially purified proteins from the mammalian cell extracts proved that SMUG1 contributed to the observed XDG activities in *Aag* knockout mice. In addition, we also found XDG activity in *Schizosaccharomyces pombe* thymine-DNA glycosylase (Thp1p), which is homologous to human thymine-DNA glycosylase (TDG), an enzyme that removes thymine from T/G pair. It was reported that Thp1p contains uracil DNA glycosylase and hypoxanthine DNA glycosylase activity (Nucl. Acids Res. 31: 2261). The cleavage analyses with the double and single oligonucleotide substrates revealed that Thp1p could remove X, I and U relatively efficiently with no preference to opposite base. The order of the Thp1p glycosylase activity for these deaminated lesions was found to be X>I>U>O (oxanine). Thp1p is the first glycosylase in the yeast that catalyses the removal of xanthine. These studies indicate that eukaryotic uracil DNA glycosylases have evolved with high structural flexibility to achieve its broader substrate specificity towards deaminated DNA bases.

P10

Unleashing FANCF/BRIP1 from BRCA1 Alters the DNA Damage Response. Litman R, Peng M, Xia J, Cantor S. UMASS Medical School, Worcester, MA, United States.

Studies on the BRCA-Fanconi anemia (FA) genes revealed that defects in common DNA damage-signalling pathways lead to multiple cancer syndromes, such as breast, ovarian, and blood cancers. Likewise, mutations in *FANCF* (formally called BACH1/BRIP1) are linked to breast and FA-cancer syndromes. *FANCF* was identified as the gene defective in the FA-J patient complementation group, and was initially linked to hereditary breast cancer. This link was based on its direct binding to BRCA1 and through the identification of two breast cancer patients with mutations in *FANCF*, which also altered its helicase activity *in vitro*. More recently, *FANCF* mutations were shown to confer a two-fold increase in the risk of developing breast cancer. Consistent with *FANCF* being an FA-gene product, *FANCF*-null (FA-J) cells demonstrate ICL-induced sensitivity and a prolonged G2/M arrest. Furthermore, *FANCF* suppression leads to ICL-induced chromosomal aberrations and dramatically reduces homologous recombination. In an effort to better understand how *FANCF* functioned in DNA damage signalling, we purified a *FANCF* complex with the goal of identifying novel interacting proteins that contribute to *FANCF*'s DNA damage signalling function. In addition to BRCA1, we identified the two subunits of the MutLa complex, PMS2 and MLH1. To categorize the various phenotypes associated with loss of *FANCF*, we have addressed the requirement of distinct *FANCF* domains for re-establishing *FANCF* function. Thus, we reconstituted FA-J patient cells with wild-type *FANCF* or mutant versions to assess the importance of BRCA1, MutLa binding and/or helicase activity for *FANCF* function. For restoring *FANCF* ICL-induced response, helicase activity, as well as MutLa binding are essential. However we found that *FANCF* binding to BRCA1 was dispensable to normalize the ICL-induced response. While this finding could suggest that BRCA1 binding is not essential for *FANCF* function, instead, we found that BRCA1 binding to *FANCF* is essential to promote both homologous recombination and checkpoint functions. Thus, unleashing *FANCF* from BRCA1 appears to be oncogenic as cells resist DNA damage, ignore checkpoint functions, and generate chromosomal instability. These data suggest that BRCA1 binding to *FANCF* is more essential than helicase activity and/or MutLa binding for *FANCF*'s tumor suppression function. These findings suggest the possibility that unleashed *FANCF* is at the heart of other cancer syndromes.

P11

Genetic Characterization of the Role of Bacterial DNA Mismatch Repair in Sodium Nitrite Mutagenesis. Deschenes SM. Sacred Heart University, Fairfield, CT, United States.

Sodium nitrite is widely used as a preservative in processed meats, but it is also used in clinical (e.g., vasodilator) and industrial settings (e.g., dyeing textiles). Sodium nitrite, and the nitrous acid (NA) it forms when dissolved in acid, indirectly cause the deamination of DNA bases to hypoxanthine (Hx), xanthine (X), and oxanine (O), resulting in mismatched base pairs and ultimately mutations. Endonuclease V, encoded by the *nfi* gene, is the primary DNA repair enzyme for correcting these mismatches, although other DNA repair pathways such as base excision repair are known to play secondary or redundant roles in preventing the accumulation of NA-induced mutations. By virtue of its major function in detecting and repairing mismatched bases, mismatch repair (MMR) may also serve as a redundant DNA repair activity during NA mutagenesis. Indeed, previous studies have suggested that MMR only plays a minor role in correcting Hx-containing mismatches, but its ability to act on mismatches containing X or O has not been reported. We are using a simple genetic approach to test our hypothesis that MMR is redundant to endonuclease V in preventing NA-induced mutations. Our strategy depends on the well-established *E. coli lacZ* reversion assay which reports both mutation spectra and frequencies resulting from mutagen exposure. Null mutations in *mutS*, *nfi*, or both *mutS* and *nfi* were introduced into strains CC106 and CC102, which report the NA-induced A:T→G:C and G:C→A:T mutations, respectively, as well as in other *lacZ* reversion strains. Standard NA treatment protocols could not be used for these studies because they are performed on overnight cultures of *E. coli*; MMR is non-functional in cells that are not actively replicating DNA. Instead, bacterial cultures are being exposed to either sodium nitrite or NA while actively growing under either micro-aerobic or aerobic conditions, respectively. Our preliminary studies suggest that *mutS*-deficient CC102 and CC106 do not exhibit significant elevations in sodium nitrite-induced *lacZ* reversion frequencies under micro-aerobic growth conditions, supporting our hypothesis. The results of ongoing experiments on CC102, CC106, and other *lacZ* reversion strains lacking either or both *mutS* and *nfi* will be presented and discussed.

P12

Xanthine DNA Glycosylase Activity in SMUG1 DNA Glycosylase from *Geobacter Metallireducens*. Mi R, Cao W. Clemson University, Clemson, SC, United States.

Single-strand selective monofunctional Uracil-DNA glycosylase (SMUG1) belongs to family 3 of uracil DNA glycosylase superfamily. Previously it was thought SMUG1 exclusively exists in eukaryotic systems, however, homologs have been found in some bacterial species. Here, we report DNA glycosylase activities of SMUG1 from *Geobacter Metallireducens* (Gme). Gme SMUG1 shares 54% identities with human SMUG1 in amino acid sequences. Like human SMUG1, it is a bona fide uracil DNA glycosylase, exhibiting activity against both double-stranded and single-stranded uracil-containing DNA. In addition, Gme SMUG1 also contains significant xanthine DNA glycosylase activity for both double-stranded and single-stranded DNA. In this study, we used site-directed mutagenesis to construct mutants based on the highly conserved motifs among uracil DNA glycosylase superfamilies. The DNA glycosylase activities of these mutants are being characterized by biochemical assays. Interestingly, methionine substitution at H210 position of motif 2 caused a loss of xanthine DNA glycosylase activity while remained active on some uracil-containing substrates. These data suggest that (1) the conserved key residues in SMUG1 family are important for recognition of variety of deaminated bases; (2) SMUG1 is no longer regarded as a eukaryotes-only DNA repair enzyme; (3) bacterial SMUG1 may involve in repair of both pyrimidine and purine deaminated bases.

P13

Development and Characterization of an Antibody Against DNA Interstrand Crosslinks. McClendon TB^{1,2}, Niedernhofer LJ^{1,2}. ¹Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States, ²University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, PA, United States.

DNA interstrand crosslinks (ICLs) are adducts covalently linking two bases on opposite DNA strands. ICLs are cytotoxic as they prevent strand separation and therefore DNA replication and transcription. Because crosslinking agents are so cytotoxic to replicating cells, these drugs are commonly used in cancer chemotherapy. Despite the widespread clinical use of crosslinking agents, the mechanism of ICL repair in mammalian cells is poorly understood and enhanced repair may contribute to tumor resistance to therapy. The goal of this research is to develop an antibody that specifically recognizes psoralen DNA ICLs in genomic DNA of cells. This antibody will provide an essential tool for probing the mechanism of ICL repair in mammalian cells. Psoralens are furocoumarins that intercalate in DNA and form covalent adducts between thymines on opposite strands after photoactivation with UV-A (PUVA). To create an antigen of crosslinked DNA, double-strand (ds) calf thymus (CT) DNA was sonicated into 200-800 bp fragments. The DNA was then exposed to either 300 μ M 8-methoxypsoralen (8-MOP) or 4'-hydroxymethyl-4,5',8-trimethylpsoralen (HMT), followed by UV-A (54 kJ/m²). The DNA was dialyzed to remove non-covalently bound psoralen and concentrated by ethanol precipitation. The extent of crosslinking was determined by heat denaturing the DNA and quantifying the relative amount of ds and single-strand (ss) DNA by electrophoresis, hydroxyapatite chromatography with UV spectrophotometry, and PicoGreen[®] fluorescence. Optimized conditions yielded greater than 98% nondenaturable DNA. Milligram quantities of the 8-MOP and HMT antigen (crosslinked CT-DNA fragments) were serially injected into rabbits and chickens to produce antisera. Slot blot analysis of the antisera showed dose-dependent reactivity with the antigen and no cross-reactivity with unmodified ss or dsDNA. Reactivity of the antisera with other DNA substrates (*e.g.*, DNA containing psoralen monoadducts) is currently being tested. The antibody will then be used to detect ICLs in cells exposed to psoralen photoactivated with a UV-A laser and to identify DNA repair proteins that colocalize with ICLs.

P14

The Relation Between DNA Damage and Hyperglycemia in KK-Ay Mice. Hashimoto K^{1,2}, Takasaki W¹, Yamoto T¹, Manabe S¹, Sato I², Tsuda S². ¹Medicinal Safety Laboratories, Daiichi Sankyo Co., Ltd., Fukuroi, Shizuoka, Japan, ²Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan.

Reactive oxygen species are generated in diabetes mellitus and are considered to be one of causes of DNA damage. However, the relation between DNA damage and hyperglycemia is unclear. This study was conducted to evaluate the relation between DNA damage and hyperglycemia and the involvement of reactive oxygen species in DNA damage in hyperglycemia. KK-Ay mice and C57BL/6J mice were used as diabetic and non-diabetic model animals, respectively. DNA damage was examined using comet assays at pH 9, 12.1 and 13 in the liver, kidney and peripheral lymphocytes of these mice and the blood glucose level was monitored. The blood glucose level in the KK-Ay mice was higher than that in the C57BL/6J mice. DNA migration in the comet assay at pH 13 increased in the liver and kidney in KK-Ay mice compared with C57BL/6J mice, but DNA migration did not increase at pH 9 or 12.1. These results indicated that alkali-labile sites were accumulated in the KK-Ay mice compared with the C57BL/6J mice. To evaluate the effects of the blood glucose-lowering action on DNA damage, the KK-Ay mice were treated for eight weeks with sulfonylurea hypoglycemic agents, gliclazide (20, 50 mg/kg/day) or glimepiride (0.5, 5.0 mg/kg/day). Blood glucose levels decreased in the gliclazide- and the glimepiride-treated groups compared with the control groups. DNA migration at pH 13 was decreased in the liver and/or kidney, but not in the lymphocytes. Additionally, the KK-Ay mice were treated for eight weeks with a diet supplemented with 0.01 or 0.1% d- α -tocopherol acetate. Blood glucose levels did not change in the groups treated with d- α -tocopherol acetate at any doses. DNA migration at pH 13 was decreased in the kidney, but not in the liver or lymphocytes. These results suggest that DNA damage in the liver and kidney of KK-Ay mice is caused by hyperglycemia and that reactive oxygen species are involved in the hyperglycemia-induced DNA damage.

P15

The Role of Common Base Excision Repair Variants in Cancer Susceptibility. Yamtich J, Speed WC, Straka E, Kidd K, Sweasy JB. Yale University, New Haven, CT, United States.

The mammalian base excision repair (BER) pathway is responsible for the repair of 20,000 endogenous DNA lesions per cell per day¹. This repair is important for the maintenance of genomic integrity and protection of cells from cancer. DNA polymerase beta (pol β) has both polymerase and dRP lyase functions. Mutations in pol β have been identified in cancer; however, common polymorphisms of pol β are also found in healthy individuals. Growing evidence suggests that non cancer-associated DNA repair variants are important in the complex phenotype of cancer predisposition. For pol β , three amino acid substitutions, Gln8Arg, Arg137Gln, Pro242Arg, have been reported as polymorphisms². In assessing their global frequency distributions, we included common non-coding SNPs in and flanking the POLB gene for a total of 11 sites typed in approximately 2,500 individuals from populations worldwide. These SNPs showed strong linkage disequilibrium. African populations had 3-4 common (>10%) haplotypes, while all non-African populations had a single common (generally >80%) haplotype and some populations had 1-2 others at >5%. The common non-African haplotype occurred at 20% or less in African populations. The Gln8Arg variant was not seen in any of the individuals worldwide. The 137Gln and 242Arg alleles were generally low frequency worldwide. Further resequencing of DNA repair genes in African individuals is warranted, as our results showed that several haplotypes common in African populations are rare or unobserved outside of Africa. Next, to determine the role of these variants in individual cancer susceptibility, we purified pol β variant proteins containing these polymorphisms. In an *in vitro* steady-state primer extension assay we found that the Arg137Gln variant shows decreased activity and strand displacement synthesis compared to the common form. These results suggest that BER is affected when alternative forms of pol β are expressed, and accuracy measurements are currently underway. If common SNPs do result in aberrant BER, this could lead to altered cancer predisposition and response to chemotherapeutic agents. 1) Lindahl, T (1993) *Nature* 362, 709-7152. 2) Mohrenweiser, HW *et al.* (2002) *Cancer Epidemiol Biomarkers Prev.* 11, 1054-1064.

P16

Use of a shRNA CHO Cell WRN Knockdown to Investigate Mammalian Recombination Pathways. Rahn JJ, Della Coletta L, Lowery M, Adair GM, Nairn RS. University of Texas MD Anderson Cancer Center Science Park Research Division, Smithville, TX, United States.

The repair of DNA is essential in promoting both genome stability and survival. While DNA repair pathways in yeast are well understood for the most part, analogous pathways in mammalian systems are still being characterized. Werner's syndrome is a rare autosomal recessive condition resulting in premature aging, overall genetic instability and predisposition to cancer. Werner's syndrome patients have mutations in the Werner's helicase gene (WRN) resulting in the production of a non-functional truncated protein. WRN is a member of the RecQ helicase family and has been shown to interact with a wide range of proteins involved in DNA replication, repair and stability. While all the recombination functions of WRN in humans are not precisely understood, its potential yeast homologues Sgs1 and Srs2 have been shown to be involved in recombinational repair of double strand breaks by regulating pathway choice for gene conversion vs. crossover outcomes. To investigate the potential role of WRN in mammalian cell mitotic recombination, a stable shRNA WRN knockdown CHO cell line was established and WRN knockdown was confirmed by western analysis. Sensitivity to the crosslinking agent mitomycin C was observed in the knockdown cell line, suggesting the involvement of WRN in DNA interstrand crosslink (ICL) processing, possibly through recombinational repair. To study the potential role of WRN in strand invasion homologous recombination, a gene targeting assay system was developed at the hemizygous *APRT* locus in CHO AA8 cells. Gene targeting at the endogenous *APRT* locus using insertion-type vectors having blocking 3'-OH nonhomologies was performed to test the effects of WRN knockdown on recombination efficiency and the distribution of crossover vs. conversion recombinants. Results of these experiments will indicate whether WRN is involved in this pathway of homologous recombination, joining several other genes (e.g., *ERCC1*, *MSH3*) also shown to be involved in both recombination and the processing and repair of ICLs. (Supported by NIH P01 grant CA097175).

P17

Influence of Polymorphisms in DNA Repair Genes on the Initial Repair of MMS-Induced DNA Damage, as Measured by the Comet Assay. Ryk C¹, Routledge MN², Allan JM³, Wild CP², Kumar R^{1,4}, Lambert B¹, Hou S^{1,5}. ¹Department of Biosciences and Nutrition, Karolinska Institute, Huddinge, Sweden, ²Molecular Epidemiology Unit, The LIGHT Laboratories, University of Leeds, Leeds, United Kingdom, ³Epidemiology & Genetics Unit, Department of Biology, University of York, York, United Kingdom, ⁴Division of Molecular Genetic Epidemiology, German Cancer Research Centre, Heidelberg, Germany, ⁵AstraZeneca R&D Sodertalje, Safety Assessment, Genetic Toxicology, Sodertalje, Sweden.

In this study we used the single cell gel electrophoresis method (comet assay) to study the functional impact of seven polymorphisms in six different DNA repair genes, involved in base excision repair (*XRCC1* Arg194Trp, *XRCC1* Arg399Gln, and *APEX1* Asp148Glu), homologous recombination repair (*XRCC3* Thr241Met and *NBS1* Glu185Gln), mismatch repair (*MLH1* -93 A/G), and nucleotide excision repair (*XPB* Lys751Gln). DNA samples isolated from lymphocytes of 52 healthy non-smoking volunteers were genotyped. Stimulated cells were treated with direct acting alkylating agent methylmethane sulphonate (MMS) for 15 minutes, and allowed to repair for 6, 21, 46 or 126 minutes. DNA damage was evaluated as percent damaged (normal, mild, moderate and severe) cells and as a pseudo score (PS, comparable to percent tail DNA). The mean PS was 3.2 (±1.6) in untreated cells, 54.4 (±8.1) six minutes after treatment and 39.0 (±12.1) 126 minutes after treatment. Significantly increased odds ratio (OR) for having a high PS-value was seen at all times points in cells with at least one Asp148 allele of the *APEX1*. The *XRCC3* 241Met allele was associated with a significantly increased OR for a high PS-value at the initial two time points. The *NBS1* 185Gln allele was associated with an increased OR for a high PS-value at the first time point, and a decreased OR at the following two time points. These results indicate that the initial repair of MMS-induced DNA damage is affected by polymorphisms in the *APEX1*, *XRCC3* and *NBS1* genes. Furthermore, the present experimental system may be useful for studying the functional impact of other genetic polymorphisms in DNA repair.

P18

Endonuclease V-Mediated Deamination DNA Repair: From Single-Molecule Study to Mammalian Systems. Cao W. Department of Genetics and Biochemistry, Clemson, SC, United States.

Endonuclease V (endo V) is a unique DNA repair enzyme involved in repair of deaminated DNA base lesions. Endo V is an enzyme that initiates repair of deaminated DNA bases by making an endonucleolytic incision at the 3' side one nucleotide from a base lesion. Using the technique of fluorescence resonance energy transfer (FRET) we determined the single-molecule kinetics of bacterial endo V from *Thermotoga maritima* in a catalytic cycle using a substrate of deoxyinosine-containing single-stranded DNA (ssDNA). The ssDNA was labeled with TAMRA, a fluorescence donor, while the endo V was labeled with Cy5, a fluorescence acceptor. The time lapses of FRET, resulting from the sequential association, recognition, and dissociation of the deoxyinosine by the endo V, were determined at 5.9 s, 14.5 s, and 9.1 s, respectively, in the presence of Mg²⁺. In contrast, the process of deoxyinosine recognition appeared little affected by the metal type. The prolonged association and dissociation events in the presence of the Ca²⁺-Mg²⁺ combination, as compared to that of Mg²⁺ alone, support the hypothesis that endo V has two metal binding sites to regulate its enzymatic activities. This novel single-molecule approach will be of great interest for researchers working in the DNA damage and recognition field. Previously, technical difficulties have precluded understanding of endo V-mediated DNA repair in mammalian systems. A recent technical breakthrough in my laboratory allows biochemical characterization of human endo V. Our unpublished data indicate that human endo V possesses unique DNA repair properties that are distinctively different from bacterial endo V enzymes. These studies significantly expand our understanding of repair of DNA damage caused by reactive nitrogen species.

P19

Development of a Non-Mammalian Vertebrate Model System to Investigate Mechanisms and Pathways of Genomic Instability *In Vivo*. Kuhne WW, Ding L, Dynan WS. Medical College of Georgia, Augusta, GA, United States.

Genomic instability is a phenomenon whereby radiation-related genetic damage manifests itself one or more cell generations following the generation in which the damage was inflicted. Although not fully understood at a molecular level, radiation-induced genomic instability may reflect the presence of unstable chromosomes formed by misrepair of DNA double-strand breaks. It may also reflect the non-DNA targeted effects of radiation, including persistent oxidative stress and bystander signaling. One approach for detection of genomic instability is based on the use of locus-specific genetic tests that detect non-conservative homologous recombination events. Tests have been devised in the mouse based on a naturally-occurring mutation, the pink-eyed unstable allele, and on an artificial transgene, unstable fluorescent green direct repeat (FDGR) locus. The latter contains two tandemly repeated, but incomplete, copies of the enhanced green fluorescent protein that must undergo recombination to be active (Jonnalagadda et al., *DNA Repair* (4) 594-605 (2005)). Here we describe the adaptation of the FDGR technology to a non-mammalian model system, the Japanese medaka fish (*Oryzias latipes*). As vertebrates, medaka share with humans a large complement of vertebrate-specific organs of radiobiological interest (brain, spinal cord, vasculature, digestive, excretory, and hematopoietic systems). The short life cycle of the medaka and the transparent chorion make this vertebrate model an excellent candidate for transgenerational studies and determination of tissue-specific responses to ionizing radiation. Chimeric medaka containing the FDGR transgene will be established by microinjecting embryos at the one or two-cell stage with the test cassette. A homologous recombination event can restore the full function of the test cassette through the subpathways of either single strand annealing or a gene conversion with or without crossing over. Mature chimeric medaka will be bred with wildtype medaka of the same strain to produce an F1 generation. Fluorescent green direct repeat (FDGR) positive F1 embryos will be determined by PCR-genotyping to determine the frequency of germ-line transmission. Preliminary results of the development and characterization of the transgenic fish will be reported.

P20

Profiling Key Phospho-Protein Kinetics in Different Cell Types After Low and High LET Exposure. Whalen MK, Gurai SK, Pluth JM. Lawrence Berkeley National Lab, Berkeley, CA, United States.

Signal transduction pathways are important roadways for cells to communicate both intra- and inter-cellularly within tissues and throughout the body. These communications influence cellular responses after insults and can determine cellular fate after DNA damage. One major method of cell signaling involves protein phosphorylation. Many different antibodies recognizing key radiation responsive phosphorylation events have been developed and are commercially available. Some phosphorylation events occur within minutes or seconds after exposure and are critical for cell survival, re-establishing homeostasis, and maintaining genomic stability. We have developed FACS-based assays using commercially available phospho-specific antibodies to profile cellular responses to various classes of ionizing radiation (high and low LET). To date we have profiled phosphorylation levels of gH2AX (Ser139) ATF2 (Ser490/498) and SMC1 (Ser966) phospho-proteins at a number of doses and time points following both X-ray and Fe ion exposure (1000 MeV) in human fibroblasts. We have also performed similar studies in lymphoblasts to compare and contrast with fibroblast cells response. Differences between the different phospho-proteins were noted between responses for low and high LET and the responses between the types of cells studied. Cell cycle status can also be determined at each time point and dose, to determine the relationship between cell cycle status and phosphorylation. Unique to flow cytometry, data can be extracted from collected files to determine the levels of gH2AX foci for individual cells in each phase of the cell cycle. We are currently looking at this aspect in more detail to determine what percentage of cells in various phases of the cell show high levels of damage, or gH2AX fluorescence, and how this changes with time and quality of radiation. In addition to the flow cytometry profiles we have also counted radiation induced foci (RIF) microscopically by eye using these same antibodies and cell types. These data will help us to determine how mean fluorescent levels obtained via flow cytometry compare to the more standard method of counting RIF. The variations between phospho-protein profiles after exposure to different energies of radiation as well as the variances between cell-type profiles will lend insight into the relative biological effects of radiation classes on various human tissues. The detailed findings of these investigations will be reported on.

P21

Post-Translational Modification of Enzymes Involved in Repair of Oxidative DNA Damage in *Saccharomyces cerevisiae*. Griffiths LM, Swartzlander D, Doetsch PW. Emory University, Atlanta, GA, United States.

Base excision repair (BER) is the primary repair pathway for oxidative DNA damage. In humans, increased oxidative genotoxic stress has been linked to conditions such as cancer and aging. *Saccharomyces cerevisiae* is a widely accepted eukaryotic system for the study of DNA repair as the pathways between yeast and humans are quite similar. Ntg1p and Ntg2p function as DNA glycosylases and AP lyases in yeast. Ntg1p and Ntg2p are important enzymes in initiation of BER, recognizing and removing small lesions and making incisions in the phosphodiester backbone. Thus, it is important to understand how Ntg1p and Ntg2p function and are regulated. Various lines of evidence suggest that Ntg1p and Ntg2p can be post-translationally modified by sumoylation (SUMO= small ubiquitin like modifier). Ntg1p and Ntg2p possess seven and one consensus sequences for sumo binding, [Hydrophobic] K x [ED], respectively. Recently, it has been shown that Ntg1p and Smt3p (yeast SUMO) associate. Furthermore, thymidine DNA glycosylase, a BER enzyme in humans, has been shown to be sumoylated, resulting in an increase in the rate of BER in humans. To date, a variety of functions has been shown for sumoylation. Those that might apply to Ntg1p and Ntg2p include: differential localization, ability to associate with other proteins, and modulation of enzyme activity. The goal of these studies is to determine whether or not Ntg1p and Ntg2p are sumoylated and how this modification affects the function of these enzymes. In order to determine the extent to which Ntg1p and Ntg2p are sumoylated, strains were designed with an inducible TAP-tagged Ntg1p and Ntg2p and a constitutively expressed His7- tagged Smt3p. By first pulling down sumoylated proteins from cell lysates via affinity chromatography and isolating TAP-tagged protein, sumoylated Ntg1p and Ntg2p can be purified and detected via Western blots. Current studies are aimed at determining which residues on Ntg1p and Ntg2p are sumoylated and how sumoylation modifies the function of these enzymes. Sumoylation of Ntg1p and Ntg2p suggests a new control mechanism for the regulation of BER and may be very important to understanding the regulation and cross-talk between other DNA damage management pathways and BER.

P22

Structure-Function Studies of DNA Binding By the N-terminal Domain of yPms1. Arana ME, Holmes SF, Fortune JM, Pedersen LC, Kunkel TA. National Institutes of Health/NIEHS, Research Triangle Park, NC, United States.

Eukaryotes encode multiple homologs of homodimeric *E. coli* MutL that are essential for DNA mismatch repair (MMR). These homologs form different heterodimers, including Mlh-Pms1 in yeast and MLH1-PMS2 in humans. In addition to MMR, these MutL heterodimers participate in other types of DNA repair, in cellular responses to DNA damage and in meiotic recombination, such that defects in MutL functions can have profound effects on cancer susceptibility and infertility. MutL homologs have conserved N-terminal domains and less well conserved C-terminal regions. To accomplish their many functions, MutL homologs bind and hydrolyze ATP, they bind to DNA, and they interact with other proteins. In addition, Pms1/PMS2 has endonucleolytic activity. Our study focuses on the DNA binding properties of the N-terminal domain (NTD) of yPms1. In addition to binding and hydrolyzing ATP, the yPms1 NTD binds ss- and ds-DNA. When the structure of the *E. coli* MutL NTD was solved, it was proposed to bind DNA in a positively charged cleft formed when the N-terminal domains dimerize. An additional possibility is suggested by our previous observation that the NTDs of yeast Mlh1 and Pms1 each bind to DNA independently and in the absence of detectable dimerization. This led to the present study, which attempts to identify the DNA binding surface on the yeast Pms1 NTD. We first determined the crystal structure of NTD yPms1 to 2.1Å resolution. Its electrostatic surface potential suggests that DNA may bind in a different region than that proposed for the bacterial MutL NTD. Based on this idea, we replaced several positively charged amino acids with a negatively charged glutamate in a haploid yeast strain. Mutator effects were observed at three loci, indicating loss of MMR. Correlating with these apparent MMR defects *in vivo*, several mutants have decreased DNA binding affinity *in vitro*. These structure-function analyses suggest the position of the DNA binding surface on the yPms1 NTD, towards the ultimate goal of better understanding the role of DNA binding by MutL homologs in MMR and possibly other functions.

P23

Post-Translational Phosphorylation of hMSH6 and Mismatch Recognition Activity. Kaliyaperumal S, Williams K. University of Toledo Health Science Campus, Toledo, OH, United States.

The Mismatch repair (MMR) system plays a significant role in maintaining the integrity of the genome by repairing DNA mismatches and insertion-deletion loops (IDLs) resulting from replication, recombination, and error-prone repair. In human cells, mismatches are recognized and bound by a heterodimer, hMSH2-hMSH6 (hMutS α). A second heterodimer, hMLH1-hPMS2 (hMutL α) interacts with hMutS α and is thought to act as a mediator for downstream repair proteins. Defects in MMR are associated with hereditary non-polyposis colorectal cancer (HNPCC) and sporadic neoplasias. Studies from our lab and others have shown that hMSH6 is post-translationally phosphorylated. Little information is available in regard to specific hMSH6 phosphorylation sites or functional significance. There are now several lines of evidence that the PKC family of kinases play a functional role during hMSH6 phosphorylation. Therefore, we used Phorbol 12-myristate 13-acetate (PMA), a classical PKC activator and UCN01, a staurosporin analogue that inhibits PKC in the studies described below. HeLa cells were exposed to PMA and harvested at different time points. Nuclear extracts were subjected to electrophoretic mobility shift assay (EMSA) using ³²P-labeled 69mer oligos containing a G:T mismatch. We observed an initial increase and then a subsequent decrease in G:T binding over time. We then exposed HeLa MR cells to PMA and/or UCN01. EMSA results indicate decreased binding by nuclear extracts to both G:T and O⁶-me-G:T 69mers after UCN01 treatment, as compared to PMA or untreated nuclear extracts. An *in vitro* [γ -³²P] ATP phosphorylation assay was then performed using HeLa MR nuclear extracts and G:T or O⁶-me-G:T oligos. We obtained a significant increase in concentration of [³²P]-phosphorylated hMSH6 after incubation with both PMA and G:T oligo, as compared to incubation with G:T oligo alone. Similar results were observed after incubation with PMA and O⁶-me-G:T oligo, although at a reduced intensity. Overall, we have demonstrated that PKC activation by PMA enhances hMutS α -mismatched DNA binding, and this enhanced binding involves phosphorylation of hMSH6, but not hMSH2. We are currently investigating specific sites of phosphorylation on the N-terminal of hMSH6 to determine relevance to MMR activity.

P24

DNA Damage Causes Oxidative Stress in *Saccharomyces cerevisiae*? Rowe LA, Hopkins M, Degtyareva NP, Doetsch PW. Emory University, Atlanta, GA, United States.

Cells are exposed to both endogenous and exogenous sources of reactive oxygen species (ROS). At low levels in the cell, ROS can be beneficial and aid in homeostasis for normal growth and metabolism. At higher levels, ROS can lead to impaired physiological function through cellular damage. Elevated levels of ROS have been associated with certain human diseases including: cancers, neurodegenerative disorders, cardiovascular disease, as well as the process of aging. ROS is thought to lead to these diseases by damaging DNA, proteins, and lipids. In this study we are using *Saccharomyces cerevisiae* as a model system to examine the levels of ROS that are produced in response to DNA damage in four isogenic strains. The four strains include wild type (WT), base excision repair defective (BER-), nucleotide excision repair defective (NER-), and defective in both repair pathways (BER-/NER-). The levels of a spectrum of ROS were determined using fluorescent probes (dihydrorhodamine - DHR, dihydroethidium - DHEt, N-Acetyl-3,7-dihydroxyphenoxazine - Amplex Red (APR), and 2-[6-(4'-Hydroxy)phenoxy-3H-xanthen-3-on-9yl]bezoic acid - HPF) which are specific for different types of ROS. We found that when cells are exposed to DNA damage (both spontaneous and induced) there is an increase in the amount of ROS present in the cell. We have examined the spectrum of ROS to elucidate the role ROS may be playing in the DNA damage response. To further examine this process in the cell we are examining the role of ROS-handling pathways relevant to oxidative DNA damage (superoxide dismutase (SOD1 and SOD2) and catalase (CTT1, and CTA1)) and the oxidative stress-induced transcription factor Yap1p. These results suggest that the DNA damage-induced increase in cellular ROS levels is a generalized genotoxic stress response that is likely to function in various signaling pathways.

P25

FANCD2 Monoubiquitination in ERCC1-XPF Deficient Cells in Response to Interstrand Crosslink Damage. Bhagwat NR¹, Robinson AR¹, Niedernhofer LJ². ¹Department of Human Genetics, GSPH, University of Pittsburgh, Pittsburgh, PA, United States, ²Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States.

Fanconi anemia is caused by a group of different genetically recessive protein deficiencies (FANCA, B, C, D1, D2, E, F, G, I, J, L, M and N) and is characterized by bone marrow failure and cancer predisposition. The FANCD2 and FANCI proteins are required for resistance to DNA interstrand crosslink (ICL) damage. The Fanconi anemia core complex of proteins (comprised of A, B, C, E, F, G, M and L) responds to ICLs by monoubiquitinating FANCD2 and FANCI, which then form nuclear foci with numerous repair proteins at sites of DNA damage. ERCC1-XPF endonuclease is a component of the nucleotide excision repair (NER) machinery that repairs helix-distorting DNA lesions. ERCC1-XPF is a structure-specific nuclease that cuts the damaged strand of DNA 5' to the lesion in NER. ERCC1-XPF deficient cells are hypersensitive to crosslinking agents compared to other NER deficient cells. This indicates that ERCC1-XPF has a unique role in ICL repair via a mechanism that is distinct from NER. However, it remains unknown if ERCC1-XPF and a second nuclease, MUS81-EME1, function in the same ICL response pathway as the FANCD2 proteins. We show here that FANCD2 is monoubiquitinated in response to ICL damage in both ERCC1-XPF and MUS81-EME1 deficient cells. In human and mouse ERCC1-XPF mutant cells, monoubiquitinated FANCD2 levels persist at later timepoints after crosslink damage relative to wild type cells. However, the fraction of FANCD2 that is chromatin bound is reduced in mutant cells. In contrast, the kinetics and level of FANCD2 monoubiquitination is normal in MUS81-EME1 deficient cells. These data indicate that FANCD2 monoubiquitination is independent of nucleolytic processing of ICLs, but that chromatin localization of FANCD2 occurs after the crosslink is unhooked by ERCC1-XPF.

P26

Association Between Gene Polymorphisms and the DNA Repair Capacity in Young Lung Cancer Patients. Roessler U¹, Rosenberger A², Sauter W³, Bickeboeller H², Illig T⁵, Wichmann HE³, Hornhardt S¹, Gomolka M¹. ¹BfS Federal Office of Radiation Protection, Oberschleissheim, Germany, ²Department of Genetic Epidemiology, University of Goettingen, Goettingen, Germany, ³Institute of Epidemiology, GSF-Research Center, Oberschleissheim, Germany.

