light (320-400 nm). While exposure of cells to UVA light alone at 0.122 J/cm2/min for 5-45 min displayed a dose-response for mutation induction in the Tk gene, with a induced mutant frequency (MF) of 136 10-6 after 30 min of light irradiation, cells received RP alone at 25-250 ug/ml for 4 hrs did not increase MF. Treatment of cells with 0.5-25 ug/ml RP under UVA light for 30 min produced a synergistic mutagenicity effect. At 25 ug/ml RP with UVA exposure (12% of relative total growth), the induced MF was about 3-fold higher than that for UVA exposure alone. Similar results were obtained from AR treatment. While AR itself was not mutagenic, it induced mutation induction in the cells when exposed concomitantly to UVA light. To elucidate the underlying photomutagenic mechanism of RP, we determined the loss of heterozygosity (LOH) at four microsatellite loci spanning the entire chromosome 11 for RP/UVA-induced mutants. Ninety three percent of the mutants lost the Tk+ allele at Tk locus, and 84% of the damage extended to 6cM in chromosome length. These results suggest that RP and AR are photomutagenic in mouse lymphoma cells through a clastogenic action.

2219 GENETIC TOXICOLOGICAL STUDIES FOR LUPINUS TERMIS ALCOHOLIC EXTRACT.

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Lupin species have been widely cultivated for human consumption and used in traditional medicine. The Lupin specie, Lupinus termis has been widely cultivated in Sudan and used as an aqueous extract to treat a variety of dermatological disorders. Lupinus termis seeds also have been used as a skin softener and in a variety of cosmetic preparations. Previous studies have shown the effectiveness of the alcoholic extract of Lupinus termis seeds in the treatment of chronic eczema. In the current study, the mutagenic and/or clastogenic potential of the alcoholic extract of Lupinus termis seeds was investigated using the Mouse Lymphoma Assay and the Micronucleus Test. In Mouse Lymphoma assay, L5178Y TK+/- cells were treated for 4 hrs with a range of concentrations of the alcoholic extract, in the presence and absence of S9. Benzo(a)pyrene and 4-Nitro-quinoline-1-oxide were the positive controls. In Micronucleus test, Balb/C male mice were treated with the 50% LD50 of the alcoholic extract. Mitomycin C (2 mg/Kg) was used as the positive. Mice were sacrificed 24 hrs and 48 hrs after treatment, the bone marrow was flushed and cell suspensions were prepared to determine the incidence of micronucleated polychromatic erythrocytes. The results obtained in these genetic toxicological assays showed that there were no differences between experimental and negative control samples. These results corroborate and complement the previously obtained results for the Salmonella typhimurium Reverse Mutation Assay. This genetic toxicological study suggests that under the experimental conditions, the alcoholic extract of Lupinus termis seeds does not has either mutagenic nor clastogenic potential.

2220 TAXOL INDUCES MUTATIONS IN THE TK GENE OF L5178Y/TK+/- MOUSE LYMPHOMA CELLS THROUGH A MITOTIC NON-DISJUNCTION MECHANISM.

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Taxol (paclitaxel) is a potent anti-cancer drug that binds to tubulin and inhibits cell division. Taxol has been shown to induce chromosome aberrations in human lymphocytes *in vitro* and to induce micronuclei in mice. However, Taxol is not mutagenic in the Ames test or the CHO/Hprt gene mutation assay. In this study, we evaluated the mutagenicity of taxol in the Tk+/- 3.7.2C mouse lymphoma assay (MLA). Three independent experiments were performed using 4-hour treatment. Taxol induced a dose-related increase in mutant frequency. The results were similar in the three experiments with a 0.75 ug/ml Taxol-induced mutant frequency of 163 x 10-6 at 30% relative total growth in one experiment. Forty two percent of the mutants from this treatment were small colony mutants. Loss of heterozygosity (LOH) analysis of 73 mutants from control cultures and 160 mutants (both small and large colony) from taxol-treated cultures was conducted using four microsatellite loci spanning the entire chromosome 11. Almost all (99%) of the taxol-induced mutants lost all 4 microsatellite markers, indicating the loss of the entire chromosome 11b containing the Tk+ allele. Only 22% of the mutants from untreated control cultures showed the same microsatellite pattern. These data suggest that taxol induces mutations in the MLA via a mitotic non-disjunction mechanism. This adds to the weight of evidence that the MLA can detect aneugens.

