ABSTRACTS
POSTERS

(* Indicates competing Posters)

MOLECULAR DOSIMETRY OF METHYLATED DNA ADDUCTS IN THE MAJOR ORGANS OF MICE FOLLOWING ACUTE TREATMENT WITH 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK), C.J.G. Chang[1], C.K. Lee[2], B.G. Brown[2], J.W. Lineberry[2], V., Polhill[2], J.B. Polhill[2] and D.J. Doolittle[1,2]. [1]Dept. Physiology and Pharmacology, Bowman Gray School of Medicine and [2]Division of Research and Development, R.J. Reynolds Tobacco Co., Winston-Salem, N.C.

The tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), has been reported to induce lung adenomas in A/J mice. The initial step in NNK-induced lung tumorigenesis is thought to be the formation of methylated DNA bases leading to DNA mutations. Among those methylated DNA bases, O6-methylguanine (O6MeG) is recognized as the major promutagenic DNA adduct, inducing primarily GC to AT mispairing, whereas N7-methylguanine (7MeG) is reported to be the most prevalent methylated base after methylation. The stability of target molecules, sample preparation, and quantitation was monitored and optimized. We then monitored the molecular dosimetries of O6MeG and 7MeG in vital organs within the first 24 hrs after treatment (Experiment I) and the dose-response relationship 4 hrs after treatment (Experiment II). In Experiment I, mature female A/J mice were administered NNK (10 μmole/mouse, IP) and were sacrificed at 0, 2, 4, 8, 12 or 24 hrs after treatment. In Experiment II, mice received NNK at doses of 0, 2.5, 5, 7.5 or 10 μmole/mouse and were sacrificed 4 hrs after treatment. DNA from major organs was extracted, acid-hydrolyzed, and analyzed for O6MeG and 7MeG using HPLC with UV and fluorescence detection. O6MeG was detected in the lung and liver of all the mice and in the kidney of 50% of the mice receiving NNK treatment, but was absent in brain and heart; 7MeG was found only in liver. The concentration of lung and liver O6MeG and liver 7MeG reached its maximum 2 hrs post-NNK treatment and remained elevated throughout the 24 hrs. At the dose of 10 μmole/mouse, the average O6MeG concentrations in liver, lung, and kidney of O6MeG-positive animals, were 108, 13, and 20 pmole/μmole of guanine, respectively, and the average 7MeG concentration in liver of NNK-treated mice was 467 pmole/μmole of g anine (Gua). Four hours after NNK administration, the O6MeG in lung and liver and 7MeG in liver demonstrated a dose-response relationship. The intraassay and interassay variations were 0.176% and 1.947% for Gua; 2.372% and 2.516% for O6MeG/Gua; and 2.374% and 11.318% for 7MeG/Gua, respectively in Experiment I. In Experiment II, the intraassay and interassay variations were 0.903% and 5.335% for Gua; 3.29% and 4.897% for O6MeG/Gua; and 1.39% and 4.946% for 7MeG/Gua, respectively. These results indicate that factors other than the initial concentration of methylated adducts are critical in the tumorigenesis of NNK in A/J mice, since lung was the only target organ for NNK-induced tumorigenesis in A/J mice. (Supported by RJR-Leon Golberg Toxicology Fellowship.)

P2 * MUTANT FREQUENCIES IN LIVERS OF FEMALE *lacI* TRANSGENIC "BIG BLUE" $B6C3F_1$ MICE FOLLOWING CHLOROFORM INHALATION, C.S. Sprankle, M.V. Templin, A.A. Constan, D. Wolf, L.J. Pluta, L. Recio, B.A. Wong, and B.E. Butterworth, Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, NC, 27709.