Approximately 5-10 % of the total population is discussed to be radiosensitive. These individuals bear a higher risk to develop cancer. This may be due to defects in genes, responsible for the integrity of the genome or for DNA repair processes. The LUCY- (lung cancer in the young)-study was designed to investigate a possible interaction between the phenotyp "radiosensitive" and lung cancer patients younger than 50 years. In contrast to older patients, the early onset of the disease is most likely promoted by genetic components. Goal of the project is to identify genes which may contribute to an increase of radiation sensitivity *in vitro*. In a case control study, initial DNA damage was measured after gamma irradiation (0- 4 Gy) and DNA repair capacity was determined after 10 to 60 min via comet assay in 100 patients and 100 healthy controls. Single nucleotide polymorphism (SNP) analysis of DNA repair genes *XRCC1-G280A*, *XRCC1-G399A* and *XPB-G312A* was performed by MALDI- TOF-MS. No difference between total cases and controls was observed for induction of DNA damage, nor for DNA repair capacity. However, a small group of patients carrying a variant of the *XRCC1* gene at position 280 demonstrated reduced DNA repair capacity. Tested in a linear model the genetic impact was significant ($p = 0.04$) and displayed the highest effect size compared to other risk factors. Immortalized lymphoblastoid cell lines from these patients showed decreased survival after irradiation. The *XRCC1-G280A* genotype influences DNA repair capacity in young lung cancer patients. This is possibly due to interaction with genes harboured by lung cancer patients predisposing for cancer.

P27

Tumor-Associated Variants of DNA Polymerase β and a Functional Link to Cancer Etiology. Donigan KA, Sweasy JB. Yale University, New Haven, CT, United States.

Maintenance of genome stability is essential for cellular survival; cells must be able to contend with the high rate of daily DNA damage. Such damage occurs at a rate of 10,000 lesions per cell every day (1). The base excision repair (BER) pathway is critical for resolution of abasic sites and damaged bases. DNA polymerase β (Pol β) serves to preserve genome integrity by filling DNA gaps that result from excision of damaged bases during BER. Approximately 30% of human tumors express Pol β variants not identified in normal tissue or controls (2). Such variants may function aberrantly in BER, resulting in higher levels of somatic mutation that may affect key growth control genes and lead to cancer. This work aims to investigate the activity and fidelity of cancer-associated mutants D160N and S229P to reveal possible underlying molecular mechanisms that relate to the etiology of cancer. We have found that the D160N variant induces cellular transformation, suggesting that D160N could be linked to the onset or progression of cancer. S229P, isolated from a bladder carcinoma, shows little primer extension activity even in the presence of all four dNTPs. Thus, BER is likely compromised in cells containing this variant and this could lead to genomic instability. The relation between these cancer-associated mutants and the etiology of cancer further supports a role for Pol β in guarding genome integrity. References: 1) Lindahl, T. (1993) *Nature*. 362:709-15. 2) Starcevic D, Dalal S, Sweasy JB. (2004) *Cell Cycle* 8:998-1001.

P28

Factors Affecting DNA Repair Gene Expression Levels: Impact on the Use of mRNA Expression to Predict Acute Side Effects of Radiotherapy. Popanda O¹, Woelfelschneider A¹, Lilla C¹, Mayer C¹, Debus J², Bartsch H¹, Chang-Claude J¹, Schmezer P¹. ¹German Cancer Research Center, Heidelberg, Germany, ²Radiology, University Hospital, Heidelberg, Germany.

DNA repair is suggested to play a major role in the cellular response to ionizing radiation. Developing severe adverse reactions during radiotherapy in the normal irradiated tissue can be a limiting factor in the use of this important tool in cancer treatment. We investigated base-line expression of DNA repair and repair-related genes in normal lymphocytes of patients as a marker to characterize a patient's DNA repair activity and to identify hyper-radiosensitivity. As base-line expression might be affected by endogenous and exogenous factors, we searched for patient-related or environmental modulators of mRNA expression. In a prospective study with 405 prostate cancer patients receiving radiotherapy, 54 patients were identified who suffered from severe acute side effects during treatment. Clinical, demographic and life style data were collected together with lymphocytes before starting therapy. Cellular mRNA levels of DNA repair genes (*ATM*, *APEX1*, *BRCA1*, *BRCA2*, *ERCC1*, *MDM2*, *TP53*, and *TOP3A*) were quantified after reverse transcription by optimized, quantitative real-time-PCR. Four polymorphisms were determined in the *ERCC1*, *MDM2* and *TP53* genes. In addition, plasma concentrations of antioxidants (ascorbic acid, carotenoids and tocopherols) were measured. An up to 37-fold inter-individual variation in constitutive mRNA expression was found between patients. Influencing factors associated with base-line mRNA expression were plasma antioxidant concentrations (e.g. carotenoids) and prostatectomy. A distinct haplotype of *ERCC1* showed a significant association with decreased mRNA expression (p value = 0.001). An odds ratio of 2.1 (95% confidence interval: 0.9-4.8) was found for *MDM2* mRNA expression and radiosensitivity. In conclusion, constitutive mRNA levels measured in lymphocytes from cancer patients represent a specific steady-state level for each patient which is affected not only by transcriptional control but also by endogenous (genotype), clinical (prostatectomy), and exogenous factors (antioxidant concentrations). Thus, regulation of mRNA expression is complex and needs further analysis before mRNA levels of DNA repair genes can be evaluated as possible biomarkers for radiosensitivity. Studies were supported by "Bundesamt für Strahlenschutz", Germany.

P29

Unnatural Substrates Reveal the Importance of 8-oxoG for *In Vivo* Mismatch Recognition and Repair by MutY Glycosylase. Livingston AL¹, O'Shea VL¹, Kim T³, Kool ET³, David SS^{2,1}. ¹University of Utah, Salt Lake City, UT, United States, ²University of California at Davis, Davis, CA, United States, ³Stanford University, Stanford, CA, United States.

The oxidative DNA lesion 7,8-dihydro-8-oxo-2'-deoxyguanosine (OG) leads to transversion mutations due to facile incorporation of adenine opposite OG during DNA replication. The adenine glycosylase MutY plays an important role in preventing such mutations by excising the misincorporated A, and the lesion is fully repaired by subsequent Base Excision Repair (BER) enzymes. Several variants of the human MutY homologue, MUTYH, have been implicated in predisposing an individual to colorectal adenomatous polyps and carcinomas through inactivating somatic mutations of *APC*. This mechanism is now referred to as MUTYH-Associated Polyposis (MAP) and provides the first evidence of an inherited defect in the BER pathway leading to cancer. Importantly, an understanding of variant MUTYH forms relies upon a thorough knowledge of the wild type enzyme and its *E. coli* counterpart. Thus, to determine the importance of specific steps in the base pair recognition and base removal process of MutY in mediating repair of OG:A mismatches, we have evaluated modifications of the OG:A substrate by *in vitro* adenine glycosylase and binding assays and an *in vivo* cellular repair assay. Specifically, the OG:A substrate was modified by replacement of OG with G and of A with 3-deaza-2'-deoxyadenosine (Z3). Both modifications reduce the intrinsic rate of MutY-mediated base removal under single turnover conditions; however, only replacement of OG significantly reduces mismatch affinity. Surprisingly, OG:Z3 bps are repaired almost as efficiently as OG:A bps while G:A bps were not repaired in the cellular assay. The results from this assay correlate well with the reduced affinity and suggest that high affinity binding is critical for efficient MutY-mediated repair. However, high affinity binding alone is not sufficient to mediate repair based on the minimal cellular repair of a MutY glycosylase-resistant nucleotide, 2'-deoxy-2'-fluoro-adenosine, opposite OG. These results suggest that the ability of MutY to locate OG and intercept the OG:A mismatch prior to replication is a critical feature that dominates MutY-mediated repair *in vivo*. Notably, this is consistent with the defective recognition of OG observed by *in vitro* analysis of two MUTYH variants found in patients with MAP.

P30

Analysis of Chromosomal Structural Damage in Lymphocytes and Sperm From Hodgkin's Disease Patients Treated With MOPP/ABVD(P) Chemotherapy With or Without Radiotherapy. Frias Vazquez S^{1,2}, Salas C¹, Lopez de Lara O², Olivares JJ², Molina B², Niembro A², Lozano V³, Rivera Luna R¹, Carnevale A¹. ¹Instituto Nacional de Pediatría, Mexico DF, Mexico, ²Universidad Autonoma de la Ciudad de Mexico, Mexico DF, Mexico, ³Instituto Nacional de Cancerología, Mexico DF, Mexico, ⁴ISSSTE Coord Med Genómica, Mexico DF, Mexico.

Introduction. Anticancer treatment for Hodgkin's Disease (HD) patients MOPP/ABVD(P) includes recognized clastogens and mutagens such as Nitrogen Mustard, Oncovin, Procarbazine, Prednizone, Adriamicin, Vincristine and Dacarbazine, as well as Radiotherapy in a high percentage of patients. This treatment may affect normal somatic and germ cells, and produce chromosomal damage unbalanced or balanced. The purpose of this study is to determine structural chromosome damage in lymphocytes from HD survivors treated with MOPP/ABVD(P), analyzing chromosome structural aberrations and breaks in metaphase chromosomes, and the presence of the balanced translocation (14;18) in lymphocytes and sperm. **Methods.** Ten healthy individuals and nineteen HD survivors signed a letter of consent to participate in the study; samples of peripheral blood and semen were collected 2-20 years after anticancer treatment. To obtain the frequency of chromosomal aberrations in somatic cells, lymphocytes were cultured to obtain chromosomes; 1000 GTG banded metaphases were analyzed per sample. Real time PCR was used to detect the presence of the t(14;18) representing balanced chromosomal damage in DNA from lymphocytes and sperm of healthy and MOPP-treated individuals; every time the translocation was assayed with the following controls: TPA as housekeeping gene, positive control of the t(14;18), water as negative control. **Results.** Unstable chromosome breaks were found significantly higher in HD patients: 0.06% in healthy and 0.17% in HD patients; cells with complex stable aberrations were found in 12/19 HD patients, (frequency 1%) but not in healthy individuals. Real time PCR showed that the 14;18 balanced translocation was not present in any group lymphocytes nor sperm. **Discussion.** High frequency of structural chromosomal damage was found in HD patients after 2-20 years of treatment, however balanced damage represented by t(14;18) was not found in any patient sample. Chemotherapy /radiotherapy was genotoxic for normal lymphocytes and it is possible that the damaged cells are the hematopoietic stem cells, this may be related with the presence of second neoplasias in this patient. Project supported by CONACYT grant 099-salud.

P31

Application of Protein Footprinting, Crosslinking, and Mass Spectrometry in Mapping Ape1 Contacts During BER Progression. Yu ET, Gaucher SP, Sale K, Young MM, Hadi MZ. Sandia National Labs, Livermore, CA, United States.

Abasic (AP) DNA damage is repaired by the concerted effort of DNA repair enzymes in the Base Excision Repair (BER) pathway. The key BER enzyme, Ape1, recognizes and cleaves the DNA backbone 5' to the AP site. Preliminary biophysical characterization suggests that Ape1 undergoes conformational changes during its interaction with DNA. Following incision, the Ape1-DNA complex recruits the down-stream enzyme, Pol beta. The mechanism of DNA transfer between these enzymes is unknown. Determining residues engaged in protein-protein interactions can offer insights into how the pathway is coordinated. Using footprinting and crosslinking studies, coupled with mass spectrometric detection, our goal is to identify Ape1 inter and intramolecular contacts involved in nucleoprotein complexes formed during BER progression. Footprinting studies under conditions that mimic steps in AP recognition and catalysis were performed. In the pre-incision complex, we observed protection of lysines in known DNA binding sites, as well as those not previously shown to interact with DNA (K24, K25, K27, and K31). In the presence of the metal co-factor Mg²⁺, K24 through K31 were modified in the Ape1-DNA complex. These data suggest that Ref1 domain undergoes a conformational change upon catalysis. It is likely that Ape1 employs this switch as a timed signal to recruit other proteins. Data on the ternary (Ape1:DNA:Pol beta) complex suggest that Pol beta displaces Ape1 from DNA making minimal Ape1 contacts. Preliminary Ape1 crosslinking experiments to obtain distance constraints for molecular modeling identified crosslinks between Ref1 and the C-terminal domain of Ape1. We are now further defining APE1 contacts and the Ref1 interaction using other footprinting reagents and DNA substrate intermediates to form and freeze DNA protein complexes.

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Genetic Analysis of DNA Strand Break Repair and Loss of Heterozygosity in p53 Deficient and Wildtype Hematopoietic Stem Cells Shows Haplotype Dependent Efficiency of Repair. French JE, Parron VI. NIEHS, NIH, Research Triangle Park, NC, United States.

Mis-repair of carcinogen induced DNA damage may lead to the loss of heterozygosity (LOH) and changes in gene copy number variation (CNV) associated with the loss of tumor suppressor genes leading to cancer. Coordination of non-homologous end-joining (NHEJ) and homologous recombination (HR) pathway repair is dependent on the phase of the cell cycle, DNA replication, and expression of the p53 tumor suppressor protein. We have reported that carcinogens (ionizing radiation, benzene, melphalan, etc.) rapidly induce tumors with the LOH of the wild type *Trp53* allele in p53 heterozygous mice carrying an inherited p53 null mutation and a functioning wildtype allele. The LOH phenotype observed in ionizing radiation (IR) induced tumors is highly penetrant in the C57BL/6 (B6) mouse strain and appears low in the DBA/2 (D2) strain. IR induced *Trp53* sequence loss is the greatest in B6>B6C3F1>C3H>B6D2F1>D2. D2 alleles suppress both LOH and tumor rates. LOH in these tumors show a pattern of non-random loss of chromosome 11 (SSLP markers) as well as genome wide CNV consistent with NHEJ/HR misrepair of strand breaks. Using long-term primary cultures of initiated hematopoietic stem cells (HSC), B6 and D2 isogenic mouse HSC, we have observed significant differences in the abundance of DNA repair gene transcripts up to 3 h post-irradiation. Terminal deoxynucleotidyl transferase (TdT) assay for quantification of DNA strand breaks and the time required to resolve breaks is significantly different between these two strains. Maximum TdT fluorescence associated with breaks occurred within minutes and the time required for the resolution of TdT fluorescence was strain dependent. The data suggest that misrepair of strand breaks and LOH is a quantitative trait (polygenic) dependent upon the DNA damage repair capacity. Analysis of NHEJ repair gene haplotypes by similarity matrices indicates that allelic diversity across isogenic strains in some genes is significant but many components of this repair pathway are identical by descent. In order to identify the allelic variants (haplotypes) of genes associated with this strand break repair and LOH phenotype additional phenotyping and functional analysis of multiple isogenic strains are required. Determination of the allelic variants of genes causally related to DNA damage and repair with altered function is critical in order to understand the differences in risk due to exposure to environmental mutagens.

P33

The Acetaldehyde-Derived Adduct N2-Ethyl-2'-deoxyguanosine Blocks RNA Pol II at the Extension Step, and T7 RNA Polymerase at the Insertion Step. Cheng T-F, Brooks PJ. NIAAA NIH, Rockville, MD, United States.

The first metabolite of ethanol, acetaldehyde, is an animal carcinogen, and substantial evidence exists for causative role of acetaldehyde in the increased risk of esophageal cancer due to alcohol drinking. The primary stable DNA adduct from acetaldehyde is called N2-ethyl-2'-deoxyguanosine (N2EtdG). Its structure is shown below. Elevated levels of this adduct have been detected in the DNA of white blood cells from alcohol abusing individuals. This adduct can transiently block DNA polymerases, and is weakly mutagenic in mammalian cell systems. However, at present nothing is known about the effect of this DNA lesion on transcription by RNA polymerases (RNAPs). Therefore, we have investigated this question using a minimal system described by Kashlev and colleagues. We studied the multisubunit calf thymus RNAPII, and T7 RNAP. T7 is of interest as it is structurally and functionally homologous to mitochondrial RNAP. Since the primary human enzyme in responsible for the metabolism of acetaldehyde, ALDH2, is localized to the mitochondria, it is likely that this adduct forms in both mitochondrial as well as nuclear DNA. Methods: a 10 base RNA primer was annealed to a 38 nucleotide DNA template, such that the next template DNA base downstream of the three prime and of the primer was either dG or N2EtdG. Results: When incubated with rNTPs and calf thymus RNAPII, the undamaged template was rapidly transcribed towards the end. In contrast, using the N2EtdG containing template, at short time points (five minutes) only a single nucleotide was incorporated opposite the lesion, whereas at longer times (20 minutes) some bypass full-length transcript was observed. Using single rNTPs only CTP was incorporated opposite either dG or N2EtdG, indicating that this lesion does not stimulate transcriptional mutagenesis. In contrast, when the same primer template combinations or transcribed by T7 RNA polymerases, no detectable incorporation opposite the lesion was observed, indicating that N2EtdG prevents transcription by T7 RNA polymerase by the blocking the incorporation step. In summary, these results indicate that N2EtdG blocks both multisubunit and single subunit RNAP, but at different mechanistic steps; the multisubunit RNAPII can incorporate CTP opposite the lesion but is primarily blocked at the extension step, where his with T7 RNAP the lesion exerts its blocking effect at the insertion step. Ongoing studies are examining the mechanistic basis of this differential effect by modelling the adduct in the active site of both enzymes.

P34

The Effect of DNA Ethylation on RNA Polymerase II Transcription in the Yeast *Saccharomyces cerevisiae*. Shaheen Z., Bouska C, Ward K, Reagan M. College of St. Benedict/St. John's University, Collegeville, MN, United States.

The effects of DNA damage on transcriptional elongation has become an active area of study. The effect of template ethylation on RNA polymerase II transcription has not previously been investigated. We are investigating the effect of ethylation of genomic DNA on mRNA production *in vivo* in the yeast *Saccharomyces cerevisiae*. We have developed a system to measure the effect of DNA template damage on the production of full-length mRNAs by RNA polymerase II. We exposed yeast cells to the ethylating agent ethyl methanesulfonate (EMS), which ethylates DNA primarily at the N-7 position of guanine. We then induced the transcription of the *GAL1* and *GAL10* genes by adding galactose and measured, by Northern blot, the production of mature mRNAs post-treatment from these genes over time. A comparison of the timecourse of appearance of full-length transcripts in treated vs. untreated wild-type cells suggests that ethylation of the DNA template significantly inhibits RNA polymerase II elongation in yeast cells. We find that the higher the EMS dose, the greater the inhibition. Whether this dose-response effect reflects the greater chance of DNA in the transcribed unit being ethylated at higher doses, or whether it reflects a greater chance of the RNA polymerase arresting as it encounters more ethylated bases is being investigated with the use of various base excision repair mutants.

P35

Expression of p53 Regulated Genes in Fanconi Anemia Cells After Mitomycin C and Hydroxyurea Exposure. Martinez A¹, Hinz J^{1,2}, Gomez-Laguna L¹, Molina B¹, Acuna H¹, Frias S¹, Coleman M². ¹Instituto Nacional de Pediatría, Lab., Citogenética, Mexico, ²CMLS, Lawrence Livermore National Laboratory, Livermore CA, United States.

Fanconi anemia (FA) is a rare hereditary disease (autosomal and X-linked recessive) characterized by several congenital defects and increased risk of cancer. FA cells have high frequency of spontaneous chromosomal aberration, and it is the sensitivity to chemicals such as DEB or MMC that distinguish FA from other chromosomal instability syndromes. There are numerous studies about the FA hypersensitivity to DNA cross-linking agents, but few studies have explored the genotoxicity of FA cells to Hydroxyurea or the transcriptional response of selected DNA damage related genes in FA cells. We used QPCR to characterize the response of TP53 regulated genes in FA-A cells after MMC or HU treatments. These genes are involved in pathways such as cell cycle, DNA repair, replication, stress response, and apoptosis. We compared the transcriptional responses of FA and normal cells and found that 20 genes related to TP53 had higher levels of expression in FA cells after exposure to MMC (ABCB1, BLM, CCNB1, CDKN1A, DDB2, GADD45A, HSP8A, MDM2, MYC, NFKB1, p53, PCNA, RB1, RRM2B, SGK, SOD1, TP53I3 (PIG3), TRIAP1, TNFRS10B and XPC). Just one gene remained unchanged (NFKB1A). Following HU treatment, thirteen genes exhibited higher levels of expression (ABCB1, CCNB1, HSP8A, MYC, NFKB1, FKB1A, p53, PCNA, RB1, RRM2B, SGK, TNFRS10B and TRIAP1), while six genes had decreased expression (BLM, CDKN1A, GADD45A, MDM2, SOD1 and XPC) and two genes remained unchanged (DDB2 and TP53I3) when we compared to normal cells. Our results demonstrate that single and double strand breaks induced by HU in FA cells elicited a specific TP53-related damage response which is different from the MMC response. This transcriptional response is modified in terms of the down stream pathways and the magnitude of the transcriptional regulation.

Epigenetics, Germ Cells

P36

A High Resolution Oligonucleotide CpG Island Microarray. Foo C², Hopkins C¹, Roberts D¹. ¹Agilent Technologies, Santa Clara, CA, United States, ²UCSF, San Francisco, CA, United States.

CpG islands are stretches of high GC content DNA containing multiple CpG dinucleotides. When CpG dinucleotides within these islands are methylated, especially in promoter regions, expression of the corresponding downstream genes is often repressed. Aberrant CpG island methylation is implicated in cancer. We have developed an oligonucleotide microarray that specifically represents the CpG islands in the human genome. This microarray contains ~230,000 oligo probes tiling the 21 megabases of 27,800 CpG islands, with an average spacing between probes of 95 base pairs. The microarray is designed to be compatible with several published methods for the genome-wide detection of methylated CpG islands. To demonstrate the ability of this microarray to accurately detect methylated DNA, we performed analysis of human genomic DNA samples after methylated DNA immunoprecipitation (mDIP). Additionally, we developed and tested "spike-in" control DNA that was *in vitro* methylated to varying degrees. The mDIP method combined with CpG island microarray analysis accurately differentiated between partially and fully methylated spike-in DNAs. We then applied the whole-genome assay to the prostate cancer cell line PC3, where we detected methylated CpG islands upstream of cancer related genes including CDKN2A/p16. We extended the study to other cancer cell lines and determined relative methylation at multiple cancer related genes. Finally, using male and female embryonic lung fibroblast cell lines, we demonstrate that many CpG islands on the female X chromosome are more methylated than the corresponding islands on the male X chromosome. In comparison, methylation of CpG islands on the autosomes is essentially the same for both the male and female samples. This supports a role for CpG methylation in silencing the inactive X chromosome in females.

P37

Transgenerational Damage Induced by N-Nitrosodimethylamine (NDMA) in *Drosophila melanogaster*. Ramos-Morales P, Herrera-Bazan JJH, Munoz-Moya JA, Munoz-Hernandez A, Rivas-Martinez H, Hernandez-Bernal BR. UNAM, Mexico DF, United States.

Knowledge about the effect of genotoxins in whole organisms alert us about their mutagenic and carcinogenic activities, their ability to affect the reproductive function of genotoxins' exposed organisms or to induce malformations in their progeny. The effect of mutagenic and teratogenic compounds had been extensively studied. Recently, the interest about the possibility of additional effects induced by genotoxins in the progeny of exposed individuals has been increased. Could the parental exposition to genotoxins affects the health performance during the lifespan of their progeny? The Somatic Mutation and Recombination Test of *Drosophila* is a methodology used to screening somatic alterations induced by genotoxins. Although this assay can detect a wide spectrum of terminal genetic events, which lead to the formation of mutant spots in the adult fly, in individuals exposed to genotoxins during larval development, the somatic recombination had been found the most important mechanism involved in spots production. As a consequence, this assay detects efficiently those carcinogens that affect the integrity of the DNA. In this study we explore the induction of somatic damage in the progeny of *Drosophila* males exposed when larvae to the alkylant, promutagen N-Nitrosodimethylamine (NDMA). Methodology. Third instar, *mwh e* larvae were transferred to vials containing food supplemented with NDMA. After adults emergency, treated males were mated to virgin, *flr³/TM3, Bd^S* females. The wings of *flr³/mwh* progeny flies were put in slides and scored for mosaic spots. Results. The larval exposure to NDMA induced infertility in *mwh e* males, from 0.156 mM. In the progeny of males treated with low concentrations of NDMA, the frequency of mutant spots increases ($p < 0.05$), but the progeny from males exposed to higher concentrations and the progeny from control males showed similar frequencies of spots. The transgenerational activity of NDMA suggest that the screening of the effects of genotoxins must extend through several generations in order to improve our knowledge about their real activity. Some part of the genetic damage, which appear early in the lifespan of no exposed organisms, could happen as a consequence of the transgenerational exposure to genotoxins.

P38

Micro-RNA Regulation of Stroke Recovery. Lowings MD, Kolb B, Kovalchuk O. University of Lethbridge, Lethbridge, AB, Canada.

The impact of mRNA suppression, in part controlled by the subclass of small RNAs known as micro-RNA (miRNA), is only beginning to be understood, but it is clear that this process is an important contributor to the exquisite control mechanisms which underlie brain function. Stroke, the clinical manifestation of a disruption of the brain's oxygen supply, is one of the most common neurological lesions. Following the initial infarction, the brain undergoes a huge number of plastic changes in a short time, as the brain re-wires itself to compensate for lost function. Therapeutic intervention, using drugs such as nicotine, or an enriched environment, has been shown to increase the magnitude of behavioural recovery following a stroke. The extent of plastic change in a recovering brain suggests a role for both genetic and epigenetic regulatory mechanisms. By modulating the expression of growth factors, cytoskeletal maintenance factors, neurotransmitters and their receptors, cell cycle controls, and many others, miRNA regulation could play a key role in both the plasticity of existing neurons, and, in those therapies that show possible neurogenesis, control over the growth and differentiation of new neurons. To establish a connection between altered miRNA activity and stroke recovery, we profiled the miRNA expression of both lesioned and non-lesioned tissue in rats subjected to an experimental stroke. Initial microarray analysis reveals significant changes in the expression levels of a number of miRNAs between controls, lesioned tissue, and contralateral non-lesioned tissue, which indicates that miRNA regulation is active both at the site of an infarction as well as in otherwise uninjured areas. These miRNAs have putative binding sites on genes relating to DNA repair, DNA methylation, cell cycle control, neuronal differentiation, and apoptotic regulation, suggesting that alteration of the miRNAome plays a significant role in the brain's natural recovery from stroke. Subsets of these miRNAs show an additional modulation in response to nicotine and EGF-based recovery therapies, which indicates a potential future role for the miRNAome in pharmaceutical stroke therapy. This study will discuss the cellular and physiological repercussions of the observed changes, as well as present a model of miRNA action in stroke and stroke recovery.

P39

Assessing the Role of DNA Methylation in the Susceptibility to Develop Alcohol-Induced Liver Injury. Powell CL¹, Craig CP², Tsuchiya M², Tak WY², Threadgill DW¹, Rusyn I². ¹Department of Genetics, University of North Carolina, Chapel Hill, NC, United States, ²Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC, United States.

Alcohol exposure has been associated with global DNA hypomethylation coupled with regional hypermethylation, histone modification and chromatin remodeling. Putative mechanisms of alcohol-mediated changes in the epigenome include depletion of principal methyl donors and cofactors, and the accumulation of oxidative DNA lesions. Despite considerable insights into the mechanisms leading to alcohol-induced liver injury (ALI), little is known about how the epigenome contributes to alcohol toxicity or whether genetic variation modulates alcohol-associated epigenetic changes. Here, we hypothesize that the heterogeneity in liver injury among alcoholics can be modeled using inbred mouse strains to determine whether ALI is associated with epigenetic changes in specific genes which are dependent on genetic background. Six inbred mouse strains were exposed to alcohol subchronically. Histopathological examination of liver revealed a gradient in liver injury responses to alcohol whereby mouse strains were classified as resistant or sensitive to ALI. Global DNA methylation patterns among naïve strains sensitive to ALI were 20% less methylated compared to their resistant counterparts but became modestly hypermethylated compared to controls following alcohol treatment. If this increase in DNA methylation occurred in gene promoters, it may be a contributing factor in the down-regulation of expression in 502 genes correlated to liver pathology among sensitive strains. This gene set comprised numerous growth factors and cytokines known to be involved in regulating tissue repair. To establish if a causative link exists between DNA methylation and ALI, a sensitive mouse strain (C57BL/6J) was administered a methyl-rich diet prior to and during chronic alcohol administration. Serum ALT, a clinical marker of liver injury, along with average urine alcohol concentrations were reduced by 2-fold compared to mice given unadulterated alcohol diet. These results demonstrate that nutritional modulation of methyl donors leads to an altered phenotype of ALI presenting us with an experimental paradigm by which measures of gene expression and gene-specific methylation profiles can establish whether inherited and/or acquired epigenetic marks determines an individual's susceptibility to ALI.

P40

Cytosine Methylation in CG Repeats Increases Local Genetic Instability. Wang G, Vasquez K. University of Texas MD Anderson Cancer Center, Science Park, Smithville, TX, United States.

DNA methylation has been implicated in a variety of epigenetic processes, and abnormal methylation is the most common DNA modification in cancer cells. Both hyper- and hypomethylation have been associated in some way with chromosome breakage and translocation. The controversial conclusions may be the result of altered intracellular environments caused by global genome-wide methylation which may mask the effect of local methylation status, since methylation of CpG islands in promoters plays important roles in genetic instability by altering the pattern of gene expression. We have previously reported that sequences that are capable of forming a left-handed Z-DNA structure, which are frequently found near chromosomal breakage hotspots, caused large-scale deletions and rearrangements resulting from DNA double-strand breaks (DSBs) in mammalian cells. In this study, we determined the effect of local cytosine methylation in CG repeats on a shuttle vector, leaving the host-cell genomic DNA methylation status unaltered. We found that cytosine methylation facilitated the Z-DNA conformation at supercoiling densities insufficient to convert the un-methylated CG repeat into a Z-DNA structure. In mammalian cells, the methylated CG repeat induced a higher frequency of large deletions and rearrangements than the un-methylated CG repeat of the same length. Further, mutants induced by methylated CG repeats contained larger deletions than the un-methylated repeats. In HeLa cells extracts, methylation of CG repeats also increased the Z-DNA-induced large-scale deletions and rearrangements. Unlike the mutations detected in mammalian cells, we found that CG repeats induced small deletions within the repeat in *E. coli*, and we hypothesized that the lack of cytosine methylation in bacteria might be responsible for this difference. Surprisingly, although *in vitro* methylation also increases the CG repeat-induced mutation frequency in *MCR* deficient bacteria, the mutants are exclusively small deletions. Therefore, we conclude that local cytosine methylation of CG repeats can induce genetic instability by facilitating conformational changes in the DNA structure, resulting in DNA DSBs, large scale deletions and rearrangements that are associated with chromosome breakages found in diseases.

P41

Major Epigenetic Mechanisms of Carcinogenesis Reviewed. Chung K-T. The University of Memphis, Memphis, TN, United States.

Several major hypotheses of carcinogenesis associated with epigenetic factors are reviewed. Warburg proposed that cancer is caused by irreversible damage to the aerobic respiration capacity of normal cells; Pauling suggested that a deficiency of vitamin C was a factor of carcinogenesis; Szent-Györgyi proposed a bioelectric conductivity hypothesis. Deficiency of micronutrients such as folic acid, vitamin B₆, B₁₂, choline, methionine, iron, and zinc are factors that cause cancer. A decrease of cellular nicotinamide adenine dinucleotide (NAD⁺ and DANH) concentration is also suggested to be involved in the evolution of cancer formation. Dysfunctional gap junctional intercellular communication (GJIC) caused by environmental carcinogens was proposed to be a cause of cancer. The alteration in DNA methylation is also considered a major cause of carcinogenesis. Chromosomal abnormalities and viral infections also cause cancer. Each hypothesis, which has survived long scrutiny and many trials led to a valuable study of a particular facet in the complicated processes related to both normal and cancerous cells. It is important to study how these various hypotheses are interrelated. Integration of different hypotheses into an unified mechanism of carcinogenesis is highly desirable.

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miRNA Regulation in Human Bystander Tissue Suggests Common Bystander Regulatory Elements. Zemp FJ¹, Sedelnikova OA², Bonner WM², Kovalchuk O¹. ¹University of Lethbridge, Lethbridge, AB, Canada, ²National Cancer Institute, Bethesda, MD, United States.

Small RNA species in animals are beginning to establish themselves as important regulators of cellular homeostasis and control. In fact, the emergence of 'oncomirs' has given them a notable role in the future of oncology. Thus, as the genomic stability and epigenetic mechanisms underlying bystander effects have the potential to be regulated by small RNAs, it is the goal of this research to investigate the role of small RNAs in radiation-induced bystander effects. Artificial 3D human tissues are important tools in researching bystander effects, as these tissue models preserve the 3D geometric arrangement and communication of cells present in tissues *in vivo*. It has been previously shown that bystander effects exist in human '*in vivo*' models using 3D artificial tissue. These experiments found increased levels of apoptosis, micronuclei formation, and strand breaks in bystander tissue, as well as a global loss of DNA methylation. Here, EpiAirway (Air-100) tissues were microbeam irradiated and bystander tissues harvested at 8 hours and 1, 2, 3, 5 and 7 days post-irradiation, with mock-irradiated controls at 8 hours, 3 days and 7 days. MicroRNA microarray analysis was performed on these samples and the data was confirmed by qRT-PCR. Compared to their respective time matched controls, there were 8, 13, and 3 miRs significantly changed at 8h, 3 days, and 7 days post-irradiation. Group analysis over the entire time course revealed 54 altered miRs. Further study into the expression of the bystander and mock miRs revealed remarkably similar expression patterns. 9 groups (5 distinct and 4 subgroups) harbouring like expression patterns were formed. Detailed, within group, analysis revealed uncanny similarities between the miRs. Firstly, some groups were found in very close proximity on the same chromosome, and syntenous with putative polycistronic miRs in other organisms. Secondly, promoter analysis of the areas surrounding non-intronic miRs revealed putative promoters which have common transcription factor binding sequences. In one instance, 62 of the total 75 transcription factor binding sites between two like-regulated putative promoters was found. Groups with intronic miRs revealed that the genes in which they were located may also be regulated via similar transcription factors. Thirdly, detailed sequence analysis of miRs within the same group found similarities at the 3' and/or 5' ends, suggesting that they may be regulating similar targets. This systemic approach to analyzing our microarray has given us some novel clues to the nature of our previously studied bystander effects.

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Re-Analysis of Dose-Rate Effect After Mammalian Low Let Germ Cells Irradiation; Implication for Human Risk Assessment. Vaglenov A¹, Karadjov A², Fedorenko B³. ¹The University of Findlay, Findlay, OH, United States, ²National Centre of Radiobiology and Radiation Protection, Sofia, Bulgaria, ³Institute for Biomedical Problems, Moscow, United States.

The purpose of the study is to re-examine and evaluate data on dose-rate effects in different mammalian species and their spermatogonial stem cells susceptibility to gamma irradiation. Our results on RT reciprocal translocation yields measured after mouse, rat, Syrian hamster and rabbit spermatogonial irradiation with 1.23, 0.25, 8×10^{-2} , 7×10^{-3} , and 1×10^{-4} Gy/min fit the linear model of the dose-response relationship. Susceptibility was derived by comparing linear regression coefficients from dose-responses for each of the dose-rate tested. Genetic susceptibility to acute photon irradiation appeared to rise in the following species sequence: Macaque mulatta < Callithrix jacchus < Syrian hamster < Chinese hamster < Guinea pig < rabbit < Macaque fascicularis < mouse < man = rat < Sanguinus fuscicollis. The studied mammals clearly show a species-specific susceptibility, and in the case of chronic photon radiation, a change in sequence to: mouse = Macaque fascicularis < Syrian hamster < rat < Macaque mulatta < rabbit. It is concluded that deriving estimates from widely differing marmoset and man data for a very narrow dose range is hardly warranted and leads to overestimation of risk. As a tentative value for risk assessment purposes, we propose 3.41×10^{-4} RT/gamete/cGy, based on the comparable susceptibility of rat and man. The same value, corrected by a factor of 0.5 for decrease in effectiveness, may be used in the case of chronic photon irradiation: this is supported by evidence obtained first in rabbit as well in Macaque mulatta, showing in good agreement a 2-fold decrease of genetic damage only. Reanalysis of published data clearly shows inverse dose-rate effect in mouse with reduction from 1×10^{-4} to 1×10^{-5} Gy/min, the overall dependence of induced RT in spermatogonia are parabolically related to dose-rate, within a minimum in the same range.