2221 INCREASED GERMLINE MUTATION FREQUENCIES INDUCED BY ETHYLENE DICHLORIDE IN MALE MICE.

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The ability of genotoxic agents to disrupt or cause cessation of spermatogenesis in mammalian species including the human has been known for many years. This was generally the prediction (and diagnosis) of males undergoing vigorous chemotherapy and/or radiation therapy for cancer. However, in recent years it has become recognized that many of these individuals may become fertile again. The return to fertility after a genotoxic agent induced sterile period has not been well studied. We have developed animal models for the study of the sterilizing effects of ethylene dichloride (EDC) in male C57BL/6 mice. If a sterile period is produced in males by the killing of stem germ cells, then spermatogenesis cannot continue until a critical mass of stem germ cells is reached by the "clonal" expansion of the surviving stem germ cells. Based on this theory mutant clusters, sporadic clusters mutated stem cells, should be formed upon re-initiation of spermatogenesis. The formation of mutant clusters has been demonstrated in sperm from treated Drosophila, however due to limitations in methodologies, mutant clusters in the sperm of mammalian species, i.e. mice, has not been shown as of this date. To address this problem we used the ultra-sensitive molecular intragenic mutation assay (Needle-in-a-haystack PCR/RE/LCR assay) which is capable of one-in-a-million sensitivity. Sexually mature male C57BL/6 mice received EDC i.p. injections with dosages ranging from 5 to 40 mg/kg or vehicle and subsequently held for 45 days to allow for complete turn over of spermatogenesis. Males where then paired with females to determine fertility. Mice were sacrificed after siring two litters or determining that there was permanent sterility. The 5mg/kg male mice recovered to fertility and sired litters of pups after a sterile period of up to 5 weeks. Our results showed that there was an expansion of Bmp5 short ear mutant clusters following the return to fertility, to a frequency of one-in-ten thousand in mature sperm.

2222 MICROARRAY GENE EXPRESSION ANALYSIS REVEALS DIFFERENCES BETWEEN LARGE AND SMALL COLONY THYMIDINE KINASE MUTANTS OF L5178Y MOUSE LYMPHOMA CELLS.

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The L5178Y mouse lymphoma assay (MLA) is widely used to evaluate chemicals for their ability to induce mutation. The thymidine kinase (Tk) mutants detected in the assay fall into two categories: small colony and large colony Tk mutants. Small colony mutants grow slowly and their induction is associated with clastogenicity. Large colony mutants grow at normal rates and their induction is associated with point mutations. In order to investigate the molecular basis for differences between the large and small colony Tk mutants, microarray gene expression analysis was conducted on 4 small and 4 large colony Tk mutants. Long-oligonucleotide microarrays of 20, 000 mouse genes were used in 2-color hybridizations using a reference design. Correlation (R value) within the small and large colony groups ranged from 0.90 to 0.97. The raw data was normalized using locally weighted linear regression (LOWESS) in ArrayTrack, a database with software tools developed at the NCTR. Based on cluster analysis of the gene expression changes, the large colony mutants could be distinguished from the small colony mutants. In addition, the gene expression patterns in the large colony group of mutants were very homogeneous while the patterns from the small colony group of mutants were highly heterogeneous. The Significance Analysis of Microarray (SAM) program was then used for analysis of significant gene expression changes between the two groups (1.4-fold and false discovery rate <0.05). Genes whose expression was significantly different were mapped onto the mouse karyotype using ArrayTrack. Approximately 30% of the genes whose expression was significantly increased in the small colony mutants mapped to chromosome 11, where the Tk gene resides. These results suggest that altered expression of genes near the Tk gene is associated with the small colony phenotype.

2223 MICRONUCLEUS INDUCTION AND DNA DAMAGE IN V 79 CELLS *IN VITRO* BY DUSTS FROM HARD METAL SINTERING AND DETONATION COATING PROCESSES.