Chloroform is produced in trace amounts from chlorination of domestic water and in some industrial processes such as bleaching of paper. Typical environmental exposure is widespread but at parts per billion levels. Evidence indicates that chloroform produces cancer through a nongenotoxic-cytotoxic mode of action. Liver and kidney tumors are induced in various rodent models but only under dosing conditions that produce target organ toxicity and continual regenerative cell proliferation. An inhalation experiment was conducted with female $B6C3F_1$ *lacI* transgenic mice to determine whether chloroform is directly mutagenic or whether mutagenic events might occur secondary to long-term organ specific toxicity. The experimental design allowed comparison between long-term, low-dose and short-term, high-dose regimens that result in the same total exposures. Mice were exposed daily 6 hr/day to 0, 10, 30, or 90 ppm chloroform by inhalation, representing nonhepatotoxic, borderline, and overtly hepatotoxic chloroform atmospheres. Time points were 10, 30, 90, and 180 days. Exposures of up to 90 ppm of chloroform for 10 days failed to increase mutant frequencies in the livers of exposed mice ($p < 0.05$). Mice treated 10 days previously with daily doses of 4 mg/kg of dimethylnitrosamine via oral gavage for 4 consecutive days exhibited hepatic mutant frequencies approximately 3 times those of controls. These results are consistent with the hypothesis that chloroform is not directly mutagenic. Examination of mutant frequencies at later time points is ongoing to determine whether extended exposure periods result in an increase in mutational events that can be detected by this assay.

P3 EFFECT OF DIFFERENT CRYOPRESERVATION METHODS ON DNA MIGRATION IN THE
*** SINGLE CELL GEL (SCG) ASSAY,** A. Udumudi, M. Vasquez and R.R. Tice, Integrated Laboratory Systems, P.O. Box 13501, RTP, NC 27709.

The SCG or Comet assay is used to detect DNA damage in proliferating and non-proliferating cells using a wide variety of *in vitro* and *in vivo* test systems. Because of the potential need to handle large numbers of samples within critically short time periods and/or to transport samples from distant sites to the laboratory, we evaluated various methods for freezing different types of cells prior to processing for SCG analysis. Control and H2O2-treated human blood leukocytes were frozen in our standard mincing solution (HBSS, 20 mM EDTA, 10% DMSO) using three different methods (gradual freezing at –20°C to –70°C, rapid freezing at –70°C, and flash freezing in liquid nitrogen). As an example of *in vivo* tissues, mouse liver and spleen were collected and flash frozen in liquid nitrogen as whole tissue, or in mincing solution with and without mincing. Frozen samples were stored at –70°C for at least 1 day prior to being processed for SCG analysis. After lysis, slides were exposed to alkali (sodium hydroxide/EDTA; pH>13) for 20 min and then electrophoresed for 20 min at 25 V, 300 mAmps. 100 cells were scored per duplicate sample and the extent of DNA migration compared with samples that were processed fresh. The resulting data demonstrate that freezing cells for subsequent processing and analysis may be an acceptable method for handling large numbers of samples or for transporting cells (e.g., human blood samples) from distant sites to the laboratory. Research was supported by NIEHS through SBIR Phase II Grant No. 2-R44-ES05884-02.

P4 DETECTING GENOTOXIC ACTIVITY AGAINST HIGH MOLECULAR WEIGHT DNA USING THE
*** ALKALINE SINGLE CELL GEL (SCG) ASSAY,** M. Vasquez and R. R. Tice, Integrated Laboratory Systems, PO Box 13501, Research Triangle Park, NC 27709

For genetic toxicology, the SCG assay is performed most frequently on eukaryote cells from *in vitro* or in vivo assays. Under these conditions, the amount of DNA damage detected depends on transport across cellular/nuclear membranes, activating and deactivating intracellular enzymatic processes, levels of radical scavengers, and the repair competency of the target cell population. To eliminate cellular processes which can modulate genotoxic activity, the ability to detect damage in the high molecular weight DNA remaining from cells after lysis in the SCG assay was evaluated. Untreated human blood lymphocytes were embedded in an agarose gel matrix using the conventional slide method, and then lysed using high salt concentrations and detergents at 4°C for at least 1 hour. After lysis, the slides were rinsed free of detergents and the resulting high molecular weight DNA exposed to multiple doses of hydrogen peroxide (H_2O_2), ethyl methanesulfonate (EMS), or cyclophosphamide (CP). The H_2O_2 experiment compared levels of DNA damage induced in lysed cells versus that in live cells exposed under identical conditions (i.e., in a gel matrix for 1 hr at 5°C). For EMS and CP, the DNA from lysed cells were treated at 37°C for up to 4 hrs in the absence or presence of 10% S9 mix, respectively. Subsequently, the DNA was exposed to alkali (pH>13) for 20 min followed by 20 min of electrophoresis at 25 V and 300 mAmps. For each sample, 100 cells were scored for DNA migration by image analysis. H_2O_2 (50, 100 µM) induced a dose-dependent homogeneous increase in DNA damage in treated DNA but a highly heterogeneous response in treated live cells. In both cases, however, the maximal increase in DNA migration was similar. EMS (0.1, 1 µM) in the absence of S9 and CP (20 and 100 µM) in the presence of S9 induced a dose-dependent increase in damage to treated DNA, with greater damage occurring at 4 versus 2 hr. These data demonstrate that damage can be induced and measured in the DNA of lysed cells, independent of cellular processes which may inhibit or enhance genotoxic activity. Research was supported by NIEHS through SBIR Phase II Grant No. 2-R44-ES05884-02.