P44

Synergistic Effects of Estrogen and Ionizing Radiation on the Epigenetic Processes in the Rat Mammary Gland. Kutanzi K, Koturbash I, Kovalchuk O. University of Lethbridge, Lethbridge, Alberta, Canada.

Currently ionizing radiation (IR) is the only genotoxic agent generally accepted as a breast carcinogen. Average IR exposure doses linked to breast cancer development range widely between 0.02 and 20Gy. Estrogen is another well-known breast carcinogen with both initiating and promoting properties. Women with elevated estrogen levels are considered to be a high-risk group for breast cancer development and would likely be exposed to diagnostic IR procedures on a more frequent basis. Similarly, many women with estrogen-induced breast cancer undergo IR treatment and are exposed to relatively high X-ray doses to the healthy breast. *In vitro* application of both IR and estrogen led to the malignant transformation of normal breast epithelial cells. We have recently shown that estrogen as well as IR exposures applied separately result in profound epigenetic dysregulation in the rat mammary gland. Do those carcinogens act synergistically to promote deleterious epigenetic alterations *in vivo*? The study was aimed to address this important question. We have analyzed the nature and roles of epigenetic changes in the estrogen and IR-induced breast carcinogenesis using a rat model. The six-week-old female rats were randomly assigned to one of the following treatment groups: (i) sham treated controls; (ii) estrogen treated group; (iii) IR treated group; (iv) IR + estrogen treated group. Animals were sacrificed at 4 weeks, 12 weeks, 18 weeks after irradiation. Combined IR and estrogen exposure had cumulative effects on DNA hypomethylation. We also noted a loss of trimethylation of histone H4K20 and H3K9. Furthermore, we have found that estrogen, IR, and combined exposure resulted in significant alterations in the levels of microRNAs as compared to the age matched controls. Amongst microRNAs with altered expression were the known oncogenic microRNAs – miR-17-5p, miR-106b, miR-20a, miR23, miR-181. Experimentally confirmed targets for those miRNAs are: E2F1 and AIB1, RB1, TGFBR2, Notch1 and HES1, and Tc11 oncogene, respectively. We also noted altered expression of miR-199b which targets laminin gamma2 gene, an important component of extracellular matrix. The expression changes of the aforementioned miRNAs were confirmed by qRT-PCR.

P45

Chromodomain Helicase DNA Binding Protein 2 and DNA Damage Response Signalling. Rajagopalan S, Nagarajan P, Venkatachalam S. University of Tennessee, Knoxville, TN, United States.

Changes in chromatin structure are brought about by various factors and these changes are known to play critical role in gene regulation. Alterations in chromatin structure allow various nuclear factors to access certain regions of DNA to carry out important functions such as DNA replication, repair, recombination and transcription. The chromatin modifiers are classified broadly into two classes: histone-modifying enzymes and ATP-dependent chromatin-remodelling factors. Chromodomain helicase DNA binding proteins (CHD) are a group of highly conserved proteins sharing sequence motifs and functional domains and are classified under the group of ATP-dependent chromatin-remodelling factors. CHD2 is one of the poorly characterized CHD proteins. The human gene encoding CHD2 is mapped to chromosome15q26.2. This region has been associated with rare genetic disorders that lead to growth retardation and cardiac defects and early post natal lethality. The presence of a variety of protein domains (e.g. HGM-I and the C-terminal helicase domains) in CHD2 implicates a role for the protein in DNA repair as these domains are also represented in various DNA repair proteins. Based on the known and inferred role(s) of Chd2 we hypothesized that the Chd2 protein is involved in maintaining genomic stability via its involvement in DNA repair processes. In an effort to understand the functional role of the chromodomain helicase DNA binding protein 2 (Chd2) in mammals, we have generated a Chd2 mutant mouse model. The Chd2 protein appears to play a critical role in tumor suppression. Chd2 deficient cells exhibit growth defects indicative of cellular senescence, accumulate higher levels of the chromatin associated DNA damage response mediator, γ H2AX, and exhibit an aberrant DNA damage response after X-ray irradiation. Experiments have been initiated to identify interacting protein partners of Chd2. Additional experiments to determine the role of Chd2 in DNA damage responses are also underway.

P46

Evidence for Promoter Methylation Effects on Proximal Splicing of MLH1 mRNA in Colon Cancer Cell Lines. Tiedtke E¹, Sedwick DW², Skandalis A¹. ¹Brock University, St. Catharines, ON, Canada, ²Case Western Reserve University, Cleveland, OH, United States.

Disruption of the repair gene human MutL homologue 1 (hMLH1) has been linked to colorectal cancer. Epigenetic silencing via promoter hypermethylation in the absence of genetic mutations is one route to hMLH1 inactivation. Partial demethylation by treatment with 5-aza-2'-deoxycytidine (azadC) restores hMLH1 function in hypermethylated colon cancer cell lines. Although hMLH1 is alternatively spliced and alternative splicing mediates cellular information flow from DNA to protein, the extent to which the degree of promoter methylation influences splicing remains unknown. To investigate whether promoter methylation status alters splicing, the identity and frequency of hMLH1 splice variants was determined in 4 related sporadic non-polyposis colon cancer cell lines that differ in their degree of promoter methylation. These include the parental cell line Vaco432-azadC treated and 3 cell lines established from stably demethylated clones of V432-azadC for which the methylation status is known. As controls, we screened the unrelated colon cancer cell line RKO-azadC treated and the non-cancer cell line MRC5 in which hMLH1 is expressed. Results suggest that different factors may account for the alternative splicing events observed in the 5' proximal and distal halves of hMLH1 RNA in the cell lines examined. Proximally, high methylation is associated with low alternative splicing of exons 10 and 11 that belong to the MutS interaction domain, a region critical for the repair function of hMLH1. In contrast, alternative splicing of exons 16 and 17 that fall in the distal half of the gene vary in a methylation independent manner. The results suggest a potential role for promoter methylation beyond gene inactivation and raise the possibility that promoter methylation may subtly influence cellular phenotype long before the degree of hypermethylation that results in gene silencing is achieved and after azadC treatment has restored hMLH1 expression.

Mutagenesis, Carcinogenesis

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Genetic Dissection of Susceptibility/Resistance to Ionizing Radiation by Use of Recombinant Congenic Strain Mice. Vaglenov A¹, Kaltenboeck B², Brawner W², Carpenter D², Fortin A³, Brandhorst H², Yihang L². ¹The University of Findlay, Findlay, OH, United States, ²Auburn University, Auburn, AL, United States, ³Emerillon Therapeutics, Inc., Montreal, QC, Canada.

The research in this study aims to identify and map mouse quantitative trait loci (QTL) associated with susceptibility/resistance to X-ray exposure in recombinant congenic mouse strains (RCS) derived from A/J and C57BL/6J parental mice. This study used 9 AcB and 16 BcA strains to analyze and identify the murine quantitative trait loci (QTLs) that influence susceptibility/resistance to ionizing radiation. At 8-12 weeks of age, 404 parental A/J and C57BL/6J controls, and 390 RCS mice were used. Parental strains were irradiated in the dose range from 10 to 60 cGy to evaluate the optimal time point for phenotyping. Additionally both strains were compared at dose-rate 250 versus 10.3 cGy/min. Adaptive response, as well as diet modulations in the conditions of different dose-rate were also studied. All experiments were done with 6 MeV electron beams in Siemens Megatron 6740 Linear Accelerator. Three sets of RCS animals were sacrificed 24 h post irradiation (0, 20 and 50 cGy) and bone marrow was processed for analyze phenotype by quantifying chromosomal damages in polychromatic erythrocytes (PCE). Coded slides were scored for the incidence of micro-nucleated PCE at 1000x magnification under immersion. Approximately, 2000 PCE were scored per animal. Each group of parental or RCS (control or irradiated) contain no less than 5 mice. The optimal phenotyping time and dose are 24h after irradiation, as well as 50cGy. Linkage maps constructed with 616 informative microsatellite markers were used to identify chromosomal regions associated with susceptibility/resistance to ionizing radiation. These analyses identified first collections of chromosomal markers that best correlated with the respective outcome. Significant ($p < 0.0001$) regions were identified on chromosomes 2, 4, 6, 8, 13, 14, 19. Multiple regression analysis demonstrated that a subset of eight markers, including D2Mit401, D4Mit81, D4Mit31, D6Mit340, D8Mit131, D13Mit293, D14Mit133 and D19Mit59, accounted for 90% of the genetic variance. Three sets of data – control, after 20, as well as 50 cGy suppose strong correlation between spontaneous and induced answers to ionizing radiation. Results from 25 RCS suggest complex genetic control of susceptibility/resistance to ionizing radiation.

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Accumulation of K-Ras Mutation in Rat Colon After Azoxymethane Exposure. McKinzie PB¹, Delongchamp RR², Parsons BL¹. ¹Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR, United States, ²Division of Personalized and Nutritional Medicine, National Center for Toxicological Research, Jefferson, AR, United States.

Quantitative data describing the accumulation of oncogene and/or tumour suppressor gene mutations during carcinogenesis would aid our understanding of chemical carcinogenesis and validate the measurement of these mutations as biomarkers of cancer risk. K-Ras codon 12 GGT to GAT mutation is an oncogenic mutation frequently observed in colon tumors. Therefore, Allele-specific Competitive Blocker PCR (ACB-PCR), a method that can quantify mutant fractions (MFs) in the range of 10^{-1} to 10^{-5} , was used to study the accumulation of K-Ras codon 12 GGT to GAT mutation during azoxymethane (AOM)-induced colon carcinogenesis. At 6 weeks of age, rats were treated with a carcinogenic dose of AOM (15 mg/kg, administered in 2 weekly doses) or with the phosphate-buffered saline vehicle control. Colon tissue was harvested at 1, 8, 24, and 32 weeks post-treatment. Genomic DNA was isolated from colon tissue and used to synthesize a K-Ras PCR product encompassing codon 12. Pure mutant and wild-type K-Ras sequences were mixed to produce MF standards. ACB-PCR was then used to analyze the MF standards, negative controls, and the K-Ras PCR products in parallel, using four replicate assays. A standard curve was constructed and used to calculate the K-Ras MF in the PCR products generated from colon DNA. The mean K-Ras GAT MF in colonic DNA from control rats was 2.7×10^{-5} , 1.7×10^{-5} , 3.9×10^{-5} , and 4.4×10^{-4} after 1, 8, 24, and 32 weeks, respectively. The mean K-Ras GAT MF in colonic DNA from treated rats was 8.8×10^{-5} , 1.4×10^{-4} , 2.3×10^{-4} , and 1.3×10^{-3} , after 1, 8, 24, and 32 weeks, respectively. Overall, significant effects related to both treatment and time after exposure were observed. A statistically significant increase in K-Ras codon 12 MF was observed in treated versus control rat colon at 1, 8, and 32 weeks after dosing. These data show K-Ras codon 12 GGT to GAT mutations occur early in colon carcinogenesis and are measurable as early as 1 week after treatment. These data support ACB-PCR measurement of K-Ras mutation as a useful, early biomarker of carcinogenesis. This approach may advance the development of mathematical models of colon carcinogenesis and inform species extrapolation, given that data is being collected in a parallel study of human colon.

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Genes Involved in Inflammatory Responses Following Exposure to Cigarette Smoke in Normal Human Bronchial Epithelial (NHBE) Cells. Parsanejad R¹, Fields W^{1,2}, Steichen T², Bombick B², Doolittle D^{1,2}. ¹Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC, United States, ²Research and Development, R.J. Reynolds Tobacco Company, Winston-Salem, NC, United States.

Bronchial epithelium is frequently exposed to air pollutants. Hence, it has been hypothesized that these cells elicit inflammatory responses as the first element in pulmonary defense. Our laboratory previously reported that whole cigarette smoke induced genes involved in the cascade of inflammatory responses in NHBE cells. The purpose of this study is to evaluate changes in mRNA levels of 84 genes representing cytokines, including chemokines and interleukins, and the respective receptors over a repetitive exposure time-course to further define the inflammatory responses associated with cigarette smoke exposure in an *in vitro* lung model. NHBE cells were treated with cigarette smoke condensate (CSC) prepared from Kentucky 2R4F cigarettes (60 µg TPM/ml media, 0.2% DMSO). The transcripts were examined by real-time quantitative RT/PCR. The time course for applications of CSC was designed in 7 groups to test immediate (A & B) and early (C) responses following 0.25, 0.5, and 1 hr continuous exposures; intermediate (D, E & F) responses assessed at 4, 8, and 12 hrs following a 1 hr exposure/3 hr recovery period repeated 1 – 3 times, respectively; and late (G) response evaluated at 24 hrs and assessed with four alternating exposure/recovery periods with a final 12 hr recovery. Groups D to G exhibited three predominant gene expression responses: adaptive (return to baseline), sustained (maintained expression during treatment) and chronic (maintained expression post-treatment). Overall, 25 genes exhibited statistically significant changes: 14 genes exclusively elevated, 10 genes exclusively depressed and only IL8 exhibiting both up and down regulation in the 7 groups. Group G was the most responsive group showing modulation of expression for 18 genes. TNF was the most commonly regulated gene with significant down regulation in 5 groups (C to G). The most responsive genes were SPP1, with a 34-fold up regulation, and CXCL14, with a 23-fold down regulation, respectively. The pattern of expressed genes indicates that NHBE cells respond to CSC treatment in a time-specific manner. Our observation suggests that specific genes involved in inflammatory pathways respond to the CSC in chronic, sustained or adaptive patterns with the chronic pattern as the predominant behavior.

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Comparative Investigations on the Genotoxicity Profile of Glutaraldehyde and Formaldehyde – What Makes the Difference? Speit G, Neuss S, Schuetz P, Schmid O. Ulm University, Ulm, Germany.

Induction of DNA-protein crosslinks (DPX) has been related to mutagenicity and carcinogenicity. Glutaraldehyde (GA) and formaldehyde (FA) are efficient inducers of DPX but differences have been reported with regard to induction of other genotoxic effects, mutations and cancer. We, therefore, characterized the genotoxic and mutagenic potential of GA and FA in V79 cells. Using a modification of the alkaline comet assay we measured a concentration-related induction of DPX by both chemicals. However, the crosslinking effect of GA occurred at much lower concentrations and in a narrow range of concentrations. Removal of DPX was less efficient for GA-induced DPX than for FA-induced DPX. Induction of sister chromatid exchanges (SCE) and micronuclei (MN) already occurred at lower concentrations than the DPX-inducing effect in the case of GA, whereas SCE and MN were induced by FA only at much higher concentrations than DPX. The frequency of induced SCE and MN was higher after treatment of V79 cells with FA than after treatment with GA. Our results clearly indicate that both, GA and FA, induce DPX, SCE and MN *in vitro* in a concentration-related manner. However, characteristic differences exist with regard to the relative efficiency to induce these three endpoints. It will be discussed whether these differences can be explained by the types of DPX induced and / or differences in the cytotoxic activity of the compounds and how these differences may be related to carcinogenicity.

P51

8OG Can Lead to Ras Activation in a Mammalian Model of Transcriptional Mutagenesis. Saxowsky TT, Doetsch PW. Emory University School of Medicine, Atlanta, GA, United States.

Transcription coupled repair (TCR) acts as a safeguard against some types of DNA damage, contingent upon the ability of these lesions (often bulky) to arrest the elongating RNA polymerase. However, some of the most frequently occurring spontaneous types of damage, such as 8-oxoguanine (8OG), are efficiently bypassed by RNA polymerase *in vitro*, potentially leading to mutant transcripts by directing the incorporation of an incorrect nucleotide opposite the site of the DNA lesion. This process, called transcriptional mutagenesis (TM), could then lead to a pool of mutant proteins resulting in deleterious biological effects. We have developed a mammalian model system for analyzing TM in mouse embryonic fibroblasts either wild type in their DNA repair capacity or deficient in 8OG glycosylase (ogg) and the Cockayne Syndrome B protein (csb), enzymes important in the removal of 8OG from the genome. This system utilizes expression of the Ras oncogene from constructs in which an 8OG has been engineered into codon 61. Repair of this lesion will restore the wild type coding sequence, however bypass of this lesion during transcription (and the potential misinsertion of A opposite the 8OG) will lead to a constitutively active mutant Ras protein. Expression of this mutant Ras leads to increased activation of downstream signaling events promoting cell cycle progression, including increased phosphorylation of ERK kinase, which is monitored as the assay endpoint. Upon transfection with 8OG constructs, we observe an increase in phospho-ERK in both wild type and repair-deficient cells at the earliest time point (2h post-transfection). By 6h, this effect is absent in wild type cells, indicating that repair of the lesion has taken place. However elevated phospho-ERK is more pronounced at 6h in ogg^{-/-}csb^{-/-} cells, suggesting persistence of the lesion and continued TM. Further studies should provide valuable information regarding the involvement of 8OG and other frequently occurring spontaneous DNA lesions on replication-independent mutagenic processes and may reveal novel mechanisms contributing to events in non-dividing, growth-challenged cells that could potentially lead to escape from growth restraints during tumorigenesis.

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Comparative Toxicogenomics of Ah Receptor Mediated Hepatotoxicity in C57BL/6 Mice: Evaluation of the TCDF Toxic Equivalency Factor. N'jai AU^{1,2}, Boverhof DR^{1,2}, Burgoon LD^{1,2}, Dere E^{1,2}, Tan YS^{1,2}, Rowlands JC³, Budinsky RA³, Stebbins KE³, Zacharewski TR^{1,2}. ¹Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, United States, ²National Food Safety & Toxicology, Center for Integrative Toxicology, Michigan State University, East Lansing, MI, United States, ³The Dow Chemical Company, Midland, MI, United States.

Temporal microarray analyses were performed on hepatic tissue from orally gavaged immature female C57BL/6 mice in order to evaluate similarities and difference in gene expression profiles between 2,3,7,8-TCDD (TCDD) and 2,3,7,8-TCDF (TCDF). Time course studies out to 168 h were conducted with 300 µg/kg TCDF, 30µg/kg TCDD or sesame oil. Gene expression was monitored using cDNA microarrays containing 13,361 features, representing 8,194 genes. Empirical Bayes analysis identified 195 differentially expressed genes following treatment with TCDF. Comparison of TCDF and TCDD differential gene expression identified 116 genes which exhibited common responses; 109 common responses exhibited comparable profiles (correlation coefficients > 0.3). In general, TCDF was less potent when compared to TCDD at later time points, with maximal efficacy occurring primarily at 12 h. QRTPCR verification of Cyp1a1 and Nqo1 induction were consistent with differences in efficacy observed in microarray results. TCDF elicited hepatocytes cytoplasmic vacuolization consistent with lipid accumulation at 24 h, was more severe at 72 h, but significantly subsided by 120 and 168 h compared to TCDD. Hepatic TCDF levels were significantly lower than TCDD after 24 h, with maximal levels attained at 12 h. This was followed by a gradual decrease between 18 and 168 h, suggesting pharmacokinetic differences may account for the lower accumulation of hepatic lipids and gene expression activity elicited by TCDF at later time points. The declining TCDF responses observed after 24 h relative to TCDD, due to metabolism and/or excretion, suggests that TCDF's TEF of 0.1 may overestimate its hepatic toxicity. Funds provided by The DOW Chemical Company.

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Frequency of Micronucleated Erythrocytes in the Peripheral Blood of Juvenile Male Rhesus Monkeys, *Macaca mulatta*, Determined by Flow Cytometry. Myers M¹, Bishop M¹, Hotchkiss C³, Lin C-J¹, Chen J¹, Mattison D², Morris S¹. ¹National Center for Toxicological Research/FDA/HHS, Jefferson, AR, United States, ²National Institute of Child Health and Development/NIH/HHS, Bethesda, MD, United States, ³The Bionetics Corporation, Jefferson, AR, United States.

The *in vivo* micronucleus assay has become a widely used tool in evaluating the genotoxic potential of chemical compounds. Until recently, peripheral blood measurements of micronucleated reticulocytes (MN-RETs) were limited due to selective removal by the spleen in non-mouse species. Using the MicroFlow[®] method developed by Litron Laboratories, we have investigated the use of peripheral blood erythrocytes from the nonhuman primate rhesus monkey, *Macaca mulatta*, as a model system for genotoxic damage. We determined the normal background or "spontaneous" micronucleus frequencies in the peripheral erythrocytes from juvenile male rhesus monkeys with an age range of 1½ to 2 years of age. Briefly, peripheral blood samples from a cohort of 30 male rhesus monkeys were prepared for MN-RET scoring according to the manufacturer's protocol. In this assay, newly formed reticulocytes (RETs) are differentiated from mature normochromatic erythrocytes (NCEs) with anti-CD71-FITC (transferrin receptor). Further, populations of MN-RETs and MN-NCEs can be distinguished from RETs and NCEs by staining with propidium iodide (with RNase treatment). Prior to the analysis of the samples, daily calibration of the flow cytometer was conducted using malaria-infected erythrocytes as a biological standard. The average background or "spontaneous" micronucleus frequencies in the mature and most immature fraction of MN-RETs were 0.002% ± 0.09 [95% confidence interval (CI), 0.0018-0.0028] and 0.21% ± 0.001 [95% CI, 0.1745-0.2448], respectively. Quantification of the background frequency of micronuclei in the peripheral erythrocytes of juvenile *Macaca mulatta* provides a valuable, non-invasive tool for studies of genotoxicity in nonhuman primates. *Funded by NICHD for the Best Pharmaceuticals for Children Act of 2002. The views presented in this abstract do not necessarily reflect those of the Food and Drug Administration.*

P54

Mechanistic Investigation of a Test Article-Induced Increase in Mitotic Index in the HPBL Chromosome Aberration Assay. Mahadevan B, Nioi P, Mack M, Martin B, Sullivan E, Snyder R. Schering-Plough Research Institute, Summit, NJ, United States.

As part of a routine genotoxic screen of a test article (TA) for chromosomal aberrations using human peripheral blood lymphocytes (HPBL), a substantive dose-responsive increase in the mitotic index (MI) was observed. This was considered to be a consequence of either a proliferative response or a slowing or blocking of cell cycling. Flow cytometric analysis of cells treated with the TA indicated no change in cell number suggesting no effect on cell proliferation. Upon exclusion of phytohemagglutinin-M (PHA-M) the TA appeared to have no capacity to increase mitotic index arguing against a proliferative response. When PHA-M was included but Colcemid[®] was excluded, the TA, again, did not elevate MI. The TA exhibited a similar increased MI in the presence of another spindle fiber inhibitor, vinblastine. This suggested that the TA increased MI via a mechanism dependent on spindle fiber disruption. However, observations of the spindle assembly in TA-treated or untreated cells failed to demonstrate any obvious effects. Cell cycling was further evaluated by assessing the incorporation of 5-bromo-2'-deoxyuridine (BrdU) levels in HPBL using varying doses of the TA under several different conditions. Cells that incorporated with BrdU in the presence of Colcemid[®] were stained and scored manually and also analyzed by flow cytometry. The results from all of these experimental studies, suggest that the TA was acting via cell cycle interference rather than by enhancing proliferation.

P55

Mutagenicity and Gene Expression Changes Induced by Comfrey in Rat Liver. Mei N, Guo L, Fuscoe JC, Chen T. National Center for Toxicological Research, Jefferson, AR, United States.

Background: Comfrey is consumed by humans as a vegetable and a tea, and has been used as an herbal medicine for more than 2000 years. However, comfrey is hepatotoxic in livestock and humans, and carcinogenic in experimental animals. This concern led the USFDA to request voluntary removal of products containing comfrey from the market in 2001. Our previous study suggested that comfrey induces liver tumors by a genotoxic mechanism and that the pyrrolizidine alkaloids in the plant are responsible for mutation induction and tumor initiation in rat liver. Results: In this study, we evaluated the mutagenicity of comfrey and identified comfrey-induced gene expression profile in the livers of rats. Groups of 6 male transgenic Big Blue rats were fed a basal diet or a diet containing 8% comfrey roots, a dose that resulted in liver tumors in a previous carcinogenicity bioassay. The animals were treated for 12 weeks and sacrificed one day after the final treatment. Rats fed with comfrey displayed little weight gain after 6 weeks of feeding with comfrey. The mutant frequency in the liver *cII* gene for rats fed with comfrey was about 4-fold higher than that for the control group. G:C → T:A transversion was the major type of mutation in comfrey-fed rats, and 13% of tandem base substitutions was also observed. We also used a rat microarray containing 26,857 genes to perform genome-wide gene expression studies. Dietary comfrey resulted in marked changes in liver gene expression. When a two-fold cut off value and a *P*-value less than 0.01 were selected, 2,726 genes were identified as differentially expressed in comfrey-fed rats compared to control animals. Among these genes, there were 1,617 genes associated by Ingenuity Pathway Analysis with particular functions, and the differentially expressed genes in comfrey-fed rat livers were involved in metabolism, injury of liver endothelial cells, and liver abnormalities including liver fibrosis and cancer development. Conclusion: The gene expression profile provides us a better understanding of underlying mechanisms for comfrey-induced hepatic toxicity. Integration of gene expression changes with known pathological changes can be used to formulate a mechanistic scheme for comfrey-induced liver toxicity, mutagenicity, and tumorigenesis.

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Development of a Method for Measuring Pig-a Gene Mutants in Rat Lymphocytes Using Selection with Proaerolysin. Miura D^{1,2}, Dobrovolsky V¹, Mei N¹, Kasahara Y², Katsuura Y², Heflich R¹. ¹Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR, United States, ²TEIJIN Pharma Limited, Tokyo, Japan.

The phosphatidylinositol glycan complementation group A gene (*Pig-a*), which codes for a protein involved in the formation of the glycosylphosphatidyl inositol (GPI) anchor in cell membranes, has several characteristics that recommend it as a possible reporter of *in vivo* somatic mutation. For instance, it is located on the X-chromosome so that single mutations can result in an altered phenotype. Also, reagents exist that potentially can be used to quantify *Pig-a* mutants by both cell culture and by flow cytometry. In this study, we developed a method for the selection of *Pig-a* mutant rat lymphocytes. Proaerolysin (ProAER), which is converted at the surface of most cells to aerolysin (AER), was used as the selection agent. AER binds to the GPI anchors of membrane proteins, and forms irreversible pores in the plasma membrane, causing cell death. *Pig-a* mutant cells do not bind AER and are not affected by the toxicity of the selection agent. T-lymphocytes were isolated from rat spleens, primed with concanavalin-A, and various numbers of cells were seeded into 96-well plates and cultured for 10-12 days. The lymphocytes were either exposed to a 1 to 2-hour pulse of 0.01 to 2 nM ProAER before seeding, or were continuously treated with these concentrations of ProAER for the duration of culture. Periodically during the incubation, morphological changes in the cultured cells were monitored by microscopy. Our observations indicated that pulse treatments with ProAER had little effect on cell viability. With continuous treatments, ProAER was highly cytotoxic at concentrations ≥ 1 nM. At 1 nM, almost all cells were killed (presumably wild-type for *Pig-a*), but several growing lymphocyte clones were observed (potential *Pig-a* mutants). Using continuous selection with 1 nM ProAER, the frequency of *Pig-a* variants in untreated rats was 0.9 to 3.2×10^{-6} . By comparison, the frequency of *Hprt* mutant lymphocytes in these rats was 3.0 to 5.8-fold higher than the frequency of *Pig-a* variants. Confirmation of mutations in the *Pig-a* gene of ProAER-selected clones currently is being carried out through DNA sequencing. Our results indicate that ProAER selection may be a useful method for measuring *Pig-a* mutant frequencies in rat lymphocytes. The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

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ACB-PCR Measurement of K-Ras Codon 12 Mutation in A/J Mouse Lung Exposed to Benzo[a]pyrene: A Dose-Response Assessment. Meng F¹, Knapp G², Green T², Ross J², Parsons B¹. ¹National Center for Toxicological Research, Jefferson, AR, United States, ²Environmental Protection Agency, Research Triangle Park, NC, United States.

Benzo[a]pyrene (B[a]P) is a known human carcinogen and environmental contaminant. The direct measurement of K-Ras mutant fraction (MF) was developed as a metric with which to examine the default assumption of low dose linearity in the mutational response to B[a]P. The specific aims of this study were to determine the background level and inter-animal variability in K-Ras codon 12 TGT mutation in A/J mouse lung tissue, as well as the levels of mutation induced by exposure to B[a]P. Male A/J mice (n=10 mice, 7-9 weeks of age) received a single *i.p.* injection of 0, 0.05, 0.5, 5, or 50 mg/kg B[a]P. The mice were sacrificed 28 days after treatment and DNA isolated from a single lung of each mouse was used in a high-fidelity PCR to amplify K-Ras DNA sequence. The level of codon 12 TGT mutation in each PCR product was then determined by three replicate allele-specific competitive blocker-PCR measurements. This mutational endpoint was chosen because K-Ras codon 12 TGT mutation is frequently found in A/J mouse lung tumors induced by B[a]P. The A/J mouse lung contained relatively high spontaneous levels of K-Ras mutations. The lung tissue from control animals had a geometric mean MF of 3.88×10^{-4} , meaning on average 1 in every ~1,288 lung cells carries the mutation. The inter-animal variability in K-Ras TGT geometric mean MF was large, with the control animals having MFs ranging from 3.50×10^{-5} to 3.24×10^{-3} . The geometric mean MFs for the B[a]P treatment groups were: 3.56 $\times 10^{-4}$, 0.05 mg/kg; 6.19 $\times 10^{-4}$, 0.5 mg/kg; 2.02 $\times 10^{-3}$, 5 mg/kg; and 3.50 $\times 10^{-3}$, 50 mg/kg. Although statistically significant differences between dose groups were observed (ANOVA, P < 0.001, with Holm-Sidak method for multiple comparisons), only the 5 and 50 mg/kg dose groups were significantly higher than controls. While the data demonstrate that the K-Ras codon 12 TGT mutational response to B[a]P occurs at early times after treatment, larger treatment groups will be needed to determine the shape of the K-Ras MF dose-response at low B[a]P doses. This abstract does not necessarily reflect EPA policy.

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Temporal Hepatic Expression Profiles Induced by o,p'-DDT in the Rat. Kiyosawa N^{1,2}, Kwekel JC¹, Burgoon LD^{1,3}, Zacharewski TR^{1,3}. ¹Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, United States, ²Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd., Fukuroi, Shizuoka, Japan, ³Michigan State University, Toxicogenomic Informatics and Solutions, LLC, East Lansing, MI, United States.

Introduction: Dichlorodiphenyltrichloroethane (DDT) is an important insecticide in many countries especially for malaria vector control because of its efficacy, large margin of safety and low production cost. However, it is a persistent organic pollutant with the o, p'- enantiomer exhibiting xenoestrogen activities. Additionally, DDT is reported to cause hepatic tumors in rodents and is listed as a potential liver carcinogen in humans based on epidemiologic studies although its mechanisms of carcinogenicity have not yet been clarified. To gain insight into its potential health risks, the temporal and dose response expression patterns induced by o,p'-DDT were investigated in the rat liver. **Methods:** Immature, ovariectomized female rats were treated with 300 mg/kg DDT either once or once daily for three consecutive days by oral gavage. Liver samples were removed 2, 4, 8, 12, 18 or 24 hr after the single dose or at 72 hr after the initial dose. Total RNA was extracted from liver samples and was subjected to QRT-PCR and microarray analysis, using cDNA arrays consisting of 8500+ probes. **Results:** The induction of a number of drug metabolizing enzyme genes (Cyp2b2, Cyp3a2), cell proliferation-related genes (Ccnd1, Ccnb1, Ccnb2, Stmn1) and oxidative stress-related genes (Gclm, HO-1) were observed. Although the Cyp2b2 gene was strongly induced in a sustained manner across all time points, cell proliferation-related and oxidative stress-related genes were more transient (8 h to 24 h) returning to control levels by 72 h. **Discussion:** Strong induction of Cyp2b2 and moderate elevation in Cyp3a2 indicate the activation of nuclear receptors CAR and PXR, consistent with the gene expression changes associated with oxidative stress. Very few genes known to be regulated by the estrogen receptor (ER) were differentially expressed. These data suggest that DDT carcinogenicity observed in rodent livers is mediated by CAR activation rather than ER signaling. Since CAR regulation exhibits known species-specific differences between rodents and humans, the extrapolation of DDT carcinogenicity in rodent livers to humans warrants further investigation.

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EU Cosmetics Association's (COLIPA) Project on Development of Genotoxicity Testing in 3D Human Skin Models. Aardema MJ¹, Reisinger K², Ouedraogo G³, Curren R⁴, Krul CAM⁵, Corve R⁶, Pfuhrer S⁷. ¹The Procter & Gamble Co, Cincinnati, OH, United States, ²Phenion GmbH and Co KG, Duesseldorf, Germany, ³L'Oreal, Aulnay sous Bois, France, ⁴Institute for Invitro Sciences, Gaithersburg, MA, United States, ⁵TNO, Zeist, Netherlands, ⁶ECVAM, Ispra, Italy, ⁷Wella/Procter & Gamble, Fribourg, Switzerland.

According to the EU 7th Amendment, *in vivo* genotoxicity testing will be banned for cosmetics starting in 2009. This creates a problem since *in vivo* genotoxicity assays are routinely used to address the biological relevance of positive results obtained in the standard battery of *in vitro* genotoxicity assays and *invitro* genotoxicity assays induce a high percentage of false positive results for non-carcinogens. To address this, the EU Cosmetics Association's (COLIPA) SCAAT Project Team Genotoxicity Project Team, which consists of member companies of the Cosmetic industry, has started a program to develop approaches for eliminating/reducing animal testing for genotoxicity. One of the projects is aimed at establishing and validating new methods for genotoxicity testing in reconstructed human 3D skin models. A global, multi-lab, multi-endpoint (micronuclei and Comet), multi-tissue evaluation has been initiated. In this project, micronuclei and Comet endpoints are being assessed in 3D reconstructed human skin models including EpiDermTM (MatTek), Phenion (Henkel), and EpiSkin Full thickness (L'Oreal). Partners in this project include ECVAM (European Centre for the Validation of Alternative Methods) and the UK National Center of the 3Rs (NC3Rs).

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The Relative Ability of Cannabis and Tobacco Smoke to Induce Chromosomal Damage in Murine Pulmonary Cells. Maertens RM¹, White PA¹, Rickert W², Levasseur G³, Dougals GR¹, Desjardins S³. ¹Safe Environments Programme, Health Canada, Ottawa, ON, Canada, ²Labstat International Inc., Kitchener, ON, Canada, ³Drug Strategy and Controlled Substances Programme, Health Canada, Ottawa, ON, Canada.