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ABSTRACT Hard metal bulk dusts from a molding-sintering process and from a detonation-coating process were characterized for their ability to induce micronuclei and DNA damage in cultured V 79 Chinese hamster lung fibroblast cells. The study included the precoating mixture and overspray material from a detonation coating process, an unsintered material from a molding-sintering operation, and the tungsten carbide and cobalt ingredients from the sintering process. Doses were selected after viability testing and ranged from 0 to 200 ?g/cm2. Results indicate that the unsintered sample and pre- and post-coating detonation coating mixtures showed a positive dose-response relationship for micronucleus induction; WC alone was weakly positive for a single dose only. The overspray material from the detonation-coating process showed greater micronucleus induction than any of the other materials. Results from the DNA damage assay indicate that the three hard

metal mixtures are all capable of DNA damage, with similar dose-response curves. The addition of 10 or 20 mM N-acetyl cysteine, a general antioxidant, significantly attenuated DNA damage by all three samples. The results indicate that materials from either conventional or detonation-coating processes are capable of genotoxicity in V 79 cells *in vitro*, including DNA damage and micronucleus induction, and DNA damage is at least partially through oxidative mechanisms. The post-detonation material is a more potent inducer of micronuclei than are the predetonation and unsintered dusts, but the DNA damage potential of all three dusts are quantitatively similar.

2224 TRICHLROETHYLENE (TCE) INHALATION DID NOT INDUCE CYTOGENETIC DAMAGE IN THE RAT BONE MARROW MICRONUCLEUS TEST (MNT).

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TCE was previously reported to induce a small increase (2-3 fold) in micronucleated polychromatic erythrocytes (MN-PCEs) in the bone marrow of CD rats following a single 6h inhalation exposure. The current study attempted to replicate these findings and to correlate them to potential TCE-induced hypothermia. Male CD rats were exposed by inhalation to targeted concentrations 0, 50, 500 2500 or 5000 ppm TCE for 6h on a single day. Chamber concentrations of TCE were measured once/h. Relative body temperatures (BT) of 6 rats/group were measure pre-exposure and at 3, 19 & 48h post exposure using programmable transponders. BT was monitored at 5-min intervals 22h pre-exposure, during exposure and 19 h post-exposure in a single rat/group using radio-telemetry. Bone marrow samples were evaluated for MN formation at 19h & 48h post-exposure. Rats treated with cyclophosphamide monohydrate and sacrificed 19 hrs later served as positive controls. Actual mean chamber concentrations were 0, 53, 521, 2579 and 4677 ppm. A single 6h inhalation exposure to 5000 ppm TCE caused marked toxicity and deaths in male rats. A small, but notable drop in BT was identified via radiotelemetry at 5000 ppm during exposure but was considered inconsequential to the interpretation of the test results. No increase in MN-PCE or decrease in the relative proportion of PCE was observed in any of the TCE treatment groups. The positive control rats had significant increases in MN-PCE. In conclusion, results from the current study indicated that inhalation exposure of rats to TCE up to a maximum tolerated concentration did not induce cytogenetic damage in the bone marrow.

2225 ABILITY OF ALKYLTIN COMPOUNDS TO PENETRATE CELL MEMBRANES AND ITS RELATION TO INDUCTION OF GENOTOXIC EFFECTS IN CHINESE HAMSTER OVARY (CHO) CELLS.

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Organometals, such as tin derivatives are distributed in the environment. Human exposure to these chemicals can lead to acute and chronic intoxication. Following uptake de-/alkylation occurs that changes the bioavailability and toxicity of the tin species. In this study, the tin de-/alkylation products monomethyltin chloride (MMT), dimethyltin dichloride (DMT), trimethyltin chloride (TMT), and tetramethyltin (TetraMT) were investigated for cyto- and genotoxic effects in relation to the cellular uptake. To identify genotoxic effects micronucleus (MN) assay, chromosome analysis (CA) and sister chromatid exchange (SCE) test were used. The cellular uptake was assessed using ICP-MS analysis. The toxicity of DMT and TMT was evaluated also after forced uptake by electroporation. Our results show that the ability of organotin compounds to penetrate cell membranes modulates their genotoxicity. DMT and TMT did not induce elevated numbers of MN up to a concentration of 1 mM. Chromosome analysis and SCE revealed that DMT induced significantly elevated numbers of CA and SCE at concentration of > 1 mM. The cellular uptake of the organotin compounds was dose dependent whereas the percentage of the exposure substrate found intracellularly was always < 1%. The order of cellular uptake for the organotin compounds was: DMT > TMT > MMT > TetraMT. After forced uptake by electroporation DMT and TMT induced significantly elevated numbers of MN at concentrations of 5 and 50 microM, respectively. Altogether, our results show that the methylated tin compounds have just weak genotoxic potential. This can be explained by the poor membrane permeability of the compounds. After forced uptake of DMT and TMT, the genotoxic effects are increased. These findings suggest that methylated tin compounds are able to induce genomic damage if the membrane permeability is increased.