The prevalence of cannabis smoking is increasing among Canadian youth, and often it is perceived that cannabis smoke is less harmful than that of tobacco. However, research indicates that cannabis smoke condensate contains qualitatively the same carcinogenic chemicals as tobacco smoke. Furthermore, cannabis smoke has been shown to be more cytotoxic and mutagenic than tobacco smoke, and it is associated with various adverse pulmonary effects including chronic bronchitis, edema and mucus hypersecretion. Despite these findings, epidemiological studies have failed to establish a correlation between cannabis smoking and the development of respiratory cancers. Currently, the risks of adverse effects from cannabis smoke, as compared to tobacco smoke, are not well understood. This study examined the relative ability of cannabis and Canadian flue-cured tobacco smoke condensates to induce cytogenetic damage, measured as micronuclei, in a murine lung epithelial cell line (FE1 cells). Condensates of main- and side-stream smoke from hand-rolled cannabis and tobacco cigarettes were prepared using standard (i.e., ISO) smoking conditions, as well as "extreme" conditions designed to reflect cannabis smoking habits. Pulmonary cells were exposed to the smoke condensates for a four hour period, followed by a 28 hour growth period in the presence of cytochalasin B. Two thousand binucleated cells were scored from each treatment for the presence of micronuclei. Preliminary results indicate that cannabis samples were more cytotoxic and cytostatic than tobacco samples, as demonstrated by the lower cell proliferation indices. However, at the concentrations tested, no significant increases in micronuclei were observed in cells exposed to any of the cannabis condensates. In contrast, increases in micronuclei were observed in cells exposed to mainstream tobacco condensates smoked under both the standard and extreme conditions, and both with and without the addition of exogenous a metabolic activation mixture (i.e., rat liver S9). Although the statistical analyses are still underway, the preliminary results indicate that tobacco and cannabis smoke differ substantially in their ability to induce chromosomal damage. Future research will use DNA microarray technology to investigate the toxicological pathways induced by exposures to cannabis and tobacco smoke condensates.

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Variations in Gene Expression Copy Numbers and Enzyme Activities of Cytochrome P450 1B1 *CYP1B1* and NAD(P)H:Quinone Oxidoreductase *NQO1* Determine the Level of Benzo(A)Pyrene-DNA Adduct Formation in Mammary Epithelial Cells. Shockley M¹, Divi R¹, Channa K², Poirier M¹. ¹National Cancer Institute, NIH, Bethesda, MD, United States, ²EPA, Research Triangle Park, NC, United States.

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic in rodents, and because PAH-DNA adducts are detectable in human mammary tissue, they may contribute to breast cancer risk in humans. PAHs, including benzo[a]pyrene (BP), are activated to DNA binding species by Phase I enzymes and are detoxified by Phase II enzymes. Preliminary studies revealed up-regulation of *CYP1A1*, *1B1* and *NQO1* expression in normal human mammary epithelial cells (NHMECs) exposed to BP. To elucidate the formation of BP-DNA adducts and understand the underlying metabolism, 15 NHMEC strains and MCF-7 cells were exposed to 4 µM BP for 12h, assayed for BP-DNA adducts by chemiluminescence immunoassay, and assayed for *CYP1B1* and *NQO1* gene expression abundance (transcripts/ng RNA [tpn]) by quantitative real-time PCR (qRT-PCR). In addition, in cells exposed to 4 µM BP for 24h, *CYP1A1* and *1B1* enzyme activities (EROD assay), and dicumolol sensitive *NQO1* activity were measured. BP-DNA adduct levels, expressed as adducts/10⁸ nucleotides, were 0.2-10.6 in NHMECs and 790 in MCF-7 cells exposed to BP for 12h. In unexposed cells, *CYP1B1* gene expression (tpn) was between 569 and 3,452 in NHMECs and was 12,897 in MCF-7 cells. After 12h of BP exposure, *CYP1B1* expression increased to 4,063 - 26,777 in NHMECs and to 36,879 in MCF-7 cells. In unexposed cells, *NQO1* gene expression was between 9,004 and 22,530 in NHMECs, and was 4,962 in MCF-7 cells. After 12h of BP exposure, *NQO1* expression increased to 9,772 - 30,999 in NHMECs, and to 13,579 in MCF-7 cells. In MCF-7 cells, the ratio of *NQO1* to *CYP1B1* was very low (0.4) before and after BP exposure, and in NHMECs the same ratio was significantly higher (9.1 before BP and 1.9 after BP exposure). In unexposed MCF-7 cells, EROD activity was 4 fold higher than that found in NHMEC (strain M98016). The EROD activity increased in BP-exposed NHMECs and MCF-7 cells by 1.6-fold and 5-fold, respectively. Though BP-exposure induced *NQO1* expression in both the cell types, *NQO1* activity decreased by 45% and 13% in MCF-7 cells and NHMECs, respectively. Therefore, the high BP-DNA adduct level observed in MCF-7 cells appears to be driven by high levels of *CYP1B1* expression and enzyme activity and low levels of *NQO1* enzyme activity. Overall, these results suggest that normal human breast cells are protected from BP-DNA damage, and possibly mutagenesis, by their high capacity for detoxification and comparatively low levels of activation.

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Detection of Mutant Tumor Cancer Biomarkers by BEAMing. Karakas B¹, Elespuru RK¹, Weaver J², Diehl F³, Park BH³. ¹FDA/Center for Devices & Radiological Health, Silver Spring, MD, United States, ²FDA/Center for Drugs, Silver Spring, MD, United States, ³Johns Hopkins University Sidney Kimmel Cancer Center, Baltimore, MD, United States.

Cancer biomarkers are important for both the diagnosis of cancer and in monitoring therapeutic outcomes. However, biomarkers are often limited by sensitivity of detection in a practical biological matrix, e.g. blood. Some oncogenes (e.g., *KRAS*, *PIK3CA*) and tumor suppressor genes (e.g. *P53*, *APC*) are highly mutated in tumor tissues and cancer cells. It is known that both tumor cells and mutant tumor DNA are found in the blood of most cancer patients, depending upon tumor type and stage. Thus mutant DNA may function as biomarkers for select tumors. Standard, optimized PCR is capable of detecting approximately one mutant codon 12 *KRAS* oncogene mutation among 100 normal copies in the blood of pancreatic cancer patients, a level that may be too low for therapeutic applications. We have been optimizing a new magnetic beads/emulsion PCR technique (BEAMing) and applying it to the detection of mutant tumor DNA's. Oil/water emulsions with streptavidin-coated magnetic beads were created by vibration of samples. The BEAMING method (Diehl et al., Nature Methods 3:551-559, 2006) involved two sets of PCR reactions with tagged primers designed to immobilize the amplified products to the beads. Reaction products were detected by flow cytometry following hybridization with a specific fluorescently labeled oligonucleotide. The basis of enhanced sensitivity is the amplification of individual molecules within an emulsion bubble so that rare mutants are not competed out by wild-type DNA sequences. We have detected *PIK3CA* oncogene mutations at two hotspots of exon 9 and exon 20 (G1633A: E545K and A3140G:H1047R) found in a breast cancer cell line using the BEAMing technique. Reconstruction experiments with known amounts of normal and mutant tumor DNA spike-ins will be used to determine the limits of detection of oncogene mutations in saline and blood. These limits will help determine the utility of BEAMing in the detection of DNA-based cancer biomarkers.

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Chronic Oxidative DNA Damage Causes Chromosome Instability in Yeast *Saccharomyces cerevisiae*. Degtyareva N¹, Cheng L¹, Mieczkowski P², Doetsch P¹. ¹Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, United States, ²Department of Molecular Genetic and Microbiology, Duke University Medical Center, Durham, NC, United States.

Genetic instability is one of the common features acquired by cancer cells, enabling the development of tumors. Chromosomal instability (CIN) manifested by large-scale aberrations, such as amplifications and deletions of large segments of DNA, translocations, aneuploidy and polyploidy, characterize the majority of human malignant cells and are thought to accelerate carcinogenesis. Molecular mechanisms underlying CIN remain to be elucidated and are of profound importance for understanding of tumorigenesis. Oxidative DNA damage is also thought to play a significant role in cancer etiology. Cells have evolved several mechanisms for prevention and repair of oxidative damage. The base excision repair pathway plays a major role in the removal of oxidative DNA damage, although when this pathway is inactivated other repair pathways including nucleotide excision repair, recombinational repair, mismatch repair and translesion synthesis contribute to the handling of oxidative DNA damage. Prevention of oxidative DNA damage in cells is facilitated by several pathways enabling the scavenging of reactive oxygen species. Recent studies by other groups have demonstrated the important role of the antioxidative stress protein TSA1p in preventing large-scale chromosomal rearrangements. In the present study we employed *S. cerevisiae* as a model system to demonstrate that exceeding the cellular capacity to remove or otherwise handle oxidative DNA damage results in substantially elevated rates of CIN. In addition to utilizing of the standard methods for assessment of chromosomal rearrangements (GCR, chromosome loss and illegitimate mating assays) we employed CHEF gel analysis to karyotype large-scale chromosomal aberrations in replicative-aging haploid yeast strains with compromised DNA excision repair and/or ROS scavenging pathways under non-selective conditions. We find that chronic oxidative DNA damage leads to dramatic karyotypic changes of the genome. Employment of array comparative genome hybridization has allowed us to detect the hot spot of amplifications and deletions on chromosome II. This study provides direct, biologically relevant evidence that chronic oxidative DNA damage can rapidly overwhelm the ability of cells to maintain genome integrity.

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Differences in Lymphocyte Calpain Activity from Control and Diabetic Patients. Diaz-Villaseñor A¹, Méndez J², Ochoa E², Lara F², Zacarias R², Cebrián M³, Hiriart M⁴, Ostrosky-Wegman P¹. ¹Instituto de Investigaciones Biomédicas, UNAM, Mexico City, Mexico, ²Clínica de Diabetes, Hospital Gea González, Mexico City, Mexico, ³Sección Externa de Toxicología, CINVESTAV, Mexico City, Mexico, ⁴Instituto de Fisiología Celular, UNAM, Mexico City, Mexico.

The gene encoding calpain-10 has been linked to type 2 diabetes by positional cloning. Polymorphisms in this gene have been associated to an increased risk for the disease. Calpains participate in insulin secretion and action. Calpain-10 and 1 are involved in the secretion of insulin by pancreatic beta-cells. Exposure of pancreatic islets to calpain inhibitors results in the suppression of glucose-induced insulin secretion, whereas in adipocytes and myotubes inhibition of calpains results in a decrease in insulin-stimulated glucose uptake. The purpose of the present study was to find out if calpains have different activity and/or expression in an accessible tissue such as lymphocytes of individuals with and without type 2 diabetes. Cases and controls were recruited from the General Hospital. Subjects with insulin treatment were excluded. Anthropometric and clinic values were obtained for each donor and lymphocytes were isolated from whole peripheral blood of donors in fast. The activity of calpains was challenged with two different glucose concentrations measuring it by fluorescence. Calpain-10 mRNA was determined by real-time RT-PCR and protein expression analysis was carried by western blotting. The activity of calpains was responsive to glucose concentration only in the lymphocytes of control donors. This difference in sensitivity was not reflected in the expression of calpain-10 mRNA or in its protein. Since in lymphocytes of both patients and controls the same amount of calpain-10 was expressed, the regulation could be at the enzymatic level. This would imply that glucose is able to modulate the activity of calpains. The results presented in this study support the idea that lymphocytes are a suitable cellular model for studying glucose homeostasis, and that the enzymatic impairment occurred in type 2 diabetes not only take place in tissue directly involved in glucose homeostasis (i.e. pancreas, the liver, muscle and adipocytes), but occurring also in other type of cells such as lymphocytes. In particular, calpains are an interesting case since they participate in the pathogenesis and genetics of type 2 diabetes and the fact that they can be studied in cells that are obtained with non-invasive methods opens a broad field in the understanding of diabetes.

P65

Validation of *In Vitro* Micronucleus Study in Human Lymphocytes. Farabaugh CS, Middendorf CA, Leatherberry RS, Escobar MM, Stankowski Jr LF. Covance Laboratories, Inc., Vienna, VA, United States.

We previously reported on studies to validate the *in vitro* micronucleus (MN) assay in human peripheral blood lymphocytes (HPBLs) using the clastogens mitomycin C and cyclophosphamide. In the present study, we have extended this validation using the aneugenic agent, colchicine (COL). Since our earlier studies did not reveal any appreciable difference in response between treating whole blood (WB) and isolated HPBLs, we utilized only WB cultures here. Ten-mL cultures (containing 0.6 mL WB) were initiated (at t = 0 hr) in 15-mL centrifuge tubes in RPMI 1640 containing 2% phytohemagglutinin M (PHA-M), and grown at 37°C in a humidified atmosphere of 5% CO₂ in air. Cultures were treated (at t = 24 hr) with 10 – 40 ng/mL COL for 3 hr with and without S9, and for 20 hr without S9 only. Water was evaluated as the concurrent solvent control. Treatment was ended by centrifugation and washing in media, and the cultures were re-incubated in fresh media. Cytochalasin B was added (6 µg/mL, final; at t = 45 hr) and the cultures were harvested at 72 hr. Slides were stained with Giemsa and May-Grunwald. For each culture, 200 total cells were scored for cytotoxicity (cytochalasin B blocked proliferation index, CBPI), and 400 binucleated cells were scored for the frequency of MN (%MN-BN). Decreases in CBPI, to ~60% control values, were observed in the 3-hr treatments with and without S9, but the concentrations evaluated were far too high in the 20-hr treatment without S9. Statistically significant, dose-dependent increases in %MN-BN, to approximately 45- and 28-fold control values, were observed using 3-hr treatments with and without S9, respectively (the concentrations evaluated in the 20-hr treatment were far too toxic, at all concentrations, to allow accurate scoring). Additional confirmatory studies are in progress, as well as others to evaluate another aneugen, vinblastine.

P66

Major DNA Repair Pathways Influencing Transcriptional Mutagenesis in *Escherichia coli*. Clauson CL, Weiss B, Doetsch PW. Emory University, Atlanta, GA, United States.

Most cells in nature that are exposed to DNA damaging agents are not undergoing continuous rounds of replication, but are frequently engaged in transcription. Thus, it is important to understand the nature of RNA polymerase (RNAP) encounters with DNA damage, and the resulting biological consequences. It has been shown *in vitro*, with RNAP from various species, that the transcription machinery will stall at bulky DNA lesions; however, smaller, spontaneously occurring damages, such as uracil or 8-oxoguanine (8OG), are readily bypassed. This can lead to a population of mutant mRNAs, and mutant protein production, a process we have termed transcriptional mutagenesis (TM). TM may have biological importance in bacteria as a strategy to adapt to stressful environments. TM could also be important in mammals, as a route for generating mutant proteins, as most cells in tissues are not undergoing continuous cycles of DNA replication and cell division. It is possible that TM could have roles in human diseases such as cancer or prion-based neurodegenerative conditions. We are using a luciferase reporter assay in *E. coli* to study which DNA repair pathways influence TM caused by uracil and 8OG in bacteria. We have found that components of transcription-coupled nucleotide excision repair (TC-NER) and components of the base excision repair (BER) are involved in 8OG-mediated TM. In addition, hydrolytic AP endonucleases involved in processing abasic sites during base excision repair (BER) are not required to prevent TM, which could have implications for BER of 8OG in general. We have also found that components of the BER and TC-NER pathways are involved in uracil-mediated TM. We have determined that the major AP endonucleases are required for prevention of TM caused by uracil, most likely due to the fact that glycosylases involved in removal of uracil do not have an associated AP lyase activity (unlike several other DNA N-glycosylases/AP lyases). This also implies that while uracil is mutagenic per se, it is also mutagenic because of the abasic sites that are created as repair intermediates. Through investigation of the role of the DNA repair pathways that influence TM, we have been able to advance our understanding of TM, and DNA repair in general.

P67

Relative Expression of BAX Splice Variants Upon Radiation Exposure in Human Lymphoblastoid Cells. Banda M, Thomas R, Tucker J. Wayne State University, Detroit, MI, United States.

BAX (BCI2 associated X) is a pro-apoptotic protein that has at least six known splice variants that are expressed in various cell lines and tissues. How these splice variants precisely regulate apoptosis is unclear. BAX is known to respond to radiation, which is known to induce apoptosis. We determined the relative expression of BAX sigma, beta, and delta splice variants in response to 0 to 400 cGy ionizing radiation at 1, 4, 8, 12 and 24 hours post-exposure in the normal human lymphoblastoid GM15510 cell line using real time PCR. The expression values were normalized using GAPDH (glyceraldehyde 3-phosphate dehydrogenase) that was previously shown to be a good control gene in response to radiation in lymphoblastoid cells. Normalization was done using Relative Expression Software Tool (REST). Our results indicate that there is differential expression of these splice variants with time since exposure to radiation and also to some extent to different doses. The sigma variant expression is up-regulated earlier than beta and delta variants, i.e. after one hour. The beta variant has a transient 3-7 fold increase in expression four hours after exposure. The delta variant expression is elevated only at eight hours, when the other variants are minimally expressed. The beta variant lacks a transmembrane domain and is expressed in cytosol. The delta variant lacks the BH3 (BCL2 homology) domain that is known to be important for dimer formation. The detailed structural and functional analysis of these splice variants has not yet been studied. Because the proteins produced from these splice variants differ in their structure, their interactions with other proteins or regulators of apoptosis are likely to differ. The differential expression of these variants may suggest that the expression of a particular splice variant may influence the expression of other splice variant(s), perhaps by interacting with other proteins or regulators of apoptosis to induce programmed cell death. Alternative splicing that is known to be important regulatory mechanism in higher eukaryotes may control the regulation of BAX splice variants expression to induce apoptosis upon radiation exposure.

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Further Development and Validation of the *In Vitro* Micronucleus Assay in the EpiDermTM 3D Reconstructed Human Skin Model. Hu T¹, Mun GC², Kaluzhny Y³, Hayden P³, Barnett B¹, Wilt N³, Klausner M³, Curren RD², Aardema MJ¹. ¹Procter & Gamble, Cincinnati, OH, United States, ²Institute for In Vitro Sciences, Gaithersburg, MD, United States, ³MatTek Corporation, Ashland, MA, United States.

We have developed a novel *in vitro* human skin micronucleus assay using the 3D EpiDermTM skin model (MatTek Corp, Ashland, MA) for potential use in genotoxicity assessment as a replacement for *in vivo* genotoxicity assays that will be banned starting in 2009 according to the EU 7th Amendment to the Cosmetics Directive. This model has the advantage of better representing the complexities of *in vivo* human dermal exposures. Previously, we have shown dose-related increases in both cytotoxicity and micronuclei induction for several model genotoxins as well as negative results for dermal non-carcinogens. We have now further developed and validated the model. A dose response study with cytochalasin B demonstrates that 3 ug/ml is optimal for this assay. Results for additional chemicals showing intra- and inter-laboratory reproducibility are provided. Micronuclei and metabolism gene analyses with different tissue donors demonstrate that the assay is reproducible among multiple donors. Based on the results generated in our three laboratories, we have developed initial success criteria for the conduct of this assay. This novel assay continues to show promise as a relevant human "*in vivo*-like" genotoxicity model to replace current *in vivo* genotoxicity tests.

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DUN1-Mediated Pathway That Controls Ribonucleotide Reductase Induction is Required for Both Aflatoxin B1 (AFB1)-Associated Sister Chromatid Exchange (SCE) and Mutation in *Saccharomyces cerevisiae* (Yeast). Fasullo MT^{1,3}, Sun M³, Egner P². ¹State University of New York at Albany, Albany, NY, United States, ²Johns Hopkins University, Baltimore, MD, United States, ³Ordway Research Institute, Albany, NY, United States.

Introduction: The mycotoxin aflatoxin B1 (AFB1) is an extremely potent liver carcinogen produced by *Aspergillus flavus* that contaminates food supplies in tropical regions. Epidemiological data indicate that the p53 Ser249 mutation present in hepatocellular carcinoma correlates with AFB1 exposure. The genetic control of AFB1-induced mutation and genomic instability phenotypes is not well understood. However, AFB1-associated mutation frequencies in yeast are enhanced in *rad51* mutants, defective in homologous recombination, suggesting that a common AFB1-associated DNA adduct could stimulate either recombination or mutation. We asked whether there are particular checkpoint genes that channel DNA damage tolerance pathways towards recombination vs. mutation. **Methods:** We have designed yeast strains that can detect AFB1-associated SCE and mutation events. These yeast strains contain plasmids expressing human cytochrome P450 genes (CYP1A1 or CYP1A2) and the corresponding human oxido-reductase (hOR). **Results:** Microarray experiments have revealed that 15 DNA repair genes, including *RAD51*, *MLH1*, and *SRS2*, are induced in following AFB1 exposure in concentrated diploid cells (Keller-Seitz et al., 2004). Interestingly, in log phase cells, genes encoding ribonucleotide reductase (RNR) subunits are also induced (Guo et al., 2006). In yeast, RNR induction is controlled by the *MEC1* (ATR)-dependent signaling pathway, which includes Rad53 and Dun1. Activation of Rad53 (CHK2) requires *MEC1*. *MEC1*, *RAD53*, and *DUN1* are all required for AFB1-associated mutation and SCE. Interestingly, *DUN1* is not required for UV-associated AFB1-associated mutation or SCE. **Discussion:** We speculate that the AFB1-associated foramidopyrimidine (FAPY) and N7-guanine may impede DNA replication and subsequently stimulate SCE or mutation. After yeast were exposed to 50 μ M AFB1 for four hours, we detected N7-guanine AFB1 DNA adducts and FAPY derivatives in both the wild type and *rad53* mutant strains, using mass spectroscopy (LC/ESI/MS). Thus, the genetic requirements of AFB1-associated mutagenesis and recombination involve checkpoint genes that induce dNTP levels, which are not required for UV-associated mutation.

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Gene Expression Profiles in Normal Human Mammary Epithelial Cells (NHMECs) Exposed to Benzo(a)pyrene (BP) in the Presence or Absence of Chlorophyllin. John K^{1,2}, Keshava C³, Richardson DL², Weston A^{1,2}, Nath J¹. ¹West Virginia University, Morgantown, WV, United States, ²National Institute for Occupational Safety and Health, CDC, Morgantown, WV, United States, ³National Center for Environment Assessment, USEPA, Washington, DC, United States.

A panel of six NHMEC strains developed from breast tissue discarded at reduction mammoplasty was exposed to the ubiquitous carcinogen BP, both in the presence or absence of the chemopreventive agent chlorophyllin. Three exposure regimens were used: T1- solvent control (24h), T2- BP alone (24h), T3- 24h pretreatment with chlorophyllin followed by BP and chlorophyllin together (24h). Hu-Gene 133A arrays (Affymetrix, Santa Clara, CA) were used for expression analysis and the data analyzed using Microarray Suite 5.0 and Cluster and Tree View software. Genes altered by three fold or greater were considered for pathway analysis. Cross-talk among immune response genes were determined using Pathway Studio (Ariadne Genomics, Rockville, MD) and ArrayXPath software. A total of 49 genes were altered in T2 of which 43 were up-regulated and six down-regulated. A total of 125 genes were altered in T3 of which 103 were up-regulated and 22 down-regulated. The only gene up-regulated by more than three fold in all six cell strains was *CYP1B1*. Five immune response genes altered in T2 exhibited 2248 interactions involving a total of 1485 other genes. Twenty-four immune response genes altered in T3 exhibited 5782 interactions involving a total of 2299 other genes. Various immune response genes altered in T2 and T3 shared statistically significant associations ($p < 0.05$) with various pathways including: Biocarta, GenMAPP, PharmGKB and KEGG. These studies begin to define a role for carcinogen-induced immune response genes in human chemical carcinogenesis, and further suggest that the modulatory role of chlorophyllin in PAH mediated carcinogenesis may be mediated in part by interactions involving genes and pathways altered by BP exposure.

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ECVAM Retrospective Validation of the Micronucleus Test *In Vitro*. Corvi R¹, Albertini S², Hartung T¹, Hoffmann S¹, Maurici D³, Pfuhrer S⁴, van Benthem J⁵, Vanparys P⁶, ¹ECVAM, IHCP, JRC of the European Commission, Ispra, Italy, ²F. Hoffmann-La Roche, Basel, Switzerland, ³EFSA, Parma, Italy, ⁴Cosmital, P&G, Marly, Switzerland, ⁵RIVM, Bilthoven, Netherlands, ⁶Johnson & Johnson Pharmaceutical, Beerse, Belgium.

In the past decade several studies comparing the *in vitro* chromosome aberration test (CAT) and the micronucleus test (MNT) *in vitro* were performed. Although a high correlation was observed, no formal validation of the micronucleus *in vitro* assay was carried out. Therefore, the European Commission's European Centre for the Validation of Alternative Methods (ECVAM) established a working group to pool together the existing data, which would be useful to evaluate the validity of the MNT *in vitro* on the basis of ECVAM's modular validation approach. The primary focus was to evaluate the potential of the MNT *in vitro* as alternative to the standard CAT *in vitro*. In a first step, the working group evaluated the available data and came to the conclusion, that two publications met the criteria for a retrospective validation according to the criteria defined by the group [von der Hude et al. Mutation Research 468 (2), 137-163 (2000); Lorge et al., Mutation Research, 607(1), 13-36 (2006)]. These two studies were evaluated in depth (including a re-analysis of raw data) and provided the information required to assess the reliability (reproducibility) of the test. For the assessment of the concordance between the MNT *in vitro* and the CAT *in vitro*, additional published data were taken into consideration. On the basis of a peer review of the retrospective validation, the ECVAM Scientific Advisory Committee (ESAC) endorsed the conclusion that the MNT *in vitro* is a scientifically valid alternative to the CAT *in vitro* for genotoxicity testing. The *in vitro* MNT already gained widespread international interest, as it offers significant advantages over the *in vitro* CAT. It is less expensive and time-consuming, requires less investment in training, allows a greater statistical power, and has the potential to enhance the basic package of '*in vitro*' tests to detect aneuploids. The successful validation of the MNT *in vitro* led to EU regulatory acceptance and to the quick integration of the test in the REACH legislation for use as part of the tier 1 genotoxicity test battery. Furthermore, the validation study will support the finalisation of the test guideline and its regulatory acceptance by the Organisation for Economic Co-operation and Development (OECD).

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Mutagenicity and Loss of Heterozygosity (LOH) of Microsatellites Markers in Chromosome 11b of Mouse Lymphoma Cells Treated With Taxol. Schisler MR, Wood AJ, Geter DR, Gollapudi BB, Seidel SD. The Dow Chemical Company, Midland, MI, United States.

There is currently some uncertainty regarding the ability of the mouse lymphoma assay to detect mutagenicity induced by compounds causing spindle disruption. Some authors have reported positive findings in a microtiter version of this assay after a long (24-h), but not a short (4-h) treatment. The present investigation examined mutagenicity of taxol, a mitotic spindle inhibitor and chemotherapeutic agent, in this assay system using standard soft-agar cloning methodology and a short treatment time (4-h). Tk^{-/-}-3.7.2C L5178Y mouse lymphoma cells were treated with various concentrations of taxol ranging from 0.2 to 5.0 µg/ml and trifluorothymidine-resistant Tk^{-/-} mutants were selected following a 2 day expression period. Taxol induced a dose-related increase in mutant frequency (MF) over the solvent (DMSO) control, with the attainment of a biologically significant increase (MF:192 in treated vs. 74 x 10⁻⁶ in control) at 2.4 µg/ml which resulted in a relative total growth of 15%. A slight increase in MF at a lower concentration (1.8 µg/ml, 105 x 10⁻⁶) was not considered significant according to currently accepted criteria. To gain insight into the mechanisms responsible for this weak mutagenic activity, Tk^{-/-} mutants recovered from control and taxol-treated cultures (1.8 and 2.4 µg/ml) were analyzed for loss of heterozygosity (LOH) in chromosome 11b using PCR primers for microsatellite loci D11Ag12 (Tk locus) and D11Mit74 (tip of the chromosome distal to Tk locus). These results were compared to the spectrum of mutations induced by methyl methanesulfonate (MMS), a known clastogen. No difference was observed in the percent loss of Tk between treated and control mutant colonies. However, 65 and 76% of the mutants at 1.8 and 2.4 µg/ml, respectively, lost both microsatellite markers, compared to only 17% in control. Among the mutants analyzed from MMS-treated cultures, only 5% lost chromosome 11b (loss of both markers). From these data, we conclude that the weak mutagenicity of spindle inhibitors resulting from chromosomal loss is detectable in the mouse lymphoma assay using soft-agar cloning methodology following a 4-h treatment. These results are consistent with those reported by Moore et al. (The Toxicologist, 84, 455, 2005) using microtiter methodology.

P73

Legacy of Contaminant Exposure: Transgenerational Resistance Disclosed in the Proteome. *Andacht TM*¹, Oleksiak MF², Norris M¹, Winn RN¹. ¹University of Georgia, Athens, GA, United States, ²University of Miami, Miami, FL, United States.

Resistance to environmental contaminants is critical in reducing adverse health risks due to chemical exposure, and successful propagation of a species under stress. Despite many examples of populations exhibiting characteristics of resistance to environmental contaminants, the molecular mechanism behind this resistance remains poorly understood. An example is provided by a population of the estuarine fish species *Fundulus heteroclitus* inhabiting a heavily creosote-contaminated site (Atlantic Wood: AW) consisting predominately of polyaromatic hydrocarbons (PAHs). These fish are resistant to acute toxicity from sediment exposure and have persisted for over 40 generations despite heavy contaminant tissue levels and numerous adverse health effects. Elevated levels of hepatic glutathione S-transferase (GST) and the multidrug resistance protein (P-glycoprotein) have been proposed as possible mechanisms contributing to the PAH resistance in AW fish. To gain insight into the mechanisms of resistance and its transgenerational persistence, we maintained a subpopulation of AW fish in uncontaminated culture conditions, and compared liver protein expression levels of 3rd generation offspring to that of a reference population using Differential In-Gel Electrophoresis (DIGE) and mass spectrometry. Based on multivariate analyses, the liver proteome expression pattern of AW fish was clearly distinct from the reference population, with approximately 20% of proteins being differentially expressed ($p < 0.01$). Most striking were the reduction of glycolytic and oxidative stress enzymes and the increase of several GST isoforms in AW offspring. Several of these enzymes were shown to be similarly differentially expressed in the depurated parental AW subpopulation, indicating a genetically-based mechanism of resistance and adaptation. As chronic exposure to PAHs is known to induce oxidative stress, it is noteworthy that several of the differentially expressed metabolic pathways have been shown to be regulated in response to oxidative stress. These results suggest that this distinct metabolic profile of the AW fish provides the multiple levels of protection needed, from the initial exposure to subsequent cellular damage, to survive in this acutely toxic environment.

P74

Conditions Affecting Mutation Frequency in the Mouse Lymphoma Forward Mutation Assay. *Sahm J*, Luo FQ. Covance Laboratories Inc., Vienna, VA, United States.

A robust mouse lymphoma forward mutation assay requires good recovery of mutant colonies. The control mutant frequencies recommended by the Meeting of the International Workshop on Genotoxicity Testing, Mouse Lymphoma Working Group are specific ranges for both vehicle and positive control mutant frequencies. In order to consistently meet these Guidelines it is important to understand the parameters that affect the recovery of mutants. The vehicle control and positive control mutant frequencies were measured after varying a series of parameters. To determine the stability of the cell line, cells stocks were obtained from three different laboratories. All three had been independently carrying the L5178Y TK+/- clone 3.7.2C for over 10 years. When the three cultures were carried under the same conditions then treated and cloned, there was no consistent differences among the three sources of cells in the mutant control cultures (methyl methanesulfonate, MMS for nonactivation conditions and 3-methylcholanthrene, MCA for activation conditions), which demonstrates the stability of the cell line. Cell growth conditions were also investigated. The cells can be carried in a shaker incubator or roller drum or grown as stationary cultures. A single vial was removed from the freezer and split into a shaker culture and a stationary culture to avoid sample to sample variation. After growth under the two different conditions for at least one month, there was a marked difference in the mutant frequencies of vehicle control and positive control cultures both under nonactivation and activation conditions. In the vehicle control cultures the mutant frequencies were up to twice as high in the shaker cultures as those observed in the stationary cultures. Positive control cultures, both with MMS and MCA were also higher in the shaker cultures. Other parameters tested were cleansing and recovery conditions, time cells were grown in culture once thawed from frozen cultures, sources of agar and serum, frequency of subculture and density of cells at dosing.

P75

Mitochondrial DNA Sequence Variants in Mice Treated Transplacentally with AZT. Kunugita N¹, Mittelstaedt RA², Von Tungeln LS², Beland FA², Heflich RH². ¹University of Occupational and Environmental Health, Kitakyushu, Japan, ²National Center for Toxicological Research, Jefferson, AR, United States.

The prophylactic use of 3'-azido-3'-deoxythymidine (AZT; zidovudine) during pregnancy reduces the transmission of HIV-1 from infected mothers to their infants. The drug, however, has severe, toxic side-effects and previous studies suggest that mitochondria and mitochondrial DNA (mtDNA) are major targets for the toxicity. In order to investigate the effect of transplacental AZT treatment on mtDNA sequence variation, female C57BL/6N mice were mated with C3H males and pregnant dams were treated by gavage daily on gestational days 12 until parturition with 240 mg/kg of the drug. On postnatal days (PNDs) 1, 28 and 180 the B6C3F1 offspring were euthanized and heart and leg muscle were collected. DNA was isolated from the tissues and segments of mtDNA encoding selected tRNA genes and flanking regions were amplified by PCR. Mixtures of wild-type and altered sequence in the form of heteroduplex molecules were detected by denaturing high performance liquid chromatography (DHPLC). A mtDNA segment containing genes for tRNAs 7 through 11 and another segment containing the tRNA 16 gene were analyzed from both heart and muscle DNA from each animal. Of 10 AZT-treated PND 1 animals, tRNA 7-11 variants were seen in 3 mice and tRNA 16 changes were found in 1; differences seen in heart DNA also were detected in the muscle DNA from the same animal. Of 16 control animals, no sequence alterations were seen in heart DNA for either segment; 1 alteration was found in muscle DNA for tRNA 16. The tRNA 7-11 variant frequency for the AZT-treated mice was significantly higher than for the controls. PND 28 mice had a similar pattern of sequence alteration as the PND 1 animals. In PND 180 mice, sequence alterations were seen in DNA from AZT-treated and control animals at nearly the same frequency. All of the variants for each fragment had similar DHPLC chromatographic patterns. Our results indicate that in utero exposure of mice to AZT results in an increase in mtDNA variants in young, but not older mice. The consistent occurrence of mtDNA variants in heart and muscle from the same mouse suggests that AZT exposure may be selecting pre-existing polymorphisms whose frequency increases with age. This hypothesis is being tested by sequencing the variants.

P76

DNA Copy-Number Instability in Chronic, Low-Dose Gamma-Irradiated TK6 Lymphoblastoid Clones. Kimmel RR¹, Agnani S¹, Yang Y¹, Jordan R¹, Schwartz JL². ¹Fred Hutchinson Cancer Research Center, Seattle, WA, United States, ²University of Washington, Seattle, WA, United States.

Genomic instability that might occur early during chronic, low-dose radiation exposures may be traceable in radiogenic versus spontaneous cancers. Using a human 18K cDNA microarray-based comparative genome hybridization protocol, we measured DNA copy-number changes at over 14,000 loci in nine low-dose ¹³⁷Cs_γ-irradiated (10 cGy/day x 20 days; 0.007 cGy/min) and nine unirradiated TK6 clones, and estimated locus-specific copy-number differences between them. Irradiation induced copy-number hypervariability at thousands of loci across all chromosomes, with a 7-fold increase in low-level, randomly-positioned DNA gains. Recurrent gains at 42 loci occurred among irradiated clones and were distributed non-randomly across the genome, with the highest densities in 3q, 13q and 20q at sites that were hypodiploid without irradiation. Another non-randomly distributed set of 96 loci exhibited relative recurrent gains from a hypodiploid state to a diploid state, suggesting hemizygous-to-homozygous transitions. Frequently recurring losses at 57 loci were concentrated on the single X-chromosome, but sparsely distributed at 0-2 loci per autosome. These results suggest induced mitotic homologous recombination as a possible mechanism of low-dose radiation-induced genomic instability. Genomic instability induced in TK6 cells resembled that seen in radiogenic tumors, and suggests a way that radiation could induce genomic instability in pre-neoplastic cells.

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An *In Vitro* Alternative System for Genotoxic Screening of Compounds. Mahabir AG, Schaap MM, Hendriksen CFM, van Steeg H, van Benthem J. National Institute of Public Health and the Environment, Bilthoven, Netherlands.

The assessment of the potential genotoxicity of chemicals is important both for verification and confirmation of intrinsic mutagenicity and for establishing the mode of action of chemical carcinogens. Genotoxicity has two different endpoints: gene mutations such as base pair substitution, frame shifts, deletions and insertions, and chromosome aberrations, which in turn can be structural and/or numerical. Our goal was to develop an *in vitro* genotoxicity test using mouse embryonic fibroblasts (MEFs) isolated from the pUR288 plasmid mice harboring the *lacZ* gene of *E. coli* as a reporter. The pUR288 plasmid mouse model was chosen to supply the MEFs because with this model not only gene mutations but also chromosomal rearrangements, considered as the result of chromosomal breaks, can be detected. Consequently, with this *in vitro* test both genotoxic endpoints are covered in one single test. To increase the sensitivity also MEFs of pUR288 plasmid mice were crossed with homologous recombination repair deficient mice (*Rad54* and/or *Rad54B* deficient). Both *repair-proficient* and *repair-deficient lacZ* MEFs were exposed to compounds which predominantly induce gene mutations (B[a]P and N-AcO-2-AAF) or structural chromosome aberrations (bleomycin and mitomycin C). The *in vitro* micronucleus assay was used as a classical control for detection of clastogens. Both the mutagens as well as the clastogens induced an increase in the mutant frequency in *lacZ*⁺ MEFs. An increased mutant frequency in *lacZ*⁺*rad54*^{-/-}*rad54B*^{-/-} MEFs compared to the results in *lacZ*⁺ MEFs was only found for mitomycin C. The present results indicate that an *in vitro* genotoxicity test with *lacZ*⁺ MEFs is suitable to study both the induction of gene mutations and structural chromosome aberrations after chemical exposure. The use of homologous recombination repair deficient MEFs to increase the sensitivity of the test appeared not essential. This work was supported by ZonMw.

P78

Regulation of *Saccharomyces cerevisiae* DNA Polymerase eta transcript and protein. Pabla R, Rozario D, Siede W. UNT Health Science Center, Fort Worth, TX, United States.

Most types of DNA damage block replication fork progression during DNA synthesis because replicative DNA polymerases are unable to accommodate altered DNA bases in their active sites. To overcome this block, eukaryotic cells employ specialized translesion synthesis (TLS) polymerases with more relaxed active sites, which can insert nucleotides opposite damaged bases. In particular, TLS by DNA polymerase eta is the major pathway for bypassing UV photoproducts. One inevitable consequence of Y-family polymerases ability to synthesize across DNA lesions is their overall reduced fidelity, even on undamaged templates. Therefore, a tight regulation of damage tolerant polymerases is speculated. Pol eta when inactivated causes Xeroderma Pigmentosum-Variant (XP-V), a syndrome which predisposes humans to enhanced UV sensitivity and skin cancer. Methods: Northern blot analysis was carried out to look at the message. Western blot analysis was carried out to detect any changes in the protein amounts after UV damage and two more stress conditions. Cells were synchronized in G1 phase by using a yeast pheromone alpha-factor. Anti-Ub immunoprecipitation was carried out to detect any covalent modification of the protein. Survival assays and mutagenesis assays were carried out to study a ubz mutant (had a point mutation in a domain responsible for ubiquitination of Rad30p). Ub-agarose pull down was carried out to determine affinity of WT and mutant protein for ubiquitinated substrates. Results: Our findings show that an increase in the message of RAD30 gene encoding Polymerase eta (Pol η) after UV treatment is not reflected at the protein level. Given this polymerase is of utmost importance in the S phase of the cell-cycle, no increase was found in the protein amounts in S phase. IP results show that Pol eta is constitutively ubiquitinated. A ubz mutant which is defective in ubiquitination fails to interact with ubiquitinated substrates. Moreover, the ubz mutant makes cells more UV sensitive and confers higher mutagenicity. Discussion: We have discovered that Pol eta is constitutively monoubiquitinated and degree of monoubiquitination shows cell cycle dependence and is also affected by UV damage. We have also found that rad30 allele defective in ubiquitination renders cells more UV sensitive and confers higher mutagenicity after UV damage. This study shows that ubiquitination of Pol eta is essential for survival of the cells after UV damage.

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Genotoxicity of Ten Cigarette Smoke Condensates in Four Test Systems: Comparisons Among Assays and Condensates. DeMarini DM¹, Gudi R², Szkudlinska A², Rao M², Recio L³, Kehl M³, Kirby PE⁴, Polzin G⁵, Richter PA⁵. ¹US EPA, Research Triangle Park, NC, United States, ²BioReliance, Rockville, MD, United States, ³ILS, Durham, NC, United States, ⁴SITEK, Rockville, MD, United States, ⁵CDC, Atlanta, GA, United Kingdom.

The particulate fraction of cigarette smoke, cigarette smoke condensate (CSC), is genotoxic in many short-term *in vitro* tests and is carcinogenic in rodents. However, no study has evaluated a set of CSCs prepared from a diverse set of cigarettes and produced with different smoking machine regimens in a variety of short-term genotoxicity tests. Here we report on the genotoxicity of 10 CSCs prepared from commercial cigarettes that ranged from ultra-low tar (≤ 6.5 mg) to full flavor (>14.5 mg), a low-ignition-propensity commercial cigarette, a commercial cigarette marketed as a "low-nitrosamine" cigarette, a reference cigarette blended to be representative of a U.S. low tar cigarette, and experimental cigarettes constructed of single tobacco types. All cigarettes were machine smoked by the Federal Trade Commission (FTC) method, and one was also smoked by the Massachusetts intense method. The CSCs were tested in the presence of Aroclor-induced, rat-liver S9 mix in the *Salmonella* plate-incorporation assay using the frameshift strains TA98 and YG1041, in the micronucleus and comet assays in L578Y/*Tk*⁺-7.3.2C mouse lymphoma cells, and assayed for chromosomal aberrations in CHO-K₁ cells. All 10 CSCs were mutagenic in both strains of *Salmonella*, and their potencies, ranging over 7 fold, ranked similarly between the two strains. All 10 CSCs induced micronuclei with a 3-fold range in their potency. All CSCs but one induced DNA damage (comet assay), and their potencies ranged over 20-fold. All CSCs but one induced chromosomal aberrations, and their potencies ranged over 4-fold. The smoking-machine conditions did not produce a consistent effect on the genotoxic potency of the CSCs across the assays. There was no relation among the genotoxic potencies of the CSCs across the assays, and a qualitative advantage of the addition of the other assays to the *Salmonella* assay was not supported by our findings. Because of compensatory smoking habits and other factors, measurements of mutagenic activity of CSCs in any of the assays used in the present study have qualitative, but not quantitative value in assessing risk of cigarette types and cigarette smoking to human health. [Abstract does not necessarily reflect the policy of the US EPA or CDC.]

P80

The Newly Defined Acceptance and Evaluation Criteria for the Mouse Lymphoma Assay and Their Impact and Relevance. Poth A, Kunz S, Wollny E. RCC Germany, Rossdorf, Hessian, Germany.

In the present study historical data from 313 mouse lymphoma studies (microwell method) performed in the years 2003-2005 at RCC were investigated in respect to the newly acceptance and evaluation criteria as defined in the workshop reports of the IWGT working group. First the defined acceptable background mutant frequencies for negative/vehicle ($50-170 \times 10^{-6}$) were investigated for these studies. In 23 studies either the vehicle control or the solvent control were out of the defined range where 19 studies were beyond and 4 studies below the range. Secondly our data were investigated in respect to the newly defined acceptance criteria for the positive controls (GEF, IMF300) and compared to our previously used criteria (mutation factor of 2.0). MMS, used at RCC as a positive control without S9 mix in the pulse and in the prolonged treatment, indicate that in 94 studies at least one of the newly defined criteria could not be fulfilled, even if the mutation factor of 2 was reached or exceeded. A further investigation includes test items which were classified as genotoxic by the mutation factor approach compared to the newly defined evaluation criteria for the definition of a positive effect. In 37 out of 126 studies (29%) performed in 2005 at RCC, a positive result was obtained. 20 studies showed a positive response at pulse treatment, 17 at the prolonged treatment. In 4 studies the global evaluation factor 126 could not be reached even if the mutation factor of 2.0 was reached or exceeded (from 2.0 to 3.0). The current presentation will discuss the impact and the relevance of the newly defined acceptance and evaluation criteria for the mouse lymphoma test based on RCC's historical data and eventually a re-consideration of certain definitions.

P81

Abasic Sites are Strong Blocks to Multisubunit RNA Polymerases at the Incorporation and Extension Steps, and Incorporation Follows the "A-rule". Cheng TF, Brooks PJ. NIH, Rockville, MD, United States.

A major source of genetic instability is the release of purine bases from DNA by spontaneous hydrolysis of the glycosidic bond. During DNA replication, abasic sites are considered to be non-instructional DNA lesions, and many replicative DNA polymerases (DNAPs) incorporate A opposite abasic sites (the "A-rule"). Earlier studies showed that abasic sites could be bypassed by prokaryotic (T7 and *E. coli*) RNA polymerases (RNAPs), and both enzymes followed the "A rule". In mammalian systems, Kuraoka and colleagues (*JBC* 278:7294) found that human RNAPII initiated on a tailed template could bypass an abasic site, and incorporate CTP opposite the lesion, whereas Tornaletti et al (*CRT* 19: 1215) found that an abasic site was a complete block to promoter initiated rat liver RNAPII. To address these conflicting results, we utilized the direct assembly method of Kashlev and colleagues (*Meth. Enzymol.* 371:233) to investigate the blocking effect and nucleotide incorporation opposite an model abasic site with three different multisubunit RNAPs; *E. coli* RNAP; yeast RNAPII, and calf thymus RNAPII. We found that the abasic site was a strong block to all three RNAPs, with the strongest blocking effect observed for the calf thymus enzyme. Analysis of the extension products indicated that the lesion blocked transcription at both the incorporation and extension steps. Interestingly, despite the high degree of homology between yeast and mammalian RNAPIIs, in the enzymes active site, we obtained evidence for a significant quantitative difference between yeast and calf thymus RNAPII regarding the extent of blockage at the insertion versus extension steps. Finally, experiments using individual rNTPs demonstrated that all three enzymes clearly preferred to incorporate A opposite the abasic site, indicating that all three enzymes follow the "A-rule" despite the significant difference in the conformation of the active site between multisubunit RNAPs and replicative DNAPs. Examination of the active site of yeast RNAPII suggest that, as has been proposed for replicative DNAPs, the preference for incorporation of A opposite an abasic site might be explained by preferential base stacking properties of A versus other rNTPs.

P82

The Cytotoxic Effects of Benzo[a]pyrene, Benzo[a]pyrene-7,8-dihydrodiol, and Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide on Hormone-Sensitive Prostate Carcinoma Cell Line LNCaP. Nwagbara OE, Darling-Reed SF, Abazinge M, Thomas RD, Gragg RD. Florida A&M University, Tallahassee, FL, United States.

In the United States, prostate cancer (PC) is the second leading cause of cancer death among men, exceeded only by lung cancer, and accounting for approximately 11% of male cancer-related deaths. Humans are daily exposed to increasing amounts of xenobiotic compounds. Such xenobiotics can be natural or of anthropogenic origin, including drugs, agrochemicals, phytoestrogens, and environmental pollutants. There has been an increasing concern that natural and anthropogenic chemicals such as polycyclic aromatic hydrocarbons (PAHs) may cause endocrine systems leading to adverse effects such as cancers, reproductive system abnormalities and immune system deficiencies. PAHs are considered as potential human carcinogens. In the present study we determined cell viability, DNA damage, and cell cycle alteration on human prostate carcinoma cell line after exposure to B[a]P, BDP, and BPDE. In this study, we used a established prostate carcinoma cell line PC3 to evaluate the cytotoxicity of B[a]P and its selected metabolites. We observed that the effect on cell viability on LNCaP was dose-dependent as determined by MTT assay. After 48 hours treatment, it was observed that at higher concentration of 50 μ M (B[a]P), 20 μ M (BDP), and 10 μ M (BPDE) the percent viable cells are 45%, 35%, and 30% respectively for all three compounds. The study shows that B[a]P, BDP, and BPDE induced DNA strand breaks and cell cycle arrest in prostate carcinoma cell lines in a dose-dependent manner after 48 hours treatment. DNA damage was determined by the Comet assay and while cell cycle distribution was determined by flow cytometry. Comet assay analyses revealed that at concentrations of 50 μ M, 20 μ M, 10 μ M for B[a]P, B[a]P-7,8-dihydrodiol and BPDE, induced DNA strand breaks based on the average olive tail moment score of 13, 17, 16.5 respectively. Flow cytometric analyses showed that majority of the cells were arrested at G₁ phase in which 69.43(%) for 50 B[a]P, 66.35(%) for 20 BDP, and 67.57(%) for BPDE. Collectively, the results from the present study demonstrate that B[a]P, BDP, and BPDE induced cytotoxic effects such as DNA strand breaks and cell cycle arrest cell on human androgen-sensitive prostate carcinoma cell line LNCaP. These effects may be factors that could lead to B[a]P and its selected metabolites toxicity in normal prostate.

P83

Bhas Cell Transformation Assay as a Predictor of Carcinogenicity. Poth A, Kunz S, Heppenheimer A. RCC Germany, Rossdorf, Hessian, Germany.

Carcinogenesis is a multistep process which involves sequential genetic alterations in a single target cell ultimately leading to malignant transformation and tumour development. Carcinogen-induced alterations in DNA as well as non-genotoxic mechanisms can play a causal role. *In vitro* cell transformation tests using BALB/c3T3 or C3H10T1/2 cells simulate the process of animal two-stage carcinogenesis. A limitation of these methods however is the time (4-8 weeks) required for focus formation as a morphological indicator of transformation. Thus Sasaki et. al developed the cell line Bhas 42 by transfecting BALB/c 3T3 cells with the v-Ha-ras oncogene. Using these cells, transformed foci can be efficiently induced within 2-3 weeks by treatment with initiating and promoting agents. The Bhas-system was established and seven known carcinogens and non-carcinogens with results from other cell transformation assays (SHE- and BALB/c 3T3 assay) were compared to that of the Bhas 42 assay. By adding a metabolic activation system (S9 mix) all seven compounds showed corresponding results compared to the bioassay data. By selective trypsinization separate cultures could be established from foci cells and from normal cells. Parallel staining of microfilaments and nuclei clearly displayed a dense and multilayered piling-up of cells in foci. Thus alterations in adhesion protein profiles and enzyme activities modulating cellular adhesion and migration seem to be involved in the process of focus formation. In various tumour an increased cholinesterase activity is found. Thus the cholinesterase activity was investigated by a selective stain. A higher activity and intensity was found in the transformed foci compared to normal cells. This may be a promising candidate for the identification of molecular markers leading to a more objective scoring of foci and a greater acceptance of cell transformation tests in general. Key Words: cell transformation, *in vitro* carcinogenicity, Bhas cells, biochemical markers.

P84

Genotoxicological and Ecotoxicological Evaluation of Cyanobacterial Strains Using Plant and Fish Bioassays. Laughinghouse IV HD^{1,2}, Prá D², Rieger A², Silva-Stenico ME³, Fiore MF³, Franke SIR^{4,5}. ¹John Carroll University, University Heights, OH, United States, ²Universidade de Santa Cruz do Sul – UNISC, Laboratório de Biotecnologia e Genética, Santa Cruz do Sul, Rio Grande do Sul, Brazil, ³Universidade de São Paulo, CENA, Laboratório de Biologia Celular e Molecular, Piracicaba, São Paulo, Brazil, ⁴Universidade de Santa Cruz do Sul – UNISC, Curso de Nutrição, Santa Cruz do Sul, Rio Grande do Sul, Brazil.

Pollution is a major concern in urban areas. Water pollution caused by toxic cyanobacteria (blue-green algae) is a worldwide problem and worsens with eutrophication. Due to its biological significance, genotoxicity should be a main focus for biomonitoring pollution due to the increasing complexity of the toxicological environment in which organisms are exposed. Cyanobacteria have an enormous number of bioactive compounds, most of them completely lacking toxicological data. Microcystins comprise a class of potent cyclic heptapeptidic toxins produced mainly by *Microcystis aeruginosa*. On one hand, the hepatotoxicity of microcystins has been well-documented, but on the other, information on the genotoxic effects of microcystins is relatively scarce. In our study, we evaluated the genotoxicity and ecotoxicity of aqueous extracts from two strains of *M. aeruginosa*: NPLJ-4, which contains high levels of microcystin, and NPCD-1, with high levels of aeruginopeptin, a depsipeptide which lacks toxicity studies. Five endpoints, using a plant and a fish were applied: root growth inhibition, chromosomal aberrations, mitotic divisions and micronucleus assays in *Allium cepa*, and primary DNA damage (i.e. single and double strand breaks and alkali-labile sites) as evaluated by the comet assay (Single Cell Gel Electrophoresis) in fish of the genus *Actyanax*. The microcystin content of NPLJ-4 was confirmed through ELISA, while NPCD-1 did not produce microcystins. The extracts of NPLJ-4 were diluted at 0.01, 0.1, 1, 10 ppb of microcystins; the same cell dry weight was used to dilute NPCD-1 used as a parameter for comparison, and water was used as the control. Preliminary results demonstrated that both strains inhibited root growth and induced root abnormalities. The strain rich in aeruginopeptins seemed to be more mutagenic, altering the cell cycle, as observed for other depsipeptides that have been reported mitotic arrest by altering spindle metabolism, while microcystins seem to be mitogenic. Given the lack of studies on the toxicity of cyanotoxins, other than microcystin, these findings are novel and indicate the need for future research on cyanobacterial strains negative for microcystin. The understanding of the potent genotoxicity of *M. aeruginosa* extracts will help to determine a possible link between water cyanobacteria contamination and high risk of primary liver cancer found in some areas as well as establish limits for water level for toxins not yet studied, like depsipeptides.

P85

Genomic Instability Correlates with Ability of Human Breast Epithelial Cells to Incorporate Zidovudine (AZT) into DNA. Borojerdi JP¹, Ward Y², Poirier MC¹, Olivero OA¹. ¹Laboratory of Cancer Biology and Genetics, CCR, National Cancer Institute, NIH, Bethesda, MD, United States, ²Cell and Cancer Biology Branch, CCR National Cancer Institute, NIH, Bethesda, MD, United States.

The nucleoside analog AZT, a major component of most antiretroviral drug combinations, is a transplacental carcinogen in mice and induces cell cycle arrest, micronuclei, sister chromatid exchanges and shortened telomeres in experimental models. A predicted consequence of these events is genomic instability, which we have examined here using 2 strains of normal human mammary epithelial cells (NHMECs) exposed to 0, 10 or 200 μ M AZT for 24 hours and analyzed by immunofluorescence with confocal microscopy. Cells were stained with pericentrin antibody to localize centrosomal protein, CREST antibody to localize centromeric kinetochores in micronuclei, and antibody to Aurora A to visualize mitotic spindle integrity. The NHMEC strains used, 05 and 40, were chosen because the 05 cells incorporate AZT into DNA after 24 hr of AZT exposure, and the 40 cells do not. Using the pericentrin antibody, 31.7% of NHMEC 05 cells exposed to 200 μ M AZT showed centrosomal amplification/fragmentation (≥ 2 centrosomes/cell), and 5.8% of unexposed cells showed similar amplification. The NHMEC 40 cells, showed centrosomal amplification in 7.8% and 20.0% of cells exposed to 0 and 200 μ M AZT, respectively. When micronuclei bearing intact chromosomes were examined by staining with CREST antibody in cells exposed to 0 or 200 μ M AZT, NHMEC 05 cells showed staining in 25.9% and 36.4% of micronuclei, respectively, and NHMEC 40 cells showed staining in 0% and 38% of cells, respectively. When NHMEC 05 CELLS were exposed to 10 or 200 μ M AZT and examined by immunohistochemistry using an Aurora A antibody abnormal polarity of the mitotic spindle was observed. This was confirmed by Western blot, which showed that AZT exposure induced amplification of Aurora A in NHMEC 05 cells but not in NHMEC 40 cells. Overall the study reveals that NHMEC 05 cells, which form high levels of AZT-DNA incorporation at 24 hr, also show genomic instability that includes centromeric fragmentation/amplification, micronuclei containing chromosomal material, abnormal polarity of the mitotic spindle and Aurora A amplification. The NHMEC 40 cells, which do not incorporate AZT into DNA at 24 hr, appear to be comparatively more protected from these different manifestations of genomic instability.

P86

Novel *In Vivo* Mutation Assay Based on the Endogenous Pig-A Locus. Bryce S, Bemis J, Dertinger S. Litron Laboratories, Rochester, NY, United States.

Conventional approaches for measuring *in vivo* gene mutation have tended to rely on either endogenous genes such as *hprt* or else transgenes. Each method has its advantages and disadvantages. A significant advance in the field of *in vivo* mutagenesis would be the development of an assay that exhibited the best characteristics of these distinct systems: compatibility with any mammal, and no cell culture requirement. The experiments described herein were designed to test the utility of an assay that relies on glycosylphosphatidylinositol (GPI) anchored protein deficiency as a phenotypic marker of mutation at the pig-a locus. Pig-a resides on the X-chromosome, and the product is essential for anchoring GPI-linked proteins to the cell surface. The experiments described herein focused on Sprague Dawley rat erythrocytes and reticulocytes, as sufficient numbers of these cells are readily available via low volume venous draws. Fluorescent staining methods that permitted the enumeration of GPI-anchor deficient mature erythrocytes or reticulocytes via flow cytometry were developed and evaluated with reconstruction experiments. Following cell handling optimization, male Sprague Dawley rats were treated on 3 occasions (every other day) with 100 mg ENU/kg/day or 40 mg DMBA/kg/day. Peripheral blood specimens were harvested at weekly intervals for 5 weeks. Reticulocytes and total erythrocyte populations were analyzed for GPI anchored protein expression based on anti-CD59 labeling or else a pan-GPI ligand (i.e., FLAER, a fluorescent derivative of proaerolysin). All DMBA- and ENU-exposed animals exhibited elevated frequencies of GPI anchor deficient cells (ranging from 82 to 344 x 10⁻⁶, 310 to 647 x 10⁻⁶, and 0 to 70 x 10⁻⁶ for DMBA, ENU, and vehicle control rats, respectively). The elevated frequencies observed for the genotoxicant-exposed animals were highest in the reticulocyte subpopulation, tending to peak at 4 weeks post-exposure and plateau thereafter. These preliminary data support the hypothesis that a rapid *in vivo* mutation assay can be developed around flow cytometric analysis of GPI-anchored erythrocytes.

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Spontaneous Mutation Frequencies in Spleen in the *lacI* and Φ X174 Transgenic Mouse Mutation Assays. Valentine CR, Rainey HF, Farrell JM, Shaddock JG, Dobrovolsky VN, Delongchamp RR. National Center for Toxicological Research, U.S. FDA, Jefferson, AR, United States.

A perceived shortcoming of transgenic mutation assays is the relatively high spontaneous mutant frequencies (MnFs) of bacterial transgenes compared to endogenous genes¹. We have completed a study of the mutagenic response of the Φ X174 transgene in the Malling mouse to a single dose of 40 mg/kg ENU using the same conditions as for the *lacI* transgene of Big Blue[®] mice². Recent analysis of this study used an *in vivo* cut-off of 90 plaques per aliquot and MnFs were calculated by PFUs with clonal correction to mutation frequencies (MtFs). The spontaneous MtF from the Φ X174 forward assay was $1.7 \pm 1.9 \times 10^{-6}$ and the spontaneous MnF using 6-thioguanine selection (*Hprt*) was $2.0 \pm 1.8 \times 10^{-6}$. The MtF for ENU-treated animals from the Φ X174 assay was $12.2 \pm 6.3 \times 10^{-6}$ and $24 \pm 5.1 \times 10^{-6}$ for *Hprt* MnF. We compared our results to a linear regression of MtFs for the *lacI* gene from spleen at different ages (including embryo) from published studies²⁻⁵ using corrections for independence and type of mutation (base pair substitution) based on Walker *et al.*³. After normalization for the number of reported target sites for each reporter gene (Φ X174, 55; *lacI*, 427; *Hprt*, 312), the linear trend of MtF with age intercepted the time of conception with a frequency of 1.3×10^{-8} bps per nucleotide. This was not significantly different from the mutation rate of 1.7×10^{-8} bps per nucleotide observed for the human germline⁶ ($p = 0.72$). The normalized spontaneous MtF for this Φ X174 study was not significantly lower than the *lacI* trend ($p = 0.16$) (nor was the Φ X174 response to ENU significantly less than the *lacI* response). The results suggest that the *lacI* and Φ X174 transgenes measure MtFs approximately twice that of germline mutation rates in young animals (6 weeks). *Hprt* MnFs from several studies were all significantly lower than the spontaneous mutation rate in the human germline⁶, presumably since measured spontaneous *Hprt* MnFs are the result of selection against mutants *in vivo*⁷.¹Lambert et al., 2006, *Mutat. Res.* 590:1-280. ²Skopek et al., 1995, *EMM* 26:9-15. ³Walker et al., 1996, *Cancer Res.* 20 :4654-4661. ⁴Zimmer et al., 1999, *EMM*, 33:249-256. ⁵Hill et al., 2004, *EMM*, 43:110-120. ⁶Kondrashov, 2002, *Human Mutat.* 12:27. ⁷Deubel et al., 1996, *Mutat. Res.* 351:67-77.

P88

Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human and Marine Cell Lines. Wise SS^{1,2}, Mason MD³, Holmes AH^{1,2}, Savery LC^{1,2}, Li Chen T^{1,2}, Goodale BC^{1,2}, Shaffiey F^{1,2}, Wise Jr JP^{1,2}, Craig G³, Walter RB⁴, Payne R⁵, Kerr IAR⁵, Spaulding M⁶, Wise Sr JP^{1,2}. ¹Wise Laboratory of Environmental and Genetic Toxicology, Portland, ME, United States, ²Maine Center for Toxicology and Environmental Health, Portland, ME, United States, ³Department of Chemical and Biological Engineering, University of Maine, Orono, ME, United States, ⁴Texas State University, San Marcos, TX, United States, ⁵Ocean Alliance, Lincoln, MA, United States, ⁶Ocean Foundation, Washington, DC, United States.

In recent years nanoparticles have shown great promise with a broad range of applications, one of the current applications is due to the antimicrobial effects of silver. Silver nanoparticles are currently in wide use in consumer products. However, while silver nanoparticles have a powerful ability to kill bacteria and viruses their effects on mammalian cells remains unclear. These products are already on the market thus, exposure is occurring and it is already getting into our environment. To address this issue we investigated the cytotoxicity of 15 nm silver nanoparticles in cells from several representative species; human, North Atlantic right whale, sperm whale and medaka. We found that silver nanoparticles were significantly toxic in all cell lines at low concentrations. At 0.3, 0.5, 3 and 5 $\mu\text{g}/\text{cm}^2$, there was 90, 81, 64, and 41 percent relative survival in human skin cells respectively; 96, 90, 0, and 0 in right whale skin cells respectively; 85, 82, 5, and 0 in sperm whale skin cells respectively; and 53, 30, 0, and 0 in medaka fin cells respectively. There was no induction of chromosome damage in any of the cell lines, however all cell lines showed increases in the incidence of aneuploid cells. Aneuploidy is an indication of aberrant cellular division and a precursor to chromosome instability which can lead to tumorigenesis. Further work will characterize the development and progression of aneuploid cells and determine if transformation of cells due to silver nanoparticle exposure is possible.

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Experience With a Combined *In Vivo* Micronucleus and Comet Assay Test Protocol in Mice and Rats. Recio L¹, Hobbs-Riter C¹, Shephard K¹, Baldetti C¹, Winters J¹, Caspary W², Tice R², Witt K². ¹ILS, Inc, Research Triangle Park, NC, United States, ²NIEHS/NTP, Research Triangle Park, NC, United States.

Assessment of *in vivo* genotoxicity has traditionally been confined primarily to the assessment of bone marrow genotoxicity using the micronucleus assay. Although the *in vivo* micronucleus assay has proven an effective measure to assess the potential for *in vivo* genotoxicity, sampling of a single tissue (bone marrow) to assess genotoxicity using the micronucleus assay without the assessment of genotoxicity in other target tissues is clearly limited. The *in vivo* alkaline (pH>13) comet assay that detects a broad spectrum of DNA damage enables the investigation of genotoxic effects in almost any tissue following administration of test agents. We are evaluating a combined test protocol using the *in vivo* micronucleus and comet assay using four model genotoxic compounds acrylamide, ethyl methanesulfonate, cyclophosphamide, and vincristine and 2 test compounds that were negative in the *in vivo* micronucleus assay, tert-butylacrylamide and N-3-dimethylaminopropyl methacrylamide. Test compounds were administered over 3 to 4 consecutive days to male B6C3F1 mice or F344 rats, and mice were killed 4 hours after the last administration. The micronucleus assay was done using flow cytometry of blood and the comet assay was done on blood leukocytes, liver, and either stomach or duodenum. For the Comet assay 3 measures were examined, % tail DNA, tail length, and Olive tail moment (OTM). For the 4 model genotoxic compounds, positive results in the Comet assay were observed for acrylamide, ethyl methanesulfonate, and cyclophosphamide in multiple tissues was observed while vincristine was negative in the comet assay. For the 2 compounds that were negative in the micronucleus assay, positive results in blood leukocytes for the Comet assay based on either % tail DNA, tail length or OTM were observed while negative results were observed in liver cells. Further studies using a 4 day dosing regimen with more test compounds are ongoing to evaluate this combined micronucleus and comet assay test protocol. This work is funded by NIEHS/NTP NO1-ES-35514.

P90

Immunohistochemical Determination and Comparison of Polycyclic Aromatic Hydrocarbon (PAH)-DNA Adduct Levels in Peripheral and Transition Zones of Human Prostate. Pratt MM¹, John K¹, Sirajuddin P¹, Ragavan N², Martin FL², Olivero OA¹, Poirier MC¹. ¹Carcinogen-DNA Interactions Section, National Cancer Institute, Bethesda, MD, United States, ²Biomedical Sciences Unit, Lancaster University, Lancaster, United Kingdom.

A fibromuscular organ interspersed with glandular elements, the prostate is conventionally divided into three regions known as the transition (TZ), central (CZ) and peripheral zones (PZ). It is unclear why prostate cancer arises mainly (>70%) in the PZ, but recent studies report significantly higher *CYP1B1* expression in this region, suggesting that epithelial cells in this zone may have a greater metabolic capacity towards xenobiotics such as PAHs. Significant associations between PAH exposure, PAH-DNA adduct formation and subsequent cancer development have been shown in various human organs. Given the preponderance of prostate cancer originating in the PZ, we hypothesized that PAH-DNA adduct levels might be higher in the PZ, compared to the TZ. Semiquantitative immunohistochemistry (IHC) using an antibody elicited against DNA modified with (+/-)-7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) was employed to stain serial sections of human prostate for PAH-DNA adducts. Parallel sections were stained with hematoxylin (to identify nuclei) or immunogen-adsorbed anti-BPDE-DNA serum (for background). The Automated Cellular Imaging System (ACIS) was used to determine the intensity of nuclear staining. IHC evaluation of stained prostate tissues revealed the presence of PAH-DNA adducts concentrated in the epithelial cells lining the glandular elements. Preliminary analysis of PZ and TZ areas from 7 human prostate samples revealed substantial inter-individual variations in PAH-DNA adduct formation as indicated by differences in nuclear staining intensity. In addition, initial quantitative analysis showed no significant variation in PAH-DNA adduct levels (mean optical density (OD)/nucleus \pm SD) between the PZ (10,360 \pm 8801 OD/nucleus) and TZ (12,534 \pm 9143 OD/nucleus) regions. These results suggest that additional factors beyond PAH-DNA adduct formation may contribute to human prostate cancer and should be investigated to understand the etiology of adenocarcinoma formation in human prostate.

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Microindels Due to Highly Error-Prone Processes. Sommer SS^{1,2}, Ki K^{1,2}, Gu D¹, Gonzalez KD¹, Hill KA^{1,3}, Scaringe WA¹. ¹City of Hope National Medical Center, Duarte, CA, United States, ²Nanhua University, Hengyang, Hunan, China, ³University of Western Ontario, London, ON, Canada.

Little is known about the nature of microindels. We present the first analysis of somatic microindels in i) an endogenous and universally transcribed mammalian gene, and ii) in human cancer. Analyses of reported TP53 microindels in cancer reveal that they occur at a frequency of about 0.3% without obvious tissue or age specificity, and have a "molecular anatomy" consistent with an endogenous etiology. TP53 microindels in cancer are remarkably similar to spontaneous microindels in the non-transcribed lacI transgene in normal Big Blue mouse tissues suggesting that the selective pressures associated with oncogenesis as well as any mutagens associated with cancers have minor effects relative to endogenous mechanisms. The molecular anatomy of microindels as a class is different from that of pure microdeletions, pure microinsertions, and tandem-base mutations, suggesting unique mechanisms. Three pairs of similar but not identical recurrences were observed: these microindels showed the identical deletion with a nearly identical insertion ("recurroids"). Microindel sequence contexts suggest diverse mechanisms including error-prone mechanisms. In contrast to microinsertions which duplicate the adjacent sequence in the overwhelming majority of cases, the inserted sequences in microindels appear to derive predominantly from nearby but not adjacent sense or antisense sequences. The data suggest that indels arise from the bypass of blocking lesions by a mechanism that utilizes nearby sense or antisense sequences to help bridge the blocked lesion. The process is highly error prone with an estimated rate of 12% per bp, about two orders of magnitude greater than that measured for Y family polymerases, although those measurements were made in the absence of a bulky blocking adduct. In conclusion, the data herein describe the molecular anatomy of somatic microindels in cancer, constrain hypotheses of their nature and origin, and are consistent with these microindels generally deriving from spontaneous highly error-prone endogenous processes.

P92

Frequency of Chromosome Aberrations After Exposure to ¹³¹I in Thyroid Cancer Patients. Hernandez-Jardines A¹, Molina B¹, Del Castillo V¹, Papadakis M³, Rivera T³, Azorin J², Frias Vazquez S¹. ¹Instituto Nacional de Pediatría, Mexico DF, Mexico, ²Instituto Nacional de Cancerología, Mexico DF, Mexico, ³Universidad Autónoma Metropolitana, Mexico DF, Mexico.