2226 RAT ERYTHROCYTE MICRONUCLEUS TEST: ROLE OF ERYTHROPOIESIS AND EFFECT OF CIGARETTE MAINSTREAM SMOKE.

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The genotoxicity of cigarette mainstream smoke (MS) has been investigated using the *in vivo* micronucleus test. According to guidelines (OECD 474), the highest dose at which chemicals are to be tested should be sublethal. Since MS from filter cigarettes at sublethal doses contains significant amounts of CO, and CO may enhance erythropoiesis, its effect on micronucleus formation was investigated. Erythropoiesis was also stimulated using erythropoietin (EPO). The MicroFlow kit (Litron Laboratories) was used for quantification of CD-71-positive reticulocytes (proportion of reticulocytes among total erythrocytes: RET) and micronucleated reticulocytes (proportion of micronucleated reticulocytes among total reticulocytes: MnRET) from rat blood. Sprague-Dawley rats (8/group) were nose-only exposed to MS from the Reference Cigarette 2R4F at concentrations up to 1250 µg TPM/l (1400 ppm CO) for 2 x 1 h/day, 4 days. RET increased from 1.2% to 2.5% ($p<0.05$) and MnRET from 0.12% to 0.28% ($p<0.05$). The non-filter Reference Cigarette 2R1 failed to increase the RET and MnRET at 1250 µg TPM/l (700 ppm CO), but supplementing this smoke with CO to 1400 ppm increased RET and MnRET ($p<0.05$). CO alone (1400 ppm) increased RET ($p<0.01$), but not the MnRET. When rats were treated with 0, 30, or 100 U/kg EPO in combination with 0-1200 µg TPM/l from the 2R4F, the EPO-treatment increased the RET from 1.1% to 6.2%. MS exposure did not increase the MnRET, whereas the EPO-treatment did (to 0.38%). The RET and MnRET were increased after treatment with a non-cytotoxic dose of cyclophosphamide (1.5 mg/kg) in combination with EPO-treatment at 30 and 100 U/kg ($p<0.05$). We have demonstrated that the proportion of MnRET correlates with the proportion of RET in rat blood, indicating that enhanced erythropoiesis influences the proportion of MnRET after exposure to MS. It is thus important to carefully examine the proportion of RET in the micronucleus test with MS. The MS-induced increase in RET (as a result of CO) may yield confounded genotoxicity results.

2227 THE EFFECT OF BREVENAL ON DNA DAMAGE IN HUMAN LYMPHOCYTES INDUCED BY BREVETOXINS 2 AND 3 (PBTX-2 OR 3).

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Brevenal is a nontoxic polyether that competes with brevetoxin for the active site on the voltage-sensitive sodium channel. Brevetoxins are polyether toxins from blooms of marine dinoflagellates and activate voltage-sensitive sodium channels and may induce fish kills, marine mammal poisoning and adverse human health effects such as respiratory irritation and airway constriction. The purpose here was to determine if brevetoxins could induce DNA damage in human lymphocytes, and if the damage could be antagonized by brevenal. Unrepaired or erroneously repaired DNA damage may result in gene mutation, chromosome aberration, and modulation of gene regulation, which have been associated with immunotoxicity and carcinogenesis. The single-cell gel electrophoresis assay, or comet assay, was used to determine and compare DNA damage following various treatments. The data shown is the tail moment which is the percentage of DNA in the tail multiplied by the length between the center of the head and tail (units of measure are arbitrary). The negative control tail moment was 28.4 (S.E.=1.5), whereas the positive control(hydrogen peroxide) was 71.8 (2.2). PbTx-2, 10-8 M was 49.4 (9.9) and PbTx-3, 10-8 M was 71.8 (11.9). Brevenal, 1 ug/ml 1 hour before the brevetoxins protected the lymphocytes from DNA damage (PbTx-2; 33.9 (1.4) and PbTx-3; 42.4 (0.8)). The tail moment for brevenal alone was 30.8 (2.6). PbTx-2 and 3 are potent inducers of DNA damage in normal human lymphocytes, which is fully antagonized by brevenal. Brevenal also competitively displaces tritiated brevetoxin in a synaptosome receptor-binding assay. Similarly, brevetoxin-induced DNA damage at 10-8 M in human lymphocytes was antagonized by brevenal. (Supported by the North Carolina Agromedicine Center and USDA/CSREES; The brevetoxins and the brevenal were provided under NIEHS grant P01 ES10594).