Introduction: Iodine-131 (¹³¹I) is widely used for diagnosis and therapy of Thyroid carcinoma, the radionuclide reaches the whole acumulate body that receives a prolonged exposure of beta and gamma irradiation during dissemination and concentration of radionuclide in thyroid tissue. The aim of this study was to evaluate the frequency of chromosomal aberrations in lymphocytes before treatment, as well as after 2 and 24 hours in oral administration of therapeutic doses of ¹³¹I to patients with thyroid carcinoma. **Methods:** Ten adult females 17-57 years old with well-differentiated thyroid cancer, accepted to participate in this study. Samples of peripheral venous blood were obtained immediately prior to the administration of 100-200mCi ¹³¹I, 2 and 24 h after treatment; measurement of radioactivity was performed using CaSO₄ thermoluminescent dosimeters activated with dysprosium. Short-term three-day lymphocyte cultures were made to obtain cells in metaphase; chromosomal aberrations were scored in 200 metaphases per sample. **Results:** The highest level of radiation was found in samples of 2 h post-treatment and there was a positive correlation between the quantity of emitted radiation and the number of chromosome aberrations ($r = 0.495$, $p < 0.01$). The frequency of chromosome aberrations per cell found in all these patients was 0.009 before treatment, and after therapy it increased up to 0.04 and 0.02, 2h and 24h, respectively ($p < 0.05$). **Discussion:** The frequency of aberrations type breaks presented a peak in samples of 2 h post-treatment, however the rejoined aberrations (dicentric, rings, radial figures, translocations) increased with the sampling time. This results suggests that breaks are originated in cells exposed to radiation and then those cells carrying breaks die, however when breaks rejoin and generate stable rejoined aberrations, the cells can survive and accumulate chromosomal damage. One interesting observation was that the pretreatment frequency of chromosomal aberrations was significantly lower than that found in healthy women, and historical data from our lab. This low frequency may be due to an adaptive response to the first diagnostic dosage of 5mCi ¹³¹I.

P93

Chemical Induced Bystander Effect: Evidence and Significance. Asur R, Thomas RA, Tucker JD. Wayne State University, Detroit, MI, United States.

Most studies of the bystander phenomenon have focused on ionizing radiation and there are very few reports of this effect following chemical exposure. The present study was aimed at evaluating the ability of chemicals to induce the bystander effect through media transfer in normal human B lymphoblastoid cells. Phleomycin, a glycopeptide antibiotic of the bleomycin family and mitomycin C, a bifunctional alkylating agent, were used in these experiments, with induction of micronuclei in cytokinesis-blocked binucleated cells as the endpoint. Optimal concentrations of these chemicals for inducing micronuclei were determined in preliminary experiments. Normal human B lymphoblastoid cells were exposed to phleomycin (100 µg/mL) or to mitomycin C (200 ng/mL) for 1 hour, and unexposed cells were used as controls. Following exposure the cells were washed twice in phosphate buffered saline followed by a third wash in complete media to remove residual chemical. The cells were then incubated in fresh media and grown at 37°C for another 4 hours. The media from these cells was considered "conditioned" and was then transferred to unexposed cells, which were cultured for 28 additional hours. Cytochalasin B was present the final 28 hours. Cells grown in media obtained from cells that had been previously exposed to phleomycin and mitomycin C each had an increase in micronuclei of ~50% compared to cells grown in media obtained from unexposed cells. These experiments show that cells exposed to phleomycin or mitomycin C both release into the media soluble factors that are capable of inducing a bystander effect. To rule out the possibility that residual chemical rather than a bystander effect was responsible for the increase in micronuclei, media from the final wash was used to culture unexposed cells. The numbers of micronuclei in these cells were comparable to those observed in the controls, indicating that there was no residual chemical in the media. This work shows promise for contributing to our understanding of the mechanisms of the bystander effect.

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DNA Damage in Lymphocytes and Buccal Mucosa Cells of Children With Malignant Tumors Undergoing Chemotherapy. Ribeiro DA¹, Minicucci EM², Camargo B³, Costa MC³, Ribeiro LR¹, Salvadori DMF². ¹Federal University of Sao Paulo - UNIFESP, Santos, SP, Brazil, ²Botucatu Medical School - Sao Paulo State University - UNESP, Botucatu, SP, Brazil, ³A C Camargo Hospital, Sao Paulo, SP, Brazil.

The aim of the present study was to evaluate DNA damage (micronucleus) in cytokinesis-blocked lymphocytes and exfoliated buccal mucosa cells from children with malignant tumors and under chemotherapy. Micronucleated cells were assessed from children before, during and after chemotherapy. A total of 21 healthy children (controls), matched for gender and age were used as control. The results pointed out higher frequencies of micronucleated lymphocytes in children with malignant tumor before any therapy when compared to healthy probands. Furthermore, it was detected an increase of micronucleated lymphocytes during and after chemotherapy when compared to the data obtained before chemotherapy. No statistically significant increases of micronucleated cells were noticed in buccal mucosa cells in all of the moments evaluated. Taken together, these data indicate that the presence of malignant tumors may increase the frequency of DNA damage in circulating lymphocytes, being these cells more sensitive for detecting chromosome aberrations caused by anti-cancer drugs. The chromosome damage found herein represents a certain degree of genomic instability, reinforcing the approach to evaluate the side health effects induced by chemotherapy as well as to contribute to the micronucleus database for understanding and/or improving this methodology.

P95

Reoxygenation Following Hypoxia Activates DNA-damage Checkpoint Signaling Pathways that Suppress Cell Cycle Progression in Cultured Human Lymphocytes. Kim BM^{1,2}, Choi JY², Kim YJ², Woo HD². ¹Cancer Research Institute, Seoul, South Korea, ²School of Public Health, Seoul National University, Seoul, South Korea.

Cellular responses to DNA damage after hypoxia and reoxygenation (H/R) were examined in human lymphocytes. Cultured lymphocytes exposed to H/R showed a lower cytokinesis block proliferation index and a higher frequency of micronuclei in comparison to control cells. Western blots showed that H/R exposure induced p53 expression; however, p21 and Bax expression did not increase, indicating that H/R did not affect p53 transactivational activity. Phosphorylation of p53 (Ser15), Chk1 (Ser345), and Chk2 (Thr68) was also observed, suggesting that H/R activates p53 through checkpoint signals. In addition, H/R exposure caused the phosphorylation and negative regulation of Cdc2 and Cdc25C, proteins that are involved in cell cycle arrest at the G2/M checkpoint. The S-phase checkpoint, regulated by the ATM-p95/NBS1-SMC1 pathway, was also triggered in H/R-exposed lymphocytes. These results demonstrate that H/R exposure triggers checkpoint signaling and induces cell cycle arrest in cultured human lymphocytes.

P96

The Development of Non-Natural Nucleotides as Adjunctive Chemotherapeutic Agents. Berdis AJ, Lee I, Zhang X, Devadoss B. Case Western Reserve University, Cleveland, OH, United States.

The inhibition of nucleic acid metabolism is a powerful chemotherapeutic target having one significant pitfall – non-selective killing of both diseased and healthy cells. Non-selective killing arguably accounts for many if not all of the associated side-effects of chemotherapy including immunosuppression, alopecia, and an elevated risk of developing secondary cancers. To combat these problems, we have developed a unique strategy to potentiate the effects of existing chemotherapeutic agents by using non-natural nucleoside analogs that are preferentially inserted opposite damaged DNA. This allows us to target translesion DNA synthesis, a leading culprit in the development of secondary cancers as well as drug resistance. As a first step toward this goal, we have synthesized and characterized a variety of 5-substituted indolyl triphosphates with the goal of modulating the efficiency of their enzymatic incorporation into DNA. *In vitro* studies indicate that several of these analogs are selectively incorporated opposite an abasic site, a unique DNA lesion that is commonly formed after exposure to DNA-damaging agents. *In situ* studies validate that several of these non-natural nucleosides show anti-proliferative and, in some instances, cytotoxic effects versus leukemia (CEM-7) and colon carcinoma (HCT116) cell lines when used in combination with sub-lethal doses of DNA-damaging agents. Furthermore, potentiation occurs in cell lines defective in both mismatch repair and p53 DNA-damage response pathways. These final features may play an important role toward improving the clinical efficacy of existing chemotherapeutic agents. Current studies are aimed at determining if cell death via typical apoptotic pathways or via mitotic catastrophe.

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Gene Polymorphism and the Risk of Cutaneous Malignant Melanoma in Brazil. Toledo Gonçalves F, RP Souza S, Eluf Neto J, Kohler P, Gattás GJF. Medical School São Paulo University, São Paulo, SP, Brazil.

The strongest risk factors for Cutaneous Malignant Melanoma (CMM), a neoplastic lesion with high mortality rate, are a family history for melanoma, multiple benign or atypical nevi, and previous melanoma. Exposure to ultraviolet radiation, sun sensitivity and immunosuppression are additional risk factors that can be modified by different genetic background. This is a case-control study that was designed to evaluate genetic risks associated to the polymorphism of detoxification enzymes (*CYP1A1/Mspl*, *CYP2E1/PstI*, *GSTM1*, *GSTT1* and *GSTP1/Bsma*), DNA repair genes (*XRCC1-194/399/Mspl* and *XRCC3/Ncol*) and the vitamin D receptor gene (*VDR/TaqI* and *FokI*). We here analyzed, by PCR-RFLP, 192 patients (97 male and 95 female, mean age 51 ± 14.16 years old) with histologically confirmed CMM and 187 cancer free controls (96 male and 91 female, mean age 48 ± 14.31 years old) admitted as in-patients or out-patients in the same hospital. No significant differences were detected in *CYP1A1/Mspl* (OR, 1.22; 95% CI, 0.36–4.12), *CYP2E1/PstI* (OR, 1.51; 95% CI, 0.75–3.01), *GSTM1* null (OR, 1.41; 95% CI, 0.94–2.12), *GSTP1/Bsma* (OR, 1.12; 95% CI, 0.60–2.14), *XRCC1-194/Mspl* (OR, 0.80; 95% CI, 0.45–1.41), *XRCC1-399/Mspl* (OR, 1.53; 95% CI, 0.71–3.28), *VDR/TaqI* (OR, 1.01; 95% CI, 0.50–2.09) and *VDR/FokI* (OR, 0.83; 95% CI, 0.40–1.76) polymorphisms, when the two populations were compared. The frequency of at least one *XRCC3* mutated allele (*XRCC3-241Met/Thr* or *XRCC3-241Thr/Thr*) was higher in patients with melanoma (60.4%) when compared to the control group (50.2%) and the difference was considered statistically significant (OR= 1.51; 95%CI:1.00–2.27). By the other side, *GSTT1null* polymorphism was more frequent in the control group (32.8%) compared to CMM patients (23.7%), apparently conferring disease protection for this group (OR, 0.63; 95% CI, 0.4–1.00). Considering that polymorphisms in DNA repair genes may alter protein function and an individual's capacity to repair damaged DNA these results suggest an increased risk of developing CMM associated with *XRCC3* polymorphism. The possible *GSTT1null* polymorphism protection for CMM should be better addressed. We are increasing our patients and control sample and repair genes polymorphisms to confirm these data. (FAPESP, CNPq and LIM40-FMUSP).

P98

Evaluation of Methylphenidate in Human Peripheral Blood Lymphocytes for Chromosomal Aberrations and Micronuclei. Doppalapudi R, Garcia R, Defensor E, Riccio E, Mirsalis J. SRI International, Menlo Park, CA, United States.

Methylphenidate (MPH) is used in the treatment of various neurological conditions, including attention deficit hyperactivity disorder (ADHD), depression and narcolepsy. Approximately 3% to 5% or more U.S. children and teenagers have been or are prescribed MPH. The potential of MPH to promote or cause cancer or other chronic toxicities in humans is a particularly serious concern because of the increasing and often long-term use of MPH in children. The genotoxic effect of MPH needs to be established to estimate the possible cancer risk in humans. MPH was not mutagenic in Ames reverse mutation assay and mouse lymphoma assay and it gave conflicting results for the induction of chromosomal aberrations in Chinese hamster ovary cells. Therefore, MPH was evaluated in human peripheral blood lymphocytes for chromosomal aberrations and micronuclei in the presence and absence of rat and human S9. Cells were exposed to MPH at 100, 400 and 800 µg/ml dose levels in the absence of metabolic activation (S9), and at 50, 100 and 200 µg/ml in the presence of S9. Cells were cultured, harvested and scored for structural chromosomal aberrations and micronuclei. There was no significant increase in chromosomal aberrations at any dose level in the presence or absence of S9. The frequency of micronuclei was 0.55% in controls and 0.52%, 1.15% and 1.10% in cultures treated with 100, 400 and 800 µg/ml dose levels, respectively, in the absence of S9. There was also an increase in micronuclei at 50, 100 and 200 µg/ml dose levels in the presence of rat (0.5% micronuclei in controls vs. 0.7%, 0.9%, and 1.35%) and human (0.6% micronuclei in controls vs. 0.9% 0.9% and 1.45%) S9. The increase in micronuclei was 2-fold at the highest dose level (200 µg/ml) in the presence or absence of S9 indicative of weak genotoxic response.

P99

A Role for Thymine Nucleotide Insufficiency in Breast Carcinogenesis? Barclay BJ¹, Murray D². ¹Planet Biotechnologies Inc., St Albert, AB, Canada, ²Cross Cancer Institute, Edmonton, AB, Canada.

Breast cancer, its prevention, diagnosis and treatment, is a major public health issue. Unfortunately, we do not yet fully understand the complex multifactorial causality of the major (i.e., sporadic) forms of this disease or of its etiology at the detailed molecular level. We do know that loss and gain of genetic material is a hallmark histopathological feature in many cases. We discuss here a potential role for thymine nucleotide insufficiency (TNI) in the etiology of sporadic breast cancer. This hypothesis is driven in part by the striking similarity between the hallmark molecular features of breast cancer and the phenomenology associated with TNI. We hypothesize that TNI, caused by a combination of genetic polymorphisms in critical genes involved in thymine nucleotide or one-carbon metabolism such as thymidylate synthetase (*TYMS*) and dihydrofolate reductase (*DHFR*), micronutrient deficiencies (e.g., in folic acid intake), and exposure to environmental toxins (such as malathion), induces the non-random loss and/or gain of genetic material throughout the human genome, leading to extensive loss of heterozygosity (LOH). Consequential deletions at tumor-suppressor loci may initiate transformation, whereas events such as episome formation can result in the amplification of proto-oncogenes and lead to disease progression. Among the genes that are amplified episomally, *TYMS* and *DHFR* are of particular interest because they would be expected to be selected for not only by the underlying TNI phenotype but also by some chemotherapeutic agents (e.g., 5-fluorouracil and methotrexate) that have been widely used in the treatment of these cancers. It has recently been shown that *TYMS* is an oncogene, and its (late) amplification may thus contribute to disease progression as well as the more well-known phenomenon of drug resistance. If supported by critical experimental tests, the hypothesis has implications for breast cancer diagnosis, treatment and prevention.

P100

Cytogenetic Analyses of Lymphocytes in Workers Occupationally-Exposed to Nickel. Camargo M, Restrepo H, Zea O. University of Antioquia, Medellin, Colombia.

Humans are exposed to carcinogenic nickel (Ni) compounds mainly from occupational sources, being the nickel refining a major concern for health effects. Despite the undisclosed nature of their molecular mechanisms, the genotoxic or carcinogenic effect of various nickel compounds demonstrated in *in vitro* cell transformation assays, animal carcinogenicity bioassays, and occupational exposure, are of great significance to try to understand the cancer risk associated with human exposure. Cytogenetic biomonitoring in blood cells of exposed persons, have been extensively employed to assess genotoxicity as a biomarker of early biological effect of such exposures, recently strengthened by the observed cancer risk predisposition of high chromosomal alterations (CAs) frequencies. In our study, we report the results of a cytogenetic biomonitoring program conducted in a large nickel refinery and with workers with at least ten years of continuous occupational exposure to nickel, including a control group of non-exposed first degree relatives. A total of 205,000 binucleated cells were analyzed for the micronucleus test, 20,700 for chromosome aberrations and 10,350 for sister chromatid exchanges. Despite the fact that the overall levels of micronuclei, sister chromatid exchanges and chromosomal aberrations were statistically similar among groups, one major finding of our study is that structural subtypes of CAs such as rearranged dicentrics, rings and multiradials showed significant differences between groups, and confined only to the high exposure subpopulation. These results suggest that CA test and not MN, can differentiate exposure subtleties, and that this type of occupational exposure may interfere with DNA repair mechanisms. It is the largest sample of nickel-exposed individuals so far analyzed in the world with these biomarkers, and the first one in lateritic ore mining and refining. (The study was supported by CerroMatoso SA and University of Antioquia).

P101

Pathogenic Mutations in PEO1 Cause Biochemical Defects in the Human Mitochondrial DNA Helicase. Longley MJ, Sharif FS, Copeland WC. NIEHS, NIH, Research Triangle Park, NC, United States.

Maintaining the integrity of the mitochondrial genome is essential for proper cellular energy metabolism, and disruption of mitochondrial DNA replication causes a range of severe mitochondrial disorders. At least 17 different missense mutations in the human *PEO1* gene, which encodes the TWINKLE mitochondrial DNA helicase, co-segregate with dominant progressive external ophthalmoplegia associated with pronounced deletions in mitochondrial DNA. In an effort to identify the molecular mechanisms leading to mtDNA deletions, we chose a biochemical approach to characterize defects in the mutant forms of the helicases. Wild type and mutant forms of *PEO1* were over-expressed in *E. coli*, and recombinant helicases were purified to near homogeneity by conventional chromatographic methods. The NTPase and DNA helicase activities of the purified proteins were assessed with a variety of DNA substrates. Reaction kinetics, cofactor requirements, and responses to inhibitors were determined for each protein. Thermal stability, DNA substrate preferences and DNA-binding strength also were determined. Sedimentation analyses were utilized to assess assembly into hexameric structures as well as physical interaction with other components of the mitochondrial DNA replication fork. The varied biochemical effects of pathogenic amino acid substitutions are discussed in the context of a model for mtDNA deletions.

P102

Benzene Induces Aneuploidy of Sex Chromosomes and Chromosome 21 in the Sperm of Occupationally-Exposed Chinese Men. Xing C^{1,2}, Eskenazi B³, Li G², Weldon RH³, Kurtovich E³, Young S³, Schmid T¹, Zhang L³, Rappaport SM³, Wyrobek AJ¹, Marchetti F¹. ¹Lawrence Berkeley National Laboratories, Berkeley, CA, United States, ²Chinese Center for Disease Control and Prevention, Beijing, China, ³University of California, Berkeley, Berkeley, CA, United States.

Benzene is an industrial chemical that is present in crude oil, gasoline, and in some adhesives, paints and paint removers. In the US, benzene is a highly regulated chemical due its ability to induce leukemia and other blood disorders at exposure levels even below 1 ppm, the Permissible Exposure Limit (8 hour) set by OSHA. There is considerable epidemiological evidence that exposure of the father to environmental or occupational agents can lead to abnormal reproductive outcomes. Benzene induces chromosomal damage in white blood cells, but it is not clear whether it induces aneuploidy in sperm or if the resulting offspring would be affected by such damage. We used fluorescence *in situ* hybridization to investigate whether benzene was associated with numerical abnormalities of chromosomes X, Y and 21 in the sperm of 33 occupationally-exposed Chinese men compared to 33 Chinese men who worked in factories with no benzene use. To assess workplace benzene exposure, participants' urinary benzene levels were measured and personal air monitoring was conducted. Air monitoring revealed that ambient levels in the exposed group ranged from below detectable to 24 ppm with a mean level of 6 ppm. Based on urinary benzene levels, the exposed group was categorized into three groups: low, moderate and high with mean \pm SD levels of 2.91 ± 1.41 , 15.21 ± 11.14 , and 142.69 ± 169.86 $\mu\text{g/L}$, respectively. The mean level in the control group was 0.19 ± 0.19 $\mu\text{g/L}$. Preliminary tests for trend showed a dose-dependent increase in disomy X ($p=0.001$), disomy Y ($p=0.002$) and total hyperhaploidy ($p=0.002$) in the sperm of exposed men. Significant differences between the high exposure group and control group were detected for chromosome 21 nullisomy among Y sperm ($p=0.04$, two-sided t-test) and Y-disomic sperm ($p=0.009$) as well as total hyper- and hypo-haploid sperm ($p=0.009$). No effect on sperm diploidy was observed. These findings suggest that exposure to benzene can induce numerical abnormalities of the sex chromosomes and chromosome 21 in the sperm of exposed men. Human sperm that are aneuploid for these chromosomes have the potential to increase the risks for chromosomally-defective children with Klinefelter and Triple X syndromes and for spontaneous abortions of paternal origin.

Genotoxins, Mutagens, Antimutagens, Biodosimetry, Risk Assessment

P103

Genotoxicity Assessment Using a GADD45a-GFP Reporter Assay in a p53 Competent Human Cell Host: Metabolic Activation, Flow Cytometry and Data Update. Walmsley RM^{1,2}, Billinton N², Jagger CP², Hastwell PW³, Knight AW², Birrell L², Cahill PA². ¹University of Manchester, Manchester, United Kingdom, ²Genronix Ltd, Manchester, United Kingdom, ³GlaxoSmithKline, Ware, United Kingdom.

Introduction: A new *in vitro* mammalian cell genotoxicity assay has been described (1) in which GFP expression is driven by regulatory elements of the human GADD45a gene in TK6 cells. The early validation study (75 compounds) revealed high sensitivity to genotoxic carcinogens coupled with high specificity. Here we report the results from detailed studies of almost 200 compounds, as well as a partial screen of the Sigma LOPAC collection. We also report modifications of the assay to allow data collection by flow cytometry, which allows the assessment of compounds exposed to S9 liver extracts. **Methods:** A simple 96 well microplate assay allows genotoxicity assessment for 4 compounds at 9 dilutions and gives results within 48 hours. LOPAC compounds were screened at 100 micromolar and 3 dilutions. Most of the fluorescence and absorbance data in this study were collected by bulk cell spectrophotometry. Flow cytometry was used in the S9 studies as this reduced the interference from coloured and fluorescent components in the liver extracts. **Results:** Data from the enlarged collection show that sensitivity and specificity remain high. In particular, we have new data from problematic compounds that are positive in the *in vitro* mammalian mutation assay (MLA), but negative in the Ames test and *in vivo* MNT and carcinogenicity studies - most were negative in the new assay. About 9% of LOPAC compounds tested positive in the assay, and these had a 10 fold higher incidence of DEREK mutagenicity alerts than the compounds with negative results. The published work had a proof of principle experiment showing metabolic activation (MA) of cyclophosphamide. The new flow cytometric S9 studies show appropriate MA for many more compounds. **Discussion:** The extended dataset reported here reinforces the value of the assay as an extension to the *in vitro* testing battery. The specificity is over 90% and sensitivity is over 80%, comparable to or better than the other *in vitro* assays. The addition of an S9 protocol has increased the spectrum of genotoxic compounds detected. The lack of specificity in the existing *in vitro* mammalian assays leads to misleading unique positive results for 'safe' compounds and these are currently distinguished from genuine positives by *in vivo* studies. This new assay identifies genuine genotoxins accurately, reducing the use of animals in simply confirming positive results. (1) Hastwell *et al*, *Mut Res* 607: 160-175, 2006.

P104

Contamination With Tritium Induced Genetic Instability. Saintigny Y, Lopez BS. Commissariat à l'Energie Atomique - IRCM, Fontenay aux Roses, France.

Because of its low disintegration energy, tritium's biological effects cannot come from external exposure but from integration of organically bound (OBT) tritium into tissue. This results in an *in situ* chronic auto-irradiation. Consequently, the energy deposition is concentrated in the sub-cellular compartment in which the OBT is incorporated. In this study, we evaluate the impact of sub-lethal doses of contamination by ³H-thymidine or tritiated water on cell survival, DNA double-strand breaks (DSB), cell cycle, mutagenesis and homologous recombination (HR), in hamster and human cell models. Using ³H-thymidine, we were able to target the cell nucleus. We show here that contamination by ³H-thymidine induced stimulation of mutagenesis at very low doses of incorporation, even in the absence of cell mortality. Moreover, we found oxidative stress induced by low doses of ³H-thymidine responsible for mutagenesis peak. At higher incorporated doses, we measure a dramatic stimulation of HR. Such a tremendous mutagenesis or HR stimulation level cannot be reached with Gamma rays external radiation with comparable dose rate. We show that DSB induced by Tritium incorporated into the DNA are processed either by the Xrcc4 (NHEJ) or the Rad51 (HR) pathways. In this study, we also measured an ATR dependent G2/M cell cycle arrest after high doses of ³H-thymidine. Our results emphasize that the biological impact of Tritium is conversely proportional to the isotope emission energy but correlate to the energy transferred to the nucleus. Taking together, the data presented here show that cell contamination with non-toxic doses of tritium may be hazardous for genetic stability. Thus, the remarkable survival of these contaminated cells associated to genetics alterations may increase the risk of: 1 - transmission of genetic modifications to the next generation and 2 - increase the risk of cancer (cancer cell should accumulated mutations and be viable to generate a tumour). Our work emphasizes the strong differences between an external ionizing radiation exposure and an internal radioactive contamination on biological consequences. The concept of dose of internal contamination in radiation protection may be re-evaluated.

P105

Antimutagenic Effects of Caffeic Acid Phenethyl Ester (CAPE) in Human Breast Cancer Cells and Relationship to Gene Expression. Lyon JL¹, Leszczynska JM¹, Wu J², Frenkel K², Klein CB¹. ¹New York University School of Medicine, Tuxedo, NY, United States, ²New York University School of Medicine, New York, NY, United States.

Caffeic acid phenethyl ester (CAPE), an active component of honeybee propolis, is known for its anti-oxidant, anti-inflammatory and chemopreventive activities in animal models. In previous work we showed that low, non-toxic doses of genistein (3.125 μ M) and lycopene (2 μ M) were effective antimutagens in breast cancer cell lines. These compounds significantly reduced (by 31-49%) elevated mutation levels that characterize human breast cancer cells MCF-7 [ER α (+)] and MDA-MB-468 [ER α (-)] cells. The current study was designed to investigate whether CAPE also exerts antimutagenic effects in these cell lines. Clonogenic growth assays showed that CAPE at doses of 3.0-30 pmol/seeded cell (0.1–1.0 μ M nominal doses) was minimally toxic to both types of cells, since the survival of MCF-7 cells ranged from 98.7% to 70.4% and that of MDA-MB-468 cells from 94% to 74% as compared to the untreated cells. However, the antimutagenic potential of CAPE in MCF-7 cells was evident at very low non-toxic doses of 0.02 and 0.05 pmol/seeded cell (0.2 and 0.5 μ M nominal doses), since mutant frequencies were dose-dependently decreased by 60.7 to 66.1%. Gene array experiments showed that treating MCF-7 cells with a non-toxic dose of 0.12 pmol/seeded cell (10 μ M) CAPE causes extensive changes in gene expression, mostly by their down-regulation. Heme oxygenase 1, an inducible stress protein that protects against oxidative damage, was increased 4.2-fold. Many other genes were down-regulated 2-3 fold by CAPE, including DNA damage inducible genes, heat shock proteins, and chaperonins. Importantly, PCNA (proliferating cell nuclear antigen), BCL-2 associated athanogene (an anti-apoptotic protein), topoisomerase I, and NF- κ B (p105) transcription factor were down-regulated 4.5-, 3.2-, 2.6-, and 2.0-fold, respectively, showing decreases in oxidative stress and cell proliferation. Cumulatively, these results show that CAPE is a potent antimutagen that inhibits cancer cell growth by decreasing oxidative stress and its consequences, as well as by down-regulation of proliferation genes and anti-apoptotic genes. [This work was supported in part by grants from the Susan G. Komen Foundation BCTR0402531 (CBK) and BCTR0600476 (KF), and by T32 ES007324 (JL).]

P106

Natural Co-Occurrence of Aflatoxin and Cyclopiazonic Acid, and Their Fungi Production of Corn Grown in Egypt. Deabes M, Amra H, Damaty E, Rowayshed G. National Research Center, Cairo, Egypt.

Corn kernels were purchased from retail market from Cairo and Giza Governorates in Egypt and examined for fungi and mycotoxin (aflatoxins and cyclopiazonic acid) contamination. Two Hundred and four *Aspergillus* spp. isolates which isolated from corn samples collected from Cairo and Giza Governorates, 63.7% of them was *A. flavus*. Concerning the frequency distribution of 84 isolates of *A. flavus* were tested for isolation of *A. flavus* 65 producing aflatoxins. The results indicated that 64.6 of the isolates were *A. flavus* producing both of aflatoxins and cyclopiazonic acid form samples of corn. The dominant fungi genera were *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* in all corn samples in both seasons. Aflatoxin and cyclopiazonic acid determined by high performance liquid chromatography (HPLC). The results showed that averages of 9.4 and 10.11 mg aflatoxins/L of the YES medium were produced by *A. flavus* isolates from corn samples. On the other hand, the results showed that averages of 12.56 mg cyclopiazonic acid /L of the YES medium were produced by *A. flavus* isolates from corn. The results indicated that 14 samples out from 60 (23.3%) and 12/60(20 %) samples from corn were contaminated with aflatoxin (B₁, B₂, G₁ and G₂) and CPA respectively in winter season. While, 20 samples out from 60 (33.3 %) and 24/60 (40.0 %) samples were contaminated with aflatoxin (B₁, B₂, G₁ and G₂) and CPA in the Giza Governorate, in summer season respectively. This represent 23.3% and 33.3 % aflatoxin positive samples for corn grain samples collected from Giza Governorate in both season. The concentration of both aflatoxin (B₁, B₂, G₁ and G₂) and CPA were ranged from (2.23 -30.5, 1.10- 17.0, 2.20-14.0, and 1.0-13) and 2.0-51.0 μ g/kg in the corn samples, in the winter season, whereas in the summer season the concentrations of both aflatoxins (B₁, B₂, G₁ and G₂) and CPA were ranged from (0.93-35.5, 2.10 – 17.0, 1.20 -20.0 and 2.0-18.0) and 2.7 -46.0, for aflatoxins and CPA, respectively. This is the first report of natural co-occurrence of CPA and aflatoxins in Egypt.

P107

Evaluation of Genotoxic Potential of Radiofrequency Electromagnetic Field (RF-EMF) in Duckweed. Cvjetko P¹, Radic S¹, Malaric K², Tkalec M¹, Pavlica M¹. ¹Faculty of Science, University of Zagreb, Zagreb, Croatia, ²Faculty of Electrical Engineering and Computing, University of Zagreb, Zagreb, Croatia.

The use of wireless communication devices has drawn considerable scientific and public attention to the issue of whether or not adverse effects result from exposure to radiofrequency radiation, especially those emitted by handheld mobiles. In the mean time, rapidly emerging modern technologies in the world of telecommunications, have led to the electromagnetic pollution which became important as much as the industrial pollution of different sources. Because of reasonable concern that electromagnetic radiation may constitute a risk to human health, many *in vitro* and *in vivo* studies have been performed in order to define the biological mechanism for the better understanding of how the above mentioned radiation exerts its effect. So far this still remains unknown. Among many different methods and techniques, the single cell gel electrophoresis, also known as the comet assay is one of the most commonly used toxicity tests to detect the unknown effect of many chemical and physical agents. The same method has also found its applicability in search for possible adverse effect of electromagnetic radiation on the integrity of DNA molecule. So far the genotoxicity evaluation of electromagnetic radiation by using the comet assay was mainly performed on animal and mammalian cells. The aim of this study was to investigate the possibility of using the alkaline protocol of the comet assay as a method for detecting DNA damage caused by radiofrequency electromagnetic fields (RF-EMF) in plant cells. Higher plants offer unique advantages as test systems such as easiness of handling, low cost, vegetative reproduction and most important good correlation to animal test systems. Duckweed plants were exposed to continuous EMF waves at field strength of 23 V/m for 4 h in the Gigahertz Transversal Electromagnetic (GTEM) cell, which excluded thermal effects. Evaluation of DNA damage in duckweed cultures exposed to 900 MHz for 4 hour has shown significant increase in DNA strand breaks compared to control sample. These findings clearly indicate that plants are suitable model system for evaluation of genotoxic potential of RF-EMF.

P108

Recent Advances Using the CD71 Based Flow Cytometric Micronucleus Analysis Method. Torous D¹, Bishop J⁴, Fiedler R⁶, Heflich R⁵, Krsmanovic L⁷, MacGregor J³, Recio L², Dertinger S¹. ¹Litron Laboratories, Rochester, NY, United States, ²Integrated Laboratory Systems, Research Triangle Park, NC, United States, ³Toxicology Consulting Services, Arnold, MD, United States, ⁴NIEHS, Research Triangle Park, NC, United States, ⁵NCTR, Jefferson, AR, United States, ⁶Pfizer, Groton, CT, United States, ⁷BioReliance, Rockville, MD, United States.

Advances have been made to the CD71 based flow cytometric (FCM) method for evaluating micronuclei (MN), and summaries from a workshop are presented here. 1) Blood MN assay in dogs: Robust responses to CP were seen in blood, even at doses with low bone marrow (BM) toxicity. Relative increases in %MN-RET in BM and blood were similar. Steady state experiments show that splenic selection against MN-RETs in blood is similar to that in rats. Data suggest that FCM scoring is efficient for integrating a chromosomal damage endpoint into routine canine toxicity tests. 2) Blood MN assay in humans: Increases in %MNRET were seen in women and in their infants who received zidovudine (AZT)-containing antiretroviral therapy prenatally; no increases were seen in those who did not receive AZT, demonstrating that transplacental AZT exposure is genotoxic in humans. 3) Blood MN assay in neonatal mice: MN were evaluated in 1 to 16 day-old mice treated neonatally and/or transplacentally with various drugs and drug combinations used to prevent mother-to-child transmission of the HIV-1 virus. Generally, the highest %MN were detected in 9 or 10 day-old mice and with drug regimens that included AZT. 4) Blood MN assay to determine mode of action: Data from studies to determine mechanisms of MN formation (clastogen vs aneugen) and pathways of response to genotoxic stress are presented, indicating that this method can be used to assess modes of action at several levels for studies in rats and mice. Funded in part by NO1-ES-35514 5) Practical threshold for MN at low doses: MN induction was studied for DNA target clastogens and a non-DNA target aneugen to evaluate dose-response relationship at low dose levels. Non-linear dose-response curves were obtained suggesting the existence of a practical threshold for these model chemicals. 6) Bone marrow MN assay in mice: Good correlation was obtained when comparing % MN-RET data from blood and BM of MMS treated mice using FCM and microscopy. This indicates that either compartment is suitable for FCM evaluation. 7) Bone marrow MN assay in rats: The CD71 method established in blood with only slight modifications can be reliably applied to fractionated BM specimens, and has been incorporated into routine BM analysis for GLP and exploratory assays.

P109

Saccharomyces cerevisiae and Probiotic Bacteria Potentially Inhibit Aflatoxins Production *In Vitro* and *In Vivo* Studies. I. With Special Reference to Their Antioxidant Activity in Albino Rat. Amra H, Rowayshed G, Damaty E, Nada S, Deabes M. National Research Center, Cairo, Egypt.

Saccharomyces cerevisiae (SC) and two strains of Lactic acid bacteria (LAB1 and LAB2) potentially inhibited *Aspergillus flavus* growth and aflatoxins production in YES liquid media. The biologically active microorganisms (SC, LAB1 & LAB2) had no toxic effects in rats when orally administered single doses of SC (10^{11} CFU ml⁻¹) and LAB1 & LAB2 (10^9 CFU ml⁻¹). Moreover, daily treatments for 15 days with the three microorganisms in liquid YES media containing Aflatoxins (2 mg/ml AFB1), produced by *Aspergillus flavus*, exhibited significant reduction in serum ALT, AST, GGT, creatinin, and BUN compared with the positive control group (*A. flavus*). Results indicated that no death occurred in any treatment groups with Aflatoxins, while it was 30% mortality in rats administered Aflatoxins-YES media only at 10 -12 days of experimental period. Blood glutathione (GSH) level significantly increased in groups treated with single-treatment of SC, LAB1 & LAB2 or with Aflatoxins containing media. However, Aflatoxins-treatment severely depleted GSH level than other treatments. The best results found in SC > LAB1 > LAB2 -YES media containing Aflatoxins. Conclusion: the tested microorganisms are safely to use as food additives or preservative due to their antioxidant actives. Our study needs further continuation in this respect.

P110

Correlation Studies Between Growth Processes in Plants and Genetic Endpoints in Mice Bone Marrow Cells. Patlolla BP¹, Sekhon BS². ¹Alcorn State University, Alcorn State, MS, United States, ²Jackson State University, Jackson, MS, United States.

Halogenated aliphatic hydrocarbons have long been regarded as a pharmacological and toxicological entity. Human exposure to complex mixtures of chlorinated compounds has been extensive and effects following long-term exposure to them can be investigated through toxicological laboratory studies. In the present study the effect of three chlorinated hydrocarbons (1,1-dichloroethane; 1,1,1-trichloroethane and 1,1,2,2-tetrachloroethane) on growth in plants and are compared with the effects of same chlorinated compounds on genetic endpoints in mice. Four different concentrations of each chemical were used in this study. Correlation coefficients (r-values) were calculated to establish the correlation and further confirmed by the Z-values. There was a strong negative correlation between the shoot length of *Zea mays* and chromosomal aberrations in mice bone marrow for 1,1-dichloroethane (r-value = -0.9400), for 1,1,1-trichloroethane (r-value = -0.9249) and for 1,1,2,2-tetrachloroethane (r-value = -0.8925). The results of this investigation indicated that observing their effects on shoot length, thus reducing the unwanted usage of animals, could monitor the harmful effects of chlorinated hydrocarbons. **Key words:** Chlorinated hydrocarbons, 1,1-dichloroethane; 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, *Zea mays*, Mice, Bone marrow cells, Chromosomal aberrations.

P111

The Effect of Age on Xenobiotic Metabolizing Enzyme Activity in Rat Liver Microsomes. Mills C¹, DeVito MJ². ¹North Carolina Central University, Durham, NC, United States, ²United States Environmental Protection Agency/NHEERL, Durham, NC, United States.

In the U.S., the number of aging adults is rapidly growing. By 2030, the number of elderly persons aged 65 and older is projected to double to 70 million. Many biochemical and physiological changes occur during the aging process which may affect the correlation between exposure, dose, and response to environmental chemicals. Normal aging of organs and systems in the body results in decreased function in numerous areas. Because the liver is a key site of metabolism, it is probable that the hepatic clearance of environmental chemicals is altered in the aging adults. Additional research is needed to determine whether older adults are at risk for having an altered sensitivity to such chemicals. The objective of this research is to illustrate that age-dependent changes in xenobiotic metabolizing enzymes may result in older adults being more susceptible to environmental chemicals or pollutants. Young and old Brown – Norway (4, 12, and 24 months) and Fisher (6, 11, 18, and 24 months) rats were sacrificed, livers were removed and hepatic microsomes were prepared. Using these hepatic microsomes, ethoxyresorufin O-deethylase (CYP1A1), methoxyresorufin O-deethylase (CYP1A2), pentoxyresorufin O-deethylase (CYP2B2), caffeine biotransformation (CYP1A2), and deltamethrin clearance (CYP2C) activity were determined. Minimal changes in enzyme activity occurred in the Brown–Norway rats at the various ages. In the Fisher rats, EROD activity significantly ($p < 0.01$) increased with age, with the 24-month-old animals having double the activity of the 6-month-old animals. PROD activity was slightly decreased in the 18 and 24 month old groups, but was not statistically significant ($p > 0.1$) CYP1A1/CYP1A2 activity (detected by the MROD assay) and CYP1A2 (detected by the caffeine biotransformation assay) showed minimal changes in enzyme activity at the different ages. Further research into the effects of aging on the metabolism of environmental chemicals may provide insight into potential altered sensitivity of the elderly to environmental pollutants. (This abstract does not represent USEPA policy.)

P112

The Sources and Genotoxic Activity of Contaminated Indoor Air. Chen G, White PA, Maertens R. Health Canada, Ottawa, ON, Canada.

Indoor air pollution is a major public health concern, particularly in developing areas. Major sources of indoor air contamination include environmental tobacco smoke, emissions from combustion of coal, biomass, incense, mosquito coils, kerosene and liquefied petroleum gas, and cooking oil fumes. This work is a review and thorough evaluation on the genotoxic hazards of indoor air and indoor emission sources. Over 500 published assessments of genotoxic activity from 63 articles were examined. The majority (i.e., 60%) of these employed the Salmonella mutagenicity assay. Other tests included *in vitro* assays with mammalian cells (15%), as well as *in vivo* animal assays and human biomonitoring studies (15%). Analysis of the indoor air Salmonella mutagenicity data in rev/m³ confirmed a statistically significant effect ($r^2=0.08$ to 0.42 , $p<0.0001$) of home location (e.g., rural or urban) and source contribution. Highest mutagenic activity values (TA98 with S9) correspond to indoor environments contaminated with emissions from coal combustion (2300.6 rev/m³), environmental tobacco smoke (770.3 rev/m³), gas stove emissions (189.6 rev/m³), and cooking fumes (106.9 rev/m³). The mutagenic activity of particles emitted by typical indoor sources revealed high values (TA98 with S9) for coal combustion (2364.2 rev/mg), environmental tobacco smoke (2364.2 rev/mg), as well as emissions from combustion of petroleum gas, wood, and home heating oil (560.2, 420.1 and 390.0 rev/mg, respectively). Analysis of over 500 matched measurements of indoor and outdoor PAH levels revealed a strong empirical relationship ($r^2=0.91$, $p<0.0001$), confirming the impact of outdoor contamination on indoor air quality. Summarized *in vitro* and *in vivo* results revealed that preparations (i.e., extracts or condensates) of polluted indoor air induce effects such as sister-chromatid exchanges, micronuclei, chromosome aberrations, DNA adducts, DNA strand breaks, and oxidative damage. A series of studies on emissions from smoky coal conducted in Xuan Wei (China) provide compelling evidence of an etiological link between human lung cancer and exposure to contaminated indoor air. Not surprisingly, ventilation appears to play a critical role in determining the genotoxic and carcinogenic hazards of indoor air.

P113

Multi-Laboratory Evaluation of an Automated, High Content *In Vitro* Micronucleus Assay. Dertinger S¹, Bryce S¹, Avlasevich S¹, Bemis J¹, De Boeck M², Van Goethem F², Beerens D², Aerts H², Collins J³, Ellis P³, White A³, Harvey J³, Rees R³, Lynch A³, Lukamowicz M⁴, Elhajouji A⁴. ¹Litron Laboratories, Rochester, NY, United States, ²Johnson and Johnson Pharmaceutical R&D, Beerse, Belgium, ³GlaxoSmithKline R&D, Ware, United Kingdom, ⁴Novartis Pharma AG, Muttenz, Switzerland.

An international, multi-lab trial was conducted to evaluate a flow cytometry-based method for scoring micronuclei in mouse lymphoma (L5178Y) cells [Avlasevich *et al.*, Environ. Molec. Mutagen. 47 (2006) 56-66]. Participating laboratories were supplied with prototype *In Vitro* MicroFlow[®] kits, and each was assigned one genotoxicant, i.e., etoposide, mitomycin C, or vinblastine, and one presumed non-genotoxicant, i.e., sucrose, dexamethasone, or staurosporine. The reference laboratory (Litron) performed experiments with each of the six agents. In each case, cells were treated continuously for 24 hrs over a range of concentrations up to 5 mg/ml, or overtly cytotoxic concentrations. Micronuclei were scored via standard microscopy and flow cytometry. In addition to enumerating micronucleus frequencies, an experimental cytotoxicity measurement that is simultaneously acquired with the flow cytometric micronucleus scoring procedure was evaluated. More specifically, latex particles served as "counting beads", and facilitated relative survival measurements that exclude the presence of dead/dying cells. For comparison purposes, several commonly used cytotoxicity endpoints were measured, including relative survival measurements based on Coulter counts. Key findings include the following: (1) significant discrepancies in top concentration selection were found when cytotoxicity measurements were based on different assays, with the counting bead approach tending to be the most sensitive for the current set of compounds. (2) Both microscopy- and flow cytometry-based scoring methods detected concentration-dependent micronucleus formation for the three genotoxic agents studied, with good agreement between the reference laboratory and the participating laboratories. (3) Whereas flow cytometric and microscopic analyses showed no significant increases for the presumed non-genotoxicants when top concentration selection was based on counting beads, significantly elevated micronucleus frequencies were observed for concentrations that were chosen based on less sensitive cytotoxicity assays. Collectively, these results indicate that rapid assessment of genotoxicity can be accomplished with a simple flow cytometric technique, and that this methodology is highly portable. Additionally, the concurrent assessment of cytotoxicity that is accomplished by the flow cytometer may help reduce the occurrence of irrelevant positive results, as it may represent a better means for choosing top concentration levels.

P114

Assessment of Mutagenicity in Hairdressers by Cytogenetic Tests. Galiotte MP¹, Kohler P¹, Mussi G², Gattás GJF¹. ¹Legal Medicine, Ethics and Occupational Health, University of Sao Paulo Medical School, São Paulo, SP, Brazil, ²Occupational Health Service, Hospital das Clínicas, São Paulo University Medical School, São Paulo, SP, Brazil.

The hairdressers are regularly exposed to potential mutagenic substances as hair dyes, waving and straightening preparations, sprays and nail products. Some reports indicate the mutagenic effect of substances in beauty products as aromatic amines, formaldehyde and ethanol. Epidemiological and occupational studies showed the occurrence of some diseases in these professionals, including cancer. In the present investigation the frequency of MN and SCGE (comet assay) was evaluated on peripheral lymphocytes of 82 women hairdressers (36 ±10 years old) from different beauty saloon from São Paulo city, Brazil, compared to a control group, 82 blood donor women (32.6 ±10 years old), with different other occupations, from the São Paulo University Hospital blood bank service. Considering the analyses of 1000 cells per individual the frequency of cells with MN (5.9 ±3.1) and the total of MN (6.2 ±3.2) observed in the hairdressers were higher ($p < 0.001$) than the observed in the control group (2.7 ±1.7 and 2.9 ±1.9, respectively). Positive results ($p = 0.005$) were also detected by analyzing 100 cells for each hairdresser (159.8 ± 71) and control (125.4 ± 64.1) by the comet assay. The frequency of cells with MN increased positively in function with the time of profession ($r = 0.22$; $p = 0.05$) suggesting individual and specific action of chronic occupational exposure. Multiple regression analysis showed that not only the profession but also the tobacco consumption were associated with the higher incidence of cells with MN and comet ($p < 0.05$). The genetic damage observed in hairdressers could be associated to the chronically exposure to the occupational environment. Some other cytogenetic and molecular tests like chromosomal aberration and xenobiotic metabolizing gene polymorphisms are under analysis. Considering that hairdressers profession is not officially regulate in Brazil, more attention should be focus in this worker not only by the government but also by a multidisciplinary team intended to suggest risk prevention and control proceedings for chemical, physical and biological agents that hairdressers are exposed (LIM-40-HC-FMUSP).

P115

GSTM1, GSTT1, GSTP1, CYP2E1, CYP1A1, and XRCC1 Gene Polymorphism and the Risk of Head and Neck Cancer in Brazil. Gattás GJF¹, Curioni OA^{1,2}, Kohler P¹, Siraque MS¹, de Carvalho MB², Wünsch-Filho V³. ¹Medical School, University of Sao Paulo, Sao Paulo, SP, Brazil, ²Heliópolis Hospital, Sao Paulo, SP, Brazil, ³School of Public Health, University of Sao Paulo, Sao Paulo, SP, Brazil.

The head and neck cancer incidence and mortality rates are high in Brazil, mainly in the southeast part of the country, associated to tobacco consume, alcohol intake, dietary habits, and HPV infection. Genetic polymorphism of xenobiotic enzymes such as CYPs (cytochrome P450), GSTs (glutathione-S-transferase), and DNA repair genes have been implicated in the individual susceptibility to head and neck cancer. We here evaluated the frequency of *GSTM1*, *GSTT1*, *GSTP1BsmA*, *CYP2E1PstI*, *CYP1A1MspI*, and *XRCC1* (Arg194Trp and Arg399Gln) polymorphism in 210 histologically confirmed head and neck cancer patients (186 men and 24 women, mean age 54.8 ± 10 years old) and 244 control subjects (225 men and 19 women, mean age 53.6 ± 11 years old) admitted as in-patients or out-patients in the same hospital, by means of PCR-RFLP methods. The known risk factors for head and neck cancer as tobacco and chronic alcohol liquor intake were confirmed. *GSTM1* null alone (odds ratio [OR], 1.9; 95% confidence interval [95% CI], 1.32-2.80) or the joint effect of *GSTM1* null and *CYP1A1* polymorphism (OR, 1.87; 95% CI, 1.01-3.46) increased the risk of head and neck cancer. Tendency to significance was observed to the association of *GSTM1* null and *CYP2E1* polymorphism (OR, 2.93; 95% CI, 0.98-8.71). The variant genotype with *XRCC1* 194Trp exhibited approximately 2-fold elevated risk (OR = 1.94, 95% CI = 1.00-3.76, $p = 0.05$) for head and neck cancer. Similar results were observed when combined *XRCC1* 194Trp variant genotype with *GSTM1* null (OR = 2.72, 95% CI = 1.09-6.78, $p = 0.03$) or *CYP2E1PstI* polymorphism (OR = 1.60, 95% CI = 1.03-2.63, $p = 0.01$). In contrast *XRCC1* 399Gln allele was associated with a protection for head and neck cancer (OR = 0.55, 95% CI = 0.35-0.86, $p = 0.009$). The gene-environmental effect revealed increased cancer risk when *CYP2E1* mutated genes were considered (OR, 11.9; 95% CI, 4.03-35.68) compared to alcohol intake of at least 40g/L/day (OR = 6.7; 95% CI, 3.60-12.50). The results suggest that both environmental and genetic factors play a role in the risk of head and neck cancers. Preventive actions should be improved with polymorphic susceptibility genes evaluation, especially in the follow up of patients already under risk like alcoholics and heavy smokers (Partially supported by FAPESP, LIM-HC-FMUSP).

P116

Mechanisms Underlying Prevention of Genomic Instability in Breast Tumor Cells by Genistein and Lycopene. Batoon AK, Leszczynska J, Klein CB. New York University, Tuxedo, NY, United States.

In previous studies we have identified several dietary antimutagens that reduce the elevated spontaneous mutagenesis levels in human breast and colon tumor cells. Genistein (3.125 μ M, 24 hrs) or lycopene (2 μ M, 24 hrs) reduce the elevated spontaneous *HPRT* mutant fractions intrinsic in the breast tumor cells MCF-7 (ER α +), MDA-MB-468 (ER α -), and BT-474 (ER α +/-) by 31% to 49%. The antimutagenic effects were similar in all three cell lines regardless of their ER α status. Coordinately, all three cell lines exhibited reduced frequencies of micronuclei. Analysis of microsatellite instability (MSI) showed that prevention of MSI at the G29672/p53 MSI marker was informative for 1-week exposures to genistein or lycopene in MCF-7 single cell clones. However, this MSI marker was not informative for MDA-MB-468 and BT-474 cells, both of which are already defective in p53 function. Previously, we reported that MCF10A non-tumor breast cells pre-treated with genistein (12.5 μ M, 24 hrs) or lycopene (20 μ M, 24 hrs) prior to X-ray exposure were protected against X-ray-induced aneuploidy. New data at the lowest antimutagenic concentrations of genistein (3.125 μ M, 24 hrs) or lycopene (2 μ M, 24 hrs) also shows protection against X-ray induced MSI (G29672/p53) in the non-tumor MCF10A cells. Apoptosis was investigated by flow cytometry as a possible mechanism of these antimutagenic agents, but was not observed at the very low dietary-relevant concentrations of genistein or lycopene utilized in this study. At higher concentrations of genistein (50 μ M, 48 hrs), a G2/M shift was observed, consistent with the literature. We have found that both genistein (3.125 μ M, 1 week) and lycopene (2 μ M, 1 week) modulate the methylation patterns of the GSTP1 tumor suppressor gene in MDA-MB-468 cells. RT-PCR studies confirm a lack of GSTP1 expression in untreated MDA-MB-468, with restoration of GSTP1 expression after genistein treatment (3.125 μ M, 1 week). Methylation studies of GSTP1 and other genes are ongoing in MCF-7 and BT-474 cells as well. In summary, genistein and lycopene both attenuate the ongoing genomic instability present in tumor cells, possibly mitigating further tumor progression. [This research was supported by the Susan G. Komen Breast Cancer Foundation BTCR0402531.]

P117

Independent Validation of the GreenScreen HC GADD45 α -GFP Genotoxicity Assay Using Fifty Proprietary and Non-Proprietary Compounds. Olaharski AJ¹, Kirchner S², Uppal H¹, Kolaja K¹. ¹Hoffmann-La Roche, Palo Alto, CA, United States, ²Hoffmann-La Roche, Basel, Switzerland.

The GreenScreen HC *GADD45 α -GFP* genotoxicity assay has been reported to be a sensitive and specific assay that is highly concordant with regulatory genotoxicity as well as rodent carcinogenicity test data. (Hastwell et al., 2006). Interestingly, the assay discriminated properly amongst Ames-negative, *in vitro* mammalian-positive carcinogens and non-carcinogens, suggesting that the system effectively distinguishes between true and false-positive *in vitro* mammalian results (Hastwell et al., 2006). A modest number of fifty proprietary and non-proprietary compounds were screened in the assay to address its robustness, sensitivity, and specificity in a blinded and independent validation study. The compound list was comprised of 20 structurally diverse Roche compounds, of which 11 were identified internally as genotoxic (screening MNT or MNT and Ames positive, none requiring metabolic activation) and 9 as non-genotoxic. The non-proprietary compounds were comprised of 9 genotoxic carcinogens, 5 genotoxic non-carcinogens, 5 non-genotoxic carcinogens, and 10 non-genotoxic non-carcinogens (one compound was removed from the analysis because it required metabolic activation). Compound testing and data analysis were conducted blindly at a contract research organization by Gentronix-trained personnel. Total concordance with the established genotoxicity assays was 63% (sensitivity of 28% (7/25) and specificity of 100% (24/24)) whereas concordance with rodent carcinogenicity was 55% (sensitivity of 21% (3/14) and specificity of 87% (13/15)), with 33% of the genotoxic carcinogens properly being identified. Fourteen compounds in this validation had previously been tested in the GreenScreen assay (Hastwell et al., 2006), thirteen of which had concordant results (93%), indicating that the assay is robust and easily transferable amongst laboratories. The results from this blinded, independent validation study indicate that the GreenScreen system is a robust and highly specific genotoxicity assay, but do not support previous findings that it is particularly sensitive for detecting genotoxicants or genotoxic carcinogens. Additional work to increase the sample size of the dataset and improve sensitivity of the assay is underway.

P118

Molecular and Biochemical Evaluation of Genetic Effects of Latex of *Calotropis procera* (Ati.) on *Aspergillus terreus*. Qari S. Biology Department, Genetics, Teachers College, Uom Al-Qura University, Makkah, Saudi Arabia.

Calotropis procera (Ati.) is a plant widely distributed in Saudi Arabia. The plant is popularly known because it produces large quantity of latex. The latex of *C. procera* extract is prescribed in folkloric medicine for the treatment of various disorders such as diabetes, cough, constipation, anti-inflammatory and rheumatism. The genotoxicity of latex of *Calotropis procera* was not demonstrated at yet. So many investigations are required as a battery of tests to establish its genotoxic effects. Dense conidial suspensions of *Aspergillus terreus* were treated with elevated concentrations of *Calotropis* latex. Samples were taken at regular intervals in each treatment and assayed for survival percentage, auxotrophic mutants, quantities of DNA and total protein and further subjected to RAPD and SDS-PAGE tests. It was found that the latex of *Calotropis procera* (milk weed) has potent lethal and mutagenic activities. Survival percentage decreased as concentration or time of exposure increased. Frequency of auxotrophic mutants increased with increase in concentration or exposure time. Most auxotrophic mutants were amino acid requiring mutants. Quantity of DNA of each mutant was significantly less than control and so on for total protein. The RAPD results demonstrated polymorphic numbers of the genetic bands, which were the electrophoretic products of PCR for all mutants compared with the wild type strain. SDS-PAGE results expressed a polymorphism of protein bands as well. All these results strongly point out the mutagenicity of the latex of *Calotropis procera*.

P119

Dose-Response and Apparent Thresholds for DNA Adducts and Cytogenetic Effects in Rats Treated With MMS. Pottenger LH, Zhang F, Schisler MR, Charles GD, Wiescinski CM, Bartels MJ, Gollapudi BB. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, United States.

The existence of thresholds for genotoxic effects is a controversial topic. This study investigated the relationship between the internal dose of methyl methanesulfonate (MMS) and the induction of micronuclei (MN) in peripheral blood reticulocytes of male F344 rats. The internal dose was quantified by analyzing methylated DNA adducts (N7methylguanine (N7MeG) and O⁶methylguanine (O⁶MeG)) in white blood cells (WBC) and hemoglobin (Hb) adducts in red blood cells, using signature peptides (SP). The rats (6/group) received four consecutive daily oral doses of 0 (vehicle), 0.5, 1, 5, 25, or 50 mg MMS/kg body weight/day (mkd). Blood samples were collected 24 hr after the last dose for the analysis of Hb and DNA (pooled samples) adducts, as well as the flow cytometric analysis for MN. Isolated DNA was acid-hydrolyzed and Hb and DNA digests were analyzed using LC/nESI-MS/MS and LC/pESI-MS/MS, respectively, as described elsewhere to determine levels of methylated-Hb (Me-Hb) SP and N7- and O⁶MeG. While repeated MMS treatment clearly increased MN frequency at the higher doses (25 and 50 mkd MMS; $p < 0.05$), treatment with the lower doses did not result in statistically significant increases in %MN-RET: 0.36 ± 0.12 ; 0.42 ± 0.16 ; 0.33 ± 0.17 ; 0.52 ± 0.25 ; 1.65 ± 0.33 ; and $5.95 \pm 1.42\%$ MN-RET, respectively, for control, 0.5, 1, 5, 25, and 50 mkd MMS. The WBC N7MeG DNA adduct data demonstrated clearly increased levels compared to control values only at doses above 1 mkd MMS, with apparent thresholds for repeated treatment with MMS at 0.5 and 1 mkd MMS: 16.82, 18.61, 18.93, 26.08, 66.51, and 108.55 N7MeG/ 10^6 nt, respectively, for control, 0.5, 1, 5, 25, and 50 mkd MMS. O⁶MeG was not quantifiable (LLQ = 0.05 O⁶MeG/ 10^6 nt) at any dose. The N-terminal Me-Hb adduct SP data demonstrated systemic delivery of MMS at all doses, with 0.14, 0.16, 0.18, 0.22, 0.40 and 0.70 μg Me-SP/mg alpha-Hb, respectively, for control, 0.5, 1, 5, 25, and 50 mkd MMS. These results demonstrate the existence of thresholds for the induction of MN *in vivo* following repeated treatment of F344 rats with the potent alkylating agent MMS.

P120

Human Biomonitoring Studies in the Indian Population Using the Alkaline Comet Assay. Dhawan AJ, Bajpayee M, Pandey A, Parmar D. Industrial Toxicology Research centre, Lucknow, UP, India.

The alkaline comet assay or single cell gel electrophoresis has found wide acceptance as a tool for biomonitoring studies due to its being a simple, sensitive, rapid and relatively inexpensive technique. Studies, using the assay were undertaken to assess the DNA damage in the Indian population. Our study, undertaken to measure the basal level of DNA damage in a normal, healthy Indian population have shown a gender-related difference with males exhibiting a significantly ($p < 0.05$) higher level of DNA damage by comparison with females. This was evident by an increase in the Olive tail moment, tail DNA (%) and tail length (μm). Our data revealed that amongst the males aged between 23-57 years, a significant increase in the DAN damage was observed in smokers by comparison with non-smokers and non-vegetarians in comparison to vegetarian. The degree of damage among smokers was related to the extent of smoking and age was also found to influence the level of DNA damage. An attempt was also made to assess the level of DNA damage in the lymphocytes of a group of people, engaged in excessive physical exercise, for almost 8-10 hours per day, as an occupation. A significant increase ($p < 0.05$) in the level of DNA damage was observed in these subjects when compared with matched controls. The study revealed stress related to excessive physical activity, to be a cause of DNA damage. A significant increase in DNA damage was also observed in women using biomass fuels for cooking. In conclusion, these studies have for the first time, revealed differences in the extent of DNA damage in the normal Indian population depending on their gender, eating and smoking habits, age and physical activity. 1) Mutation Research, 474 (1-2): 121-128, 2001. 2) Mutation Research, 520 (1-2): 83-91, 2002. 3) Mutation Research, 565 (2): 163-172, 2005. 4) Environmental and Molecular Mutagenesis 45 (5) 435-441, 2005. 5) Environmental and Molecular Mutagenesis 47(1): 25-30, 2006.

P121

Technique for Culturing *Macaca mulatta* Peripheral Lymphocytes for FISH and Classical Cytogenetic Analyses. Petibone DM¹, Morris SM², Hotchkiss CE³, Mattison DR⁴, Tucker JD¹. ¹Wayne State University, Detroit, MI, United States, ²US Food and Drug Administration, Jefferson, AR, United States, ³The Bionetics Corporation, Jefferson, AR, United States, ⁴National Institutes of Health, HHS, Bethesda, MD, United States.

The rhesus monkey (*Macaca mulatta*) has long been an important model in biomedical and behavioral research. The macaque's scientific importance is due to its 93% genetic similarity with humans and its demonstrated complex social behavior. With the recent sequencing of the rhesus genome, the role it plays in biological research is likely to become more important. However, the use of the macaque as an experimental model in cytogenetic assays has been problematic, due to the difficulty of obtaining large numbers of well-spread cells in metaphase without the use of extremely toxic mitogens such as staphylococcal enterotoxin A (SEA). Here we describe a technique for culturing and producing sufficient numbers of cells in metaphase using the common mitogens phytohemagglutinin (PHA) and concanavalin A (ConA) with a T-cell growth factor (TCGF). The PHA, ConA and TCGF act synergistically to induce rhesus T-lymphocyte division in 48 hour cultures. Using this method we have obtained a mitotic index of 12.0 \pm 2.2 metaphases/100 cells [$n=5$ animals]. Obtaining well-spread chromosomes was accomplished through the use of a 60 mM KCl hypotonic treatment for 60 minutes, 3 fixations with 3:1 methanol:acetic acid followed by suspension in glacial acetic acid and dropping the cells onto slides over a 70°C water bath. FISH painting of rhesus cells was performed with human whole-chromosome probes that labeled the following chromosome pairs for human[rhesus]: 1[1], 2q[12], 2p[13], 4[5] pairs in red and 3[2], 5[6] and 6[4] pairs in green. This probe combination, which results in 3 chromosome pairs each labeled in red and green in humans, results in 4 chromosome pairs labeled red and 3 labeled green in the rhesus macaque. All the monkey chromosomes are painted along their entire length, i.e. there are no inter-chromosomal rearrangements in these chromosomes between these species. There are a number of inversions present but these do not interfere with identification of translocations induced by environmental exposures. Using the methods described here, we show a baseline frequency of 0.02 cells with translocations/100 cells in peripheral blood lymphocytes of unexposed adolescent monkeys. These methods will add to the usefulness of the rhesus as an animal model in biomedical research.

P122

Biological Monitoring and DNA Damage Evaluation of Occupational Exposure to Organic Solvents. Groot H¹, Narváez DM¹, Ortiz S¹, Rodríguez AF¹, Torres C², Varona M³. ¹Laboratorio de Genética Humana, Universidad de los Andes, Bogotá, Colombia, ²Universidad El Bosque, Bogotá, Colombia, ³Instituto Nacional de Salud, Bogotá, Colombia.

The production and use of paints stand for the main source of occupational exposure to organic solvents. Xylene, toluene and acetone are some of the solvents usually found in paints. Human *in vivo* studies suggest that exposure to toluene does not cause genetic damage. Benzene, sometimes occurring as an impurity in the solvents, is a well-known clastogen. In the present study we established the urinary levels of benzene, toluene and xylene metabolites and evaluated the level of DNA damage in workers exposed and non-exposed to organic solvents. The study group involved 90 workers, 30 were occupationally exposed to organic solvents in paints factories and 60 non-exposed. Blood and urine samples were obtained from all individuals 48 hours before the exposure and at the end of the working week. Urine metabolites were assessed using GC-FID and HPLC-UV. DNA damage was estimated using the comet assay. The parameters evaluated were cell morphology and tail length. A questionnaire from each worker was used to create a database that was processed using Stata. Continuous variables were analyzed using mean, median and standard deviation. Student T-test was used to compare independent groups. The organic solvents used by the workers were: varsol (86,7%), thinner (63,3%), xylene (33,3%), toluene and acetone (23,0%) and benzene (3,3%), along with glues, gasoline and cleaners. The 96,7% of the exposed workers had previous occupational exposure to organic solvents. None of the workers suffered from diabetes, heart disease or cancer. The urinary metabolites levels were above the limit for meta-methylpuric (11 workers), hipuric acid and ortho-methylpuric (4 workers) and phenol (1 worker). The comet assay revealed that most cells from both exposed and non-exposed workers had low DNA damage (62% and 70% respectively). Exposed workers showed a higher percentage of medium DNA damage cells (19%) in comparison to non-exposed workers (8%) ($p=0.0007$). There was no significant difference in tail length between the two groups. In the present study, occupational exposure to organic solvents exceeded the biological industrial exposure limits in 16 out of 30 exposed workers. This exposure may be associated to higher levels of DNA damage in comparison to non-exposed workers.

P123

Failure of Antimony Trioxide to Induce Micronuclei or Chromosomal Aberrations in Rat Bone Marrow After Sub-Chronic Oral Dosing. Kirkland D¹, Whitwell J¹, Deyo J², Serex T³. ¹Covance Laboratories, Harrogate, United Kingdom, ²Eastman Chemical Company, Kingsport, TN, United States, ³Arcadis BBL, Walnut Creek, CA, United States.

Sb₂O₃ Antimony trioxide (Sb₂O₃, CAS 1309-64-4) is widely used as a flame retardant synergist in a number of household products, as a fining agent in glass manufacture, and as a catalyst in the manufacture of various types of polyester plastics. It does not induce point mutations in bacteria or mammalian cells, but similar to many other metals is able to induce chromosomal aberrations (CA) in cultured cell *in vitro*. Although no CA or micronuclei (MN) have been induced after acute oral dosing of mice, repeated oral dosing for either 14 or 21 days resulted in increased CA in one report but did not result in increased MN in another. In order to further investigate its *in vivo* genotoxicity, Sb₂O₃ was dosed orally to groups of rats for 21 days at 250, 500 and 1000 mg/kg/day. There were no clinical signs of toxicity in the ATO-exposed animals except for some reductions in bodyweight gain in the top dose group. Toxicokinetic measurements in a separate study confirmed bone marrow exposure, and at higher levels than would have been achieved by single oral dosing. Large numbers of cells were scored for CA (600 metaphases/sex/group) and MN (12000 PCE/sex/group) but frequencies of CA or MN in Sb₂O₃ treated rats were very similar to controls at all doses, and not biologically or statistically different. These results provide further indication that Sb₂O₃ is not genotoxic to the bone marrow of rodents after 21 days of oral administration at high doses close to the maximum tolerated dose.

P124

How to Setup and Qualify New High Throughput Screening Assays in a Contract Research Laboratory. Pant K, Bruce SW, Sly JE, Elias CA, Escobar PA. BioReliance, Rockville, MD, United States.

In the recent years preclinical testing has moved toward non-GLP *in vitro* screening tests, due to their predictive capacity of the standard genotox battery and the low amounts of compound needed for testing. These non-GLP screening assays are the link between the drug discovery/development interface that will allow companies to eliminate drug candidates with positive genotoxicity data. The Ames II and GreenScreen HC assays are gaining popularity as investigative and preliminary screening assays prior to performing the standard battery for genotoxicity testing. GreenScreen assay HC (GADD45d) with human cells provides means to investigate a compound's genotoxicity and cytotoxicity end points. Where as the Ames II assay gives information regarding the mutagenic aspect of a compound. These two assays complement each other. For the GreenScreen assay qualification at BioReliance, a number of known Genotoxic agents were chosen from different groups of compounds including – direct acting genotoxins, topoisomerase inhibitors, aneugens, nucleotide/DNA synthesis inhibitors, compounds negative in Ames assay but positive with *in-vitro* mammalian tests, and *in-vitro* genotoxicity negative compounds. All these compounds gave the expected results in comparison to the published data in the GreenScreen assay¹. In the Ames II assay, the test articles were – direct acting genotoxins, compounds negative with *in-vitro* genotoxicity tests, compounds requiring metabolic activation and nucleotide/DNA synthesis inhibitors. The results of Ames II assay with all these compounds were as expected in comparison to results in the standard Ames assay and the published data in the Ames II assay². The Ames II and the GreenScreen assays performed well with the known genotoxic agents. BioReliance has yet to discover how well these tests will perform with unknown or coded compounds, though informal reports from scientists at both Gentronix Ltd. and major pharma using GreenScreen HC, suggest that in 2 different collections of pharmacologically active articles, the assay gives positive results for approximately 10% of compounds. ¹Hastwell P. W. *et al.* 2006 ²Gee P. *et al.* 1998.

P125

Association of DNA Damage and the Polymorphisms of the Enzymes CYP2E1, GSTM1 and GSTT1 in a Population Exposed to Organic Solvents. Groot H¹, Rodríguez AF^{1,2}, Ortiz S¹, Narváez DM¹, Torres C³, Varona M⁴. ¹Laboratorio de Genética Humana, Universidad de los Andes, Bogotá, Colombia, ²Facultad de Medicina, Universidad de los Andes, Bogotá, Colombia, ³Universidad El Bosque, Bogotá, Colombia, ⁴Instituto Nacional de Salud, Bogotá, Colombia.

Organic solvents are highly volatile chemical compounds widely used in the industry; therefore toxicological evaluation of this type of exposure is of great concern. It is known that the inherited genetic characteristics, as well as other factors, determine the susceptibility of an individual to a toxic agent. Wherefore genetic polymorphisms of some metabolic enzymes (phase I and II) can be useful as differential susceptibility markers. The aim of this study was to determine the usefulness of the genetic polymorphisms of metabolic enzymes CYP2E1, GSTM1 and GSTT1 as susceptibility biomarkers in individuals exposed and non-exposed to organic solvents. The study population consisted of 90 workers, 30 exposed to organic solvents in paints industries and 60 non-exposed. Blood samples were obtained and genomic DNA was isolated. The CYP2E1, GSTM1 and GSTT1 polymorphisms were genotyped using PCR. The frequency of the genotypes was then correlated to the results obtained from the assessment of DNA damage level with the comet assay. The GSTT1 null genotype was somewhat more prevalent in the exposed workers (46.7%) than among the non exposed workers (33.3%), whereas the distribution of the GSTM1 null genotype was slightly similar in both groups (56.7% and 50%, respectively). In the whole study population, the frequency of GSTT1 and GSTM1 null subjects was 37.8% and 52.2% respectively. Regarding the genotype frequencies found for CYP2E1, restriction with PstI/RsaI and DraI revealed that c1c1 and DD were the most widespread genotypes among the study population and that they were less frequent in the exposed population when compared to non exposed workers. Correlation between genetic polymorphisms and DNA damage level showed that men with the c1c1 genotype have higher levels of DNA damage than women. Moreover, c1c1 individuals who had been exposed to organic solvents between 12 and 36 months showed greater DNA damage; however, the statistical analyses demonstrated no significant differences between groups. These results show evidence of the importance of susceptibility biomarkers at different levels of risk assessment, as they help to understand individual risk factors and allow making estimations about the increase of risk in subpopulations.