2228 BISPHENOL A-INDUCED DNA DAMAGE IN C57BL/6N MICE: PRACTICAL *IN VIVO* APPLICATIONS OF THE COMET ASSAY.

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Bisphenol A (4, 4'-isopropylidene-2-diphenol; BPA) is an industrial monomer used for the synthesis of polycarbonate plastics and epoxy resins. Although there is no evidence that BPA poses any known health risk to humans, animal studies suggest that

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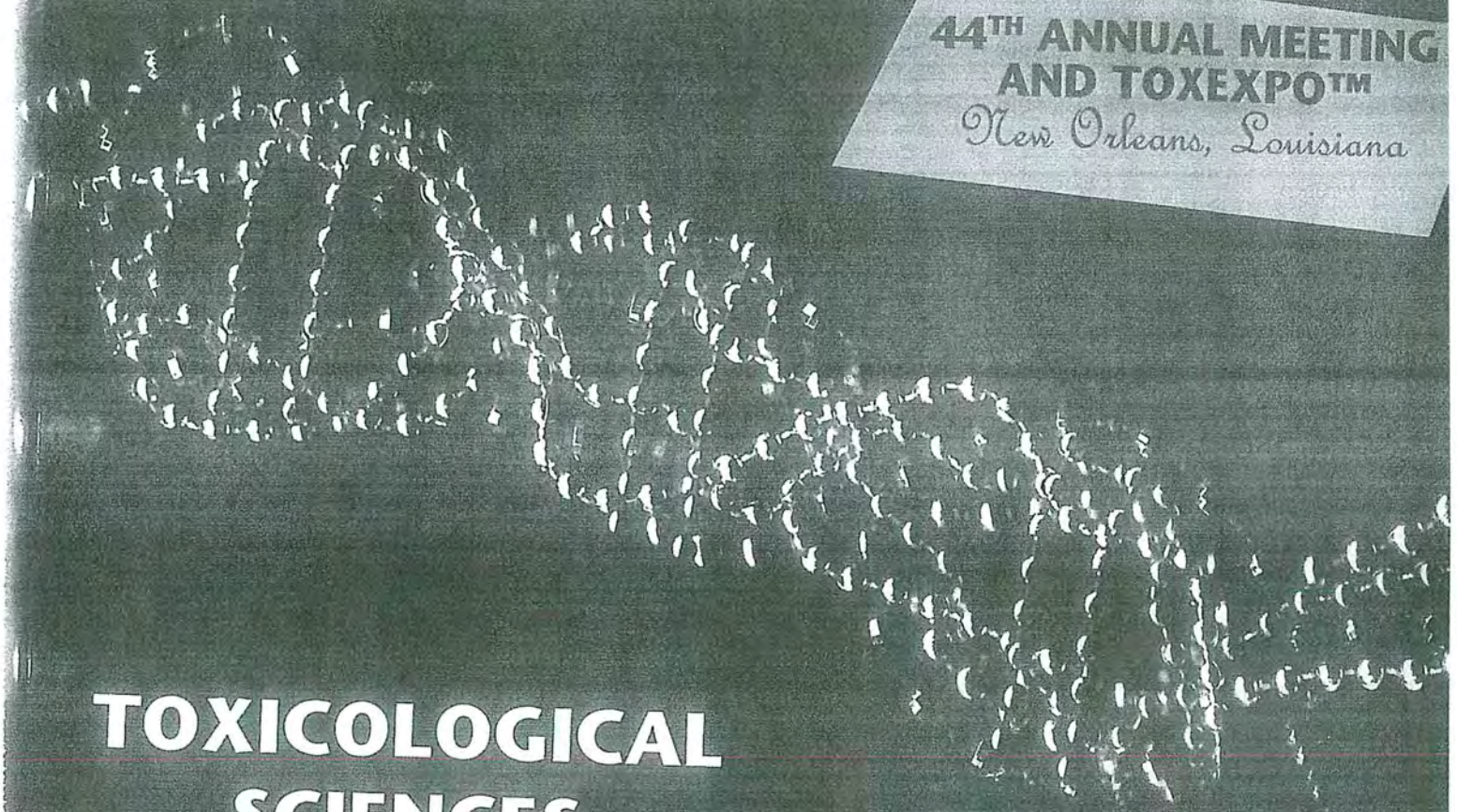

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