P126

Genomic Instability in Show Workers Modulated by Polyvitamin-Polymineral Treatment. Evaluation of Therapeutic Effect Before and After 4 Months 2 Tablets Daily Intake of Centrum Using Pancentromeric FISH. Vaglenov A¹, Lalchev S², Marcos R³, Creus A³. ¹The University of Findlay, Findlay, OH, United States, ²Medical University, Sofia, Bulgaria, ³Autonomous University of Barcelona, Barcelona, Spain.

The objective of the present study was to determine genotoxic, anti-genotoxic and therapeutic effects, radiosensitivity and induced radioresistance prior and following four months two tablets daily intake of Centrum using cytokinesis-block assay in peripheral blood lymphocytes. A part of investigated population was additionally examined using pancentromeric FISH to evaluate whether the working environment possess clastogenic or aneugenic activity. All examined 35 low exposed, 19 high exposed and 35 controls were women. Solvents exposure in the air of working area was done twice, before and after termination of the prophylaxis. Main compounds of occupational exposure were toluene 76-236 mg/m³, gasoline 360-468mg/m³ and acetone 396-764 mg/m³. There were slight increase in the frequency of BNMN between low exposed and control group and this increases was very significant in the highly exposed workers, which would indicated dose dependent levels of genotoxic risk. After four months therapeutic treatment, the frequencies of BNMN fall down for all examined groups, even lower than those obtained from the control group before therapy. Prior to treatment the *in vitro* irradiation revealed adaptive response for the highly exposed group only. After treatment a significant increases of radioresistance is observed. The mean frequency of centromere positive micronuclei was 37.2% for the control group (12 persons investigated), 67.5% for the low exposed (10 persons investigated), and 69.5% for the highly (11persons investigated) exposed workers which suppose a clear aneugenic effect of working environment on human lymphocytes. After 4 months therapy with 2 tablets daily intake of Centrum the main frequencies of centromere positive micronuclei dropped to 26.8% for the control group, 32.6% for the low exposed and 32.9% for the highly exposed workers. Thus Centrum may be used for antimutagenic and anticarcinogenic prophylaxis, protection and therapy of persons occupationally exposed to mixture of solvents.

P127

Comparative Mutagenic Effect of Structurally Similar Flavonoids, Quercetin and Taxifolin, on the Tester Strains Salmonella TA102 and E. coli WP-2. Makena P, Chung K-T. The University of Memphis, Memphis, TN, United States.

Quercetin and taxifolin are structurally similar plant polyphenols. Both have been reported to have therapeutic potential as anti-cancer drugs and antioxidants. Free radical mediated mutagenicity of quercetin and taxifolin, was evaluated in the Ames Salmonella microsomal mutagenicity assay using tester strain TA102 and *E. coli* WP-2. In the presence of rat liver S9 mix, quercetin was mutagenic to TA102 and WP-2 tester strains; whereas, taxifolin was not mutagenic to both the tester strains. In the absence of rat liver S9 and in the presence of iron (II) and NADPH, quercetin significantly induced mutations in both the tester strains; whereas, taxifolin did not. Both quercetin and taxifolin were not mutagenic to TA102 and WP-2 in the presence of NADPH alone. Incorporation of ascorbate and iron chelators such as desferroxamin and ethylenediaminetetraacetate in the test systems markedly decreased the quercetin induced mutations in both tester strains. These results suggest that quercetin could induce mutation in the presence of rat liver S9 or iron (II) plus NADPH by one electron oxidation through Fenton and redox cycling reactions to produce oxygen free radicals; whereas, taxifolin could not. The minor structural variation between the two plant polyphenols could cause a marked difference in their genotoxicities. These results provide basis for further study into the potential use of quercetin in combination of iron supplement. Our results suggest that the use of quercetin to the patients with iron disorders such as hemochromatosis as an antioxidant should be carefully evaluated.

P128

REV1 Inhibition Reduces the Incidence of Carcinogen-Induced Murine Lung Tumors. Dumstorf CD, Mukhopadhyay SM, Zacharias WZ, McGregor WM. University of Louisville, Louisville, KY, United States.

Recent advances in understanding the molecular mechanisms of mutagenesis indicate that most mutations are dependent on the activity of DNA translesion synthesis (TLS) polymerases. The impact of reducing these polymerases in mutagenesis and carcinogenesis in mouse models remains poorly defined. Recent evidence implicates REV1, a Y-family polymerase, as an integral component in the mutagenic bypass of replication blocking lesions. Current data suggest that REV1 serves as a structural component tethering other TLS polymerases at the site of damage with its polymerase function perhaps subservient in TLS. We hypothesized that a reduction in the mutagenic load, through REV1 inhibition, would reduce the incidence of lung cancer formation in mice injected with benzo[a]pyrene (B[a]P). To examine this, we developed strategies to reduce REV1 in the mouse lung using vectors encoding REV1 mRNA targeting ribozymes. These vectors were complexed with polyethylenimine, a non-viral cationic polymer, and delivered to the lung via aerosol. We observed a lowering of endogenous REV1 transcript up to 50% in the bronchial epithelium as determined by real time RT-PCR in laser capture microdissected cells. This reduction when done before B[a]P injection was found to effectively decrease the multiplicity of carcinogen-induced lung tumors from 6.4 tumors/ mouse to 3.7 tumors/ mouse. Additionally, REV1 inhibition completely abolished tumor formation in 27% of the B[a]P treated mice. The reduction of REV1 had no observable effect on the size or types of tumors in both control and treatment groups. REV1 inhibition had no influence on tumor development when given four weeks after B[a]P. These data support the hypothesis that REV1 reduction likely decreases the formation of B[a]P induced mutations and inhibits tumor formation at the initiation step of murine lung carcinogenesis. This work was supported by a grant from the Kentucky Lung Cancer Research Board and by NIH CA112197 and CA112664.

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Interaction Between Bleomycin and Aminoacridines in an Assay for Mutagenic and Recombinagenic Effects in Yeast. Hoffmann GR, Ronan MV, Sylvia KE, Tartaglione JP. College of the Holy Cross, Worcester, MA, United States.

Interactions between bleomycin (BLM) and several classes of amines have been studied with respect to genetic effects in strain D7 of *Saccharomyces cerevisiae*. Various amines modulate the genotoxicity of BLM in yeast and other organisms, and the interactions include antimutagenic effects, potentiation, and synergy, with the differences depending on the class of amines, the organism, and physiological conditions. The broad array of possible interactions of BLM with other agents is of interest as a model for studying mechanisms of antimutagenicity and potentiation. There can also be implications for BLM therapy if the DNA-damaging activity of BLM in tumor cells or nontarget cells were enhanced or diminished by other agents; such interactions may have therapeutic utility, but they also raise the possibility of unforeseen hazards. In yeast strain D7, aliphatic amines that bind in the grooves of DNA are antimutagenic, whereas aminoacridines that intercalate between the base pairs accentuate the activity of BLM. In this study we measured effects of aminoacridines on the induction by BLM of mitotic gene conversion at the *trp5* locus and reversion of the *ilv1-92* allele. The acridine compounds included simple intercalators that do not react covalently with DNA and aminoacridine derivatives that intercalate and also form adducts in DNA owing to mustard side chains or a nitro group. Procedures included cotreatment of growing yeast in medium and separate intercalation of acridines during growth followed by BLM treatment of nongrowing cells in buffer. Dose-dependent potentiation of BLM was observed with the intercalators 9-aminoacridine, quinacrine, proflavine, and acridine orange; the acridine mustards ICR-191 and quinacrine mustard; and a series of nitroacridines that includes nitracrine and Entozon. In cases in which the acridines were themselves genetically active in strain D7, there was synergy between the acridine compound and BLM. Methods have been developed to quantify the synergistic interactions.

P130

Cytotoxicity and its Impact on the *In Vivo* Comet Assay. Vasquez MZ. Helix3 Inc., Morrisville, NC, United States.

Among the various mechanistic-based techniques for evaluating the genotoxic activity of a compound, the *in vivo* comet assay is increasingly regarded as one of the more valuable approaches. Current guidelines for *in vivo* comet assay genetic toxicology testing recommend that the highest dose tested should be defined as (a) the dose that produces signs of toxicity or excessive cytotoxicity in the target organ or (b) 2000 mg/kg body weight for single or multiple treatments up to 14 consecutive days for nontoxic substances. The recommended lower dose is defined as approximately 25-50% of the highest dose. However, the guidelines were based on conventional and less sensitive *in vivo* genotoxicity tests. As a result, they do not take into consideration the higher sensitivity of the comet assay or the influence cytotoxicity may have on migration data. If cytotoxicity is not taken into consideration during comet assay study design and conduct and/or during data interpretation, there is a significant risk of the misinterpretation of data when determining the genotoxicity of tested compounds. Therefore, the ability of the assay to detect genotoxicity is dependent on the appropriateness of the dose range selected and on the determination of the effects of cytotoxicity on cells sampled. This paper includes data from sponsor supported *in vivo* comet studies performed at Helix3. The data presented are examples of the possible effects of cytotoxicity on DNA migration and the interpretation of comet assay data.

P131

Evaluation of Cytotoxicity Indicators for an *In Vitro* Alkaline Comet Assay in Primary Rat Hepatocytes: Utility in Avoiding False Positive Results. Gill S, Gealy R, Barnum J, Kraynak A, Storer R, Galloway S. Merck & Co., Inc., West Point, PA, United States.

The non-genotoxic non-carcinogenic compounds with cytotoxic potential chlorpheniramine maleate (CM), dichlorophenol (DCP), amphetamine sulphate (AS) and dithiocarbamate (DTC) and the genotoxins N-nitrosodimethylamine (DMN), etoposide (ETP), gamma radiation (γ), styrene oxide (SO) and cyclophosphamide (CP) were tested in an *in vitro* comet assay in primary rat hepatocytes. The comet assay is a sensitive measure of DNA strand breaks but careful monitoring of cell viability is needed as DNA damage may occur via cytotoxic mechanisms. The following were evaluated as measures of cytotoxicity to minimize false positives in the assay: trypan blue dye exclusion (TPDE), the neutral diffusion gel assay for double-strand breaks (ND), "hedgehog" counts, and cellular ATP content. The alkaline elution DNA strand break assay (AE), which is also susceptible to false positives due to cytotoxicity, was run in parallel using previously established criteria (1, 2). True positive results: Without concomitant cytotoxicity, statistically significant increases in % DNA in tail (%DT, comet assay) and elution rates (ER, AE assay) were noted for DMN, ETP, γ and SO. For CP, only the comet assay was positive. Potential false positives: Significant increases in %DT for AS and DTC occurred without significant cytotoxicity by TBDE, HHC or ATP. In AE, increases in ER for CM, DCP and AS were at higher doses, associated with decreased TBDE or ATP, and thus not considered positive. DTC did not increase ER at any dose. Increases in HHC were noted only for CM. These were associated with increases in %DT and decreased TBDE, and so, HHC may be a useful indicator of toxicity, but not for all compounds. ND was the most labor-intensive method. When conducted with RNase, ND did not reveal any cytotoxicity. ND without RNase correlated inversely with TBDE and/or ATP for CM, AS and DTC. Here, TBDE and ATP were the most sensitive measures of cytotoxicity, but we show they may not be sufficient to eliminate some false positives in the comet assay. Use of sensitive measures of cytotoxicity and a sufficiently high threshold for positivity may improve specificity of the *in vitro* comet assay. (1) R. Gealy, et al, Mutation Res. 629 (2007) 49-63. (2) R.D. Storer, et al. Mutation Res. 368 (1996) 59-101.

P132

Genotoxicity of Polymeric Gene Carrier, Polyethylenimine (PEI) and Polyamidoamine (PAMAM) Dendrimer, in Human Blood Lymphocytes Determined by Single Cell Gel Electrophoresis and Micronucleus Assay. Choi YJ, Kang SJ, Jeon HK, Kim YJ, Chung HW. School of Public Health, Seoul National University, Seoul, South Korea.

In polymeric nonviral gene delivery systems, cationic polymers are used to condense negatively charged DNAs, generating cationic polymer/DNA nanoparticles. The nanoparticles should be dissociated in the cell nucleus for the transcription to take place, which causes a concern that the dissociated cationic polymer might interact with chromosomal DNA and induce DNA damages. It is well-known that cationic polymers are cytotoxic; however, it has not been reported whether those polymers induce damages in the chromosomal DNA of host cell. Using two widely used cationic polymers, branched polyethylenimine (PEI) 25 kDa and polyamidoamine (PAMAM) dendrimer generation 4, we investigated the genotoxic effect of the polymers in human blood lymphocytes using single cell gel electrophoresis (SCGE) assay and cytokinesis-block micronuclei (CBMN) assay. For the alkaline SCGE assay, we exposed human lymphocytes with PEI or PAMAM for 4 h. It was found that the comet tail length progressively increased as a function of the polymer concentration, indicating that the DNA damages are induced in a dose-dependent manner. PEI and PAMAM consist of non-degradable vinyl bonds and degradable peptide bonds, respectively. Therefore, the effective concentration of PEI was about 10 times lower than that of PAMAM, which suggests that the genotoxicity be related to the degradable property of the polymers. In a CBMN assay where the cells were treated with the polymers for 72 h, the frequency of micronuclei formation was increased in a polymer-treated group, which is another evidence of the polymers' genotoxic effect. All these results indicate that the cationic polymers might induce DNA damage, and a proper control of polymer degradability is critical in reducing the genotoxic effect.

P133

Genotoxic Effect of Dichloromethane on Human Lymphocytes is Associated With the Activity of GSTT1. Olvera A, Albores A, Vega L. Cinvestav-IPN, Mexico City, Mexico.

The evidence of the carcinogenicity of dichloromethane (DCM) has been derived from studies on mutagenicity in bacteria and yeasts as well as from animal bioassays. Epidemiological studies in humans have shown that those persons that were exposed by at least 10 years to DCM developed liver and pancreas cancer, suggesting that the exposure to DCM can be associated with an increase in the risk of cancer, is worth mention that these studies have not been forceful. Cancer process can be initiated when an agent (physical, chemical or biological) is capable of generating changes and/or breaks on the DNA sequence, and tumoral progression (that generally lasts many years) reflects the evolution of these lesions. Therefore, the purpose of this work is to identify through cytogenetical techniques, if DCM is capable of generating DNA damage in human lymphocytes. To this end, cultures of entire blood of apparently healthy men between 25 and 35 years old were used; the cultures of entire blood were treated with different doses of DCM (0, 15, 30, 60, 125 y 500 ppm) for 72 h. These cultures were harvested and processed according to classical cytogenetic techniques. The frequencies of sister chromatid exchanges (SCE), mitotic index (MI) and cell proliferation kinetic (CPK) were evaluated as parameters of genotoxicity, citotoxicity and citostaticity, respectively. Also, GSTT1 activity was determined through formaldehyde production according to Bogaards et al., (1993). The activity found was between 8.4 ± 4.5 pmol of HCHO/min/mg protein in a group of 7 individuals classified as *normal*, as well as from 39.7 ± 6.3 pmol of HCHO/min/mg protein in 2 individuals classified as *susceptible*. The exposure to DCM decreased MI in a dose-dependent manner ($r = 0.90$ y 0.91 $p < 0.05$ in normal and susceptible individuals, respectively). The CPK decreased at 125 ppm of DCM, and the SCE frequency increased at 500 ppm of DCM. A significant different response was observed between the GSTT1 normal enzymatic activity group of individuals and the group with high GSTT1 enzymatic activity, thus indicating a clear susceptibility to DCM genotoxic effects. Additionally, we observed that the treatment for 15 min with 500 ppm DCM inhibited MI in a similar way that the treatment for 72 h did. Our results show that DCM is highly cytotoxic in human lymphocytes and that it has genotoxic potential even at doses within the Mexican safety limit. Due to this, a review of the established maximal limits on Mexican occupationally exposed populations is advised.

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Detection of Mutagens Derived From 3,3'-Dichlorobenzidine in River Water Flowing Through an Industrial Area in Wakayama, Japan. Ohe T¹, Watanabe T², Hasei T², Wakabayashi K³. ¹Kyoto Women's University, Kyoto, Japan, ²Kyoto Pharmaceutical University, Kyoto, Japan, ³National Cancer Center Research Institute, Tokyo, Japan.

3,3'-Dichlorobenzidine (DCB), which has been assigned to be a probable carcinogen (Group 2B) by IARC, is produced as a raw material in the manufacture of polymers and dye intermediates. In our previous paper, we identified DCB as a major indirect-acting mutagenic constituent in the water concentrate from the Waka River, which is flowing through an industrial area in Wakayama, Japan. In this study, we demonstrate the detection of mutagens derived from DCB in the water samples from the Waka River. Mutagens in the river water were adsorbed to blue rayon at the site where wastewater was discharged from chemical plants into the river. The adsorbate was highly mutagenic toward YG1024 in the presence of S9 mix and in the absence of S9 mix, inducing 440,000 and 170,000 revertants/g blue rayon equivalent, respectively. This sample was classified as extremely mutagenic both with and without S9 mix according to the mutagenic potency classification. Two mutagenic fractions, which accounted for 18% and 12% of the total mutagenicity of the water concentrate in YG 1024 with S9 mix, were separated by HPLC with a reversed-phase column followed by Sephadex LH20 chromatography. Both fractions were further separated by HPLC using reversed-phase columns. On the basis of spectral data, one mutagen in the former fraction was identified as DCB and one mutagen in the latter fraction was deduced to be 3,3'-dichloro-5-nitrobenzidine derived from DCB. Furthermore, we quantified the levels of this novel mutagen and mutagenic/carcinogenic benzidine derived from DCB via monochlorobenzidine by photodecomposition.

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Effects of Co-Exposure to Electromagnetic Fields and Clastogens on DNA Damage Determined by the Alkaline Comet Assay and Chromosomal Aberration Test. Lee M¹, Kim J-Y². ¹Department of Biology, University of Incheon, Incheon, South Korea, ²Korea Institute of Toxicology, KRICT, Daejeon, South Korea.

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation *in vitro*. Here two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks): *in vitro* alkaline comet assay and *in vitro* chromosome aberration (CA) test. For these assays, the mammalian cells alone or combined with positive mutagens were applied in an artificial mobile phone frequency EMF generator with continuous waveform at a specific absorption rate (SAR) of 4 W/kg. No direct cytogenetic effect of 835-MHz RF-EMF was found in the *in vitro* CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-Nitroquinoline 1-oxide). Taken together, this and previous our results indicate that RF-EMF alone might have no mutagenic and clastogenic potentials in three short-term genotoxicity test. However, we cannot exclude the possibility that RF-EMF might enhance DNA-damaging activity of clastogens as observed in comet assay. Since human populations are probably exposed to many genotoxic agents concomitantly with radiofrequency fields, this possibility has important implications. Thus, our results encourage further investigations in these and other exposure conditions.

P136**Automated *In Vitro* Micronucleus Scoring: Adherent CHO-K1 and V79 Cells Represent an Efficient Screening Platform.**

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The *in vitro* micronucleus assay is gaining in popularity as an early screening tool for assessing chemicals' genotoxic potential. Here we describe a flow cytometric procedure for enumerating *in vitro* micronuclei in CHO-K1 and V79 cultures. With this method, necrotic and mid/late stage apoptotic cells are labeled with the fluorescent dye ethidium monoazide. Cells are then washed, stripped of their cytoplasmic membranes, and incubated with RNase plus a pan-nucleic acid dye (SYTOX Green). This process provides a suspension of free nuclei and micronuclei that are compatible with flow cytometric analysis (488nm laser). Initial experiments were directed at developing the procedures that would allow for very efficient processing, especially in regard to cell handling/staining procedures. One significant early observation was that sequential addition of staining and lysis solutions could be made directly to multi-well plates. As these specimens were found to be compatible with flow cytometric scoring, this processing method eliminated transfer and centrifugation steps that are required for suspension cultures. Furthermore, experiments with V79 cells suggest that the resulting stained nuclei/micronuclei can be stored for several days before flow cytometric analysis has to occur. Subsequently, CHO-K1 cells were treated with mitomycin C, cyclophosphamide, etoposide or dexamethasone for 4 hrs with and without S9 activation followed by 40 hrs recovery, or else for 44 hrs of continuous exposure without S9. Key findings include the following: (1) expected responses for the genotoxic and non-genotoxic agents were observed, supporting the compatibility of attachment cell lines with the automated scoring procedure that was initially developed for suspension cell lines. (2) Attachment cells can be processed very efficiently for flow cytometric analysis of micronuclei, as the staining and lysis reagents can be added directly to plates, eliminating trypsin, transfer, and centrifugation steps. (3) A simultaneous cytotoxicity measurement, relative survival, can be accomplished by the flow cytometric procedure through the addition of fluorescent microspheres ("counting beads") directly to the SYTOX staining solution.

P137**Environmental Effects on the Quality of Microarray Gene Expression Measurements.** Fuscoe JC, Han T, Melvin CD, Desai VG, Moland CL, Scully AT. FDA/NCTR, Jefferson, AR, United States.

Ozone present in the lower atmosphere rapidly degrades fluorescent cyanine dyes used in microarray experiments. While Cy3 is relatively resistant to the effects of ozone, Cy5 is highly sensitive to inactivation. In typical a 2-color microarray experiment, the ratio of the dye intensities is used as a measure of gene expression level. Therefore, uncontrolled reduction of a microarray to one dye relative to the other will result in inaccurate and irreproducible data. Ozone in central Arkansas typically ranges between 22-46 ppb and can be as high as 100 ppb depending upon season and time of day. After a 12 min. exposure of a microarray to environmental ozone, the Cy5 intensity of hybridized "in-house" printed microarrays was reduced by 40%. Similar results were seen with microarrays manufactured by Agilent Technologies. To eliminate ozone effects on microarrays, a carbon filter was installed in the laboratory air handling system, and the airflow was balanced to exclude non-filtered air from entering the laboratory. This straightforward engineering solution reduced the ozone concentration within the microarray laboratory to a constant level of approximately 2-4 ppb. Using these new conditions, no decrease in Cy5 intensity was detected during the initial 4-6 min. required to scan a microarray. Even after a 90 min. exposure to carbon-filtered air the decrease in Cy5 intensity was only 17% compared to an 86% reduction in non-carbon-filtered air. Comparison of identically hybridized microarrays exposed either to carbon-filtered air or unfiltered air demonstrated the protective effect of ozone removal on microarray data. Experiments were also conducted to examine the effect of high humidity on microarray quality. Modest, but significant, increases in Cy5 and Cy3 signal intensities were observed after 2 or 4 hours exposure at 98-99% humidity compared to 42% humidity. In conclusion, simple installation of carbon filters in the laboratory air handling system resulted in low and consistent ozone levels. This allowed the use of Cy5 and Cy3 fluorescent dyes for the accurate determination of gene expression by microarray.

P138**Evaluation of Kava Extracts and Kavalactone Standards for Mutagenicity and Toxicity.** Whittaker P², Clarke J¹, Dunkel V², Jaeger L², Seifried H³, Betz J³, San R¹. ¹BioReliance, Rockville, MD, United States, ²FDA-CFSAN, College Park, MD, United States, ³NIH, Bethesda, MD, United States.

Kava (*Piper methysticum*) is a member of the pepper family that has been cultivated by South Pacific islanders for centuries and used as a social and ceremonial drink. Traditionally, kava extracts are prepared by grinding or chewing the rhizome and mixing with water and coconut milk. The active constituents of kava are a group of about 18 compounds collectively referred to as kavalactones or kava pyrones. Kawain, dihydrokawain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin are the six major kavalactones. Kava beverages and other preparations are known to be anxiolytic and are used for anxiety disorders. Dietary supplements containing the root of the kava shrub have been implicated in several cases of liver injury in humans, including several who required liver transplants after using kava supplements. In order to study the toxicity and mutagenicity, two commercial samples of kava and the six pure kavalactones including both *d*-kawain and *dl*-kawain, were evaluated in L5178Y mouse lymphoma cells. Neither the kava samples nor the kavalactones induced a mutagenic response in the L5178Y mouse lymphoma mutation assay with the addition of human liver S9 activation.

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The Mutagenic Hazard and Carcinogenic Risk of Complex PAH Mixtures in Contaminated Soils. Lemieux CL¹, Long A^{1,2}, Lundstedt S³, Tysklind M³, White PA¹. ¹Safe Environments Programme, Health Canada, Ottawa, ON, Canada, ²Department of Biology, University of Ottawa, Ottawa, ON, Canada, ³Department of Chemistry, University of Umeå, Umeå, Sweden.

The objective of this study was to employ an *in vitro*, mammalian cell mutation assay to evaluate the validity of current risk assessment methods employed for soils contaminated with mutagenic carcinogens (e.g., polycyclic aromatic hydrocarbons or PAHs). The strategy usually employed to assess the carcinogenic risk of such complex mixtures focuses on priority analytes and assumes that the total risk is equal to the sum of that contributed by each of the identified mixture constituents. Organic components of PAH-contaminated soils were extracted using pressurized fluid extraction, and separated on silica gel columns into non-polar neutral (PAHs) and polar aromatic fractions (oxy-PAHs and N-heterocyclics). Synthetic mixtures containing 16 priority PAHs were prepared using the results of chemical analyses. The mutagenic activities of the soil fractions, corresponding synthetic PAH mixtures and individual PAHs were assessed using the *lacZ* mutation assay in FE1 Muta™ Mouse cells. Excess lifetime cancer risk attributable to each of the soils was calculated using the standard method, as well as a novel mutagenic potency ratio (MPR) method that employs mutagenic potencies to derive estimates of carcinogenic risk. A significant, concentration-related increase in *lacZ* mutations was observed for all non-polar neutral fractions, all polar aromatic fractions, all synthetic PAH mixtures, and 5 priority PAHs. Predictions of mutagenic activity, based on additivity of individual PAHs or synthetic PAH mixtures, were greater than the mutagenic activities observed for the soil fractions themselves. In most cases, the excess cancer risk estimates for the soils that were calculated using the novel MPR method were greater than 2-fold lower than those calculated using the traditional method. The results indicate that the total mutagenic hazard and carcinogenic risk of a complex PAH mixture may be far less than that calculated using a chemical-specific method and an assumption of additivity. Thus, targeted risk assessments that focus on priority PAHs will likely provide conservative predictions of mutagenic (or carcinogenic) activity. However, routine risk assessments cannot account for the hazard/risk contributed by unidentified compounds (e.g., oxy-PAHs and N-heterocyclics).

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***In Vitro* Micronucleus and Sister Chromatid Exchange Analyses in Chinese Hamster Ovary Cells Following Exposure to Whole Cigarette Smoke.** Fowler KW, Harger KM, Morgan WT, Bombick BR, Doolittle DJ, R. J. Reynolds Tobacco, Winston-Salem, NC, United States.

The *in vitro* micronucleus (MN) and the sister chromatid exchange (SCE) assays are screening tests for chromosomal damage. The objective of this work was to compare results from these assays following direct exposure to mainstream whole cigarette smoke (MWS). Chinese Hamster Ovary (CHO-WBL) cells grown in tissue culture flasks were exposed for one hour to whole smoke from 2R4F Kentucky reference cigarettes or two commercial cigarette brands under the Federal Trade Commission (FTC) smoking regimen. For each smoking, triplicate flasks were exposed to four concentrations of whole smoke with duplicate concurrent air-exposed flasks. Four replicate smokings were performed for each cigarette. Positive responses were seen in both assays with all three cigarettes. At the highest concentration of each cigarette, both the percentage of micronuclei and the number of sister chromatid exchanges increased to approximately three times the background level, although the SCE assay showed higher responses at lower concentrations than the MN assay. There were no statistically significant differences ($p < 0.05$) between the slope values determined from regression models for the three cigarettes in either assay.

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Identification of Persistent Gene Expression BioSignatures for Acute Radiation Exposure. Kulkarni R, Thomas RA, Tucker JD. Wayne State University, Detroit, MI, United States.

Developing rapid and sensitive methods to detect and quantify exposures to low-doses of ionizing radiation is greatly desired. We are interested in identifying genes with changes in expression levels that can be used as biosignatures of low-dose radiation up to 24 hours post-exposure. Quantitative Real-Time PCR (QPCR) with Taqman chemistry was used to study temporal effects of low-dose exposure on the transcription of radiation-response genes in normal human B cell lines. GAPDH mRNA levels were used to normalize the data. Based on our preliminary work, 21 putative radiation-responsive genes were selected for more detailed analyses of the effects of both dose and time since exposure. Most of these genes exhibited transitory up-regulation of expression in the first few hours immediately after exposure, and a few genes showed elevated transcript levels up to 24 hours. Genes involved in DNA damage repair and growth arrest such as Cyclin-dependent Kinase inhibitor 1A (CDKN1A), Growth Differentiation Factor 15 (GDF15), Chemokine (C-X-C motif) ligand 10 (CXCL10) and RAD51C showed statistically significant changes in the transcript levels at all doses examined (1 to 70 cGy). GDF15, RAD51C and CDKN1A showed maximal (2 to 6 fold) increases in transcript levels 4 hours after exposure. After 24 hours, the transcript levels for RAD51C and CDKN1A were reduced to 2 to 4 fold whereas GDF15 transcript levels continued to increase up to 6 to 7 fold in an approximate dose-dependent manner. CXCL10 showed a gradual 2-fold change in transcript levels out to 8 hours and the levels remained 2 to 3 fold elevated out to 24 hours after exposure. These four gene products showed a persistent response to low-doses of ionizing radiation, suggesting that they could serve as biomarkers of radiation exposure, especially in situations where sample collection might not be immediately possible. This work shows promise for the development of biosignatures using genes that are the most persistently up-regulated after exposure to ionizing radiation.

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DNA Damage Analysis of Pakistani Agricultural Workers Exposed to Mixture of Pesticides: A Follow-up Study. Bhalli JA¹, Ali T¹, Asi AR², Khan QM¹. ¹Environmental Toxicology Lab., National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan, ²Pesticides Chemistry Lab. National Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan

Man is exposed to a great deal of environmental harms that may affect the functioning of specific biomolecules, thereby damaging health at various levels and pesticides are one of these harms. To determine whether occupational exposure to a complex mixture of pesticides results in a significant increase in the level of genetic damage, a follow-up study was designed. The aim of this study was to determine the possible DNA damage in farmers chronically exposed to pesticides while pesticide spraying in agriculture fields. Leukocytes from 47 agriculture workers exposed to pesticides and 50 control individuals were evaluated with comet assay. Exposed individuals were heavily exposed to pesticides in agriculture fields. Pesticide load was evaluated from serum samples using HPLC technique. Statistically significant difference ($P < 0.001$) in DNA damage of exposed individuals (mean \pm S.D 16.90 \pm 4.95) was observed when compared with control (6.54 \pm 1.73). Of the possible confounding factors smokers had significantly larger mean comet tail length than nonsmokers and ex-smokers for both the workers (20.26 \pm 3.53 vs 14.19 \pm 4.25, $P < 0.001$) and the controls (7.86 \pm 1.09 vs 5.80 \pm 1.59, $P < 0.001$). Age had a minimal effect on DNA damage ($P < 0.05$). Years of pesticides exposure has a positive co-relation with DNA damage in exposed individuals. More than one pesticides were detected from the serum samples of exposed individuals. Our study shows that chronic exposure to pesticides produces DNA damage in pesticide sprayers. It also suggests that this type of monitoring is valuable in recommending preventive measures for pesticide sprayers.

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Identification of Novel TP53 Regulated Genes Induced by Ionizing Radiation. Bourguet F¹, Krefft A¹, Peterson LE², Coleman MA¹. ¹Chemistry, Materials and Life Sciences, LLNL, Livermore, CA, United States, ²Dept. of Public Health. The Methodist Hospital, Houston, TX, United States.

Genome-scale expression microarray data in conjunction with DNA sequence/pattern databases were used to identify and validate gene regulatory elements that synergistically interacted with TP53 to control ionizing radiation responses. First we identified radiation responsive genes, including those that were specific for high and low dose responses based on gene expression array analysis. From the array data we used computational tools to further categorize genes in to responsive groups, which identified a cluster of genes that were identified as potentially regulated by TP53. To further expand the TP53 regulatory network several *in silico* approaches were used to identify sequence level elements and transcription factor modules shared across the group of genes. This identified thousands of TP53 elements across the human genome that were synergistic with other ionizing radiation responsive elements such as SP1 and CREB transcription factor binding sites. A total of three individual modules were found within 5 of the 33 genes. These modules predicted novel IR responsive genes. QPCR verified both known and predicted IR responses in two human lymphoblastoid cell lines. Chromatin immuno-precipitation assays were used to validate TP53 binding in the proximal promoters after exposure to both low and high doses of IR. These novel elements and modules help define new IR responsive networks. This data also suggest that proximal promoter regulatory elements may act cooperatively with TRP53 to modulate the cells response at both high and low doses of IR. This information provides the basis for identifying susceptibility regulatory factors at the sequence level that are involved in individual responses to IR exposures. This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48 with funding from the Laboratory Directed Research and Development and DOE Low Dose Radiation Research programs.

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Genotoxicity and Apoptosis Induced by Hydroquinone and Benzoquinone in HL-60 and Jurkat Cells. Kim YJ, Choi JY, Woo HD, Choi YJ, Kang SJ, Chung HW. School of Public Health, Seoul National University, Seoul, South Korea.

Although benzene exposure has been associated with hemotoxicity, the underlying molecular mechanism has not been determined. In the present study, the cytotoxicities of hydroquinone (HQ) and benzoquinone (BQ) were investigated, to determine the mechanism for myelotoxicity, encompassing oxidative DNA damage, apoptosis, and cell growth. In addition, the role of myeloperoxidase:NAD(P)H:quinone oxidoreductase (MPO:NQO1) ratios in genotoxicity was investigated using MPO-deficient T-lymphoblastic leukemia cells and MPO-rich HL-60 promyelocytic leukemia cells. Even a low level of HQ (5 μ M) increased reactive oxygen species (ROS) generation by more than 50% in Jurkat cells, whereas pretreatment of the cells with N-acetylcysteine (NAC) suppressed HQ-induced production of ROS to the control level. Dose-dependent relationships were observed for benzene metabolite exposure and micronuclei frequency or tail moment in both the HL-60 and Jurkat cells. Cells exposed to a high level of HQ (50 μ M) exhibited significantly decreased mitochondrial membrane potential (MMP) and showed increased induction of apoptosis of HL-60 and Jurkat cells, compared to the controls, whereas a low level of HQ (5 μ M) resulted in S-phase recruitment in the cell cycle distribution. The addition of NAC decreased HQ-induced apoptosis and increased the MMP, which suggests that ROS production has at least a partial effect on the induction of apoptosis. In conclusion, the MPO level is not a determining factor for benzene metabolite-induced toxicity, and different cellular responses, including apoptosis induction and increased cell growth, are induced to different extents by benzene metabolites.