HSP70-2 IS A SEX-SPECIFIC COMPONENT OF THE SYNAPTONEMAL COMPLEX IN MICE, J.W. Allen[1], D.J. Dix[1], B.W. Collins[1], B.A. Merrick[2], C. He[2], J.K. Selkirk[2], P. Poorman-Allen[3], M.E. Dresser[4] and E. M. Eddy[2]. [1]NHEERL, U.S. EPA, RTP, NC; [2]NIEHS, RTP, NC; [3]Glaxo Wellcome Inc., RTP, NC; [4]OK Med Res Fdn, OK City, OK.

Mouse and human spermatogenic cells are known to express HSP70-2, a member of the HSP70-2 family of heat shock proteins. In the present study, we have characterized the expression and localization of HSP70-2 in mouse meiotic cells. After separating spermatogenic cells into cytoplasmic and nuclear fractions, proteins were separated by two-dimensional electrophoresis and detected with HSP70-2 specific antibodies. HSP70-2 was determined to be a nuclear protein, and with immunocytological analysis was localized to the synaptonemal complex (SC). SCs from fetal mouse pachytene oocytes did not show any evidence of HSP70-2. RT-PCR analyses of gene expression confirmed this sex-specificity; *Hsp70-2* mRNA was detected in mouse testes but not ovaries. These findings suggested that HSP70-2 plays a role in the formation, maintenance, or function of the SC in spermatocytes but not oocytes. In male mice deficient for this protein due to targeted disruption of *Hsp70-2*, SC complements with structural and numerical abnormalities were observed, and meiosis was typically halted in late prophase. As HSP70-2 may be cytoprotective following exposure to genotoxic chemicals, the specificity of this protein for spermatocyte SCs could have implications for sex-specific germ cell loss, aneuploidy, and other possible outcomes of failed or aberrant synapsis/exchange. [this abstract does not necessarily reflect EPA policy.]

INTRODUCTION TO ACTIVITY PROFILES OF CARCINOGENICITY DATA, M.A Jackson[1], H.F. Stack[1] and M.D. Waters[2], [1]Integrated Laboratory Systems, RTP, NC 27709, [2]U.S. Environmental Protection Agency, RTP, NC 27711.

An activity profile graphically displays test results on multiple endpoints or parameters for individual chemical agents. This methodology has been used in the EPA/IARC Genetic Activity Profile database for a decade to present data abstracted from the published literature on the genetic and related effects of hundreds of carcinogens. The approach also has been applied to data from studies of developmental toxicity, acute and chronic toxicity, and antimutagenicity for a number of compounds. We here present preliminary results obtained in applying the activity profile methodology to NCI/NTP rodent cancer bioassays as compiled by Gold et al. (EHP 100:65-168, 1993) in the Carcinogenic Potency Database. The data plotted in the carcinogenic activity profiles are derived from the tumorigenic dose rate 50 (TD_{50}), which may be defined as the chronic dose rate in mg/kg body weight/day that would induce tumors in half of the test animals at the end of their standard lifespan adjusting for spontaneous tumors at the target site. As will be discussed, the profiles facilitate comparative analyses of the target specificity and potency of chemical analogues, the influence of route of exposure, the effects of stabilizers, etc. An Excel7 spreadsheet is used to link profiles to underlying data for both sexes of rats and mice across tissue sites and tumor types. The data linkage provides access to details of specific histopathology of tumor sites, duration of the study, dosing, and other information provided in the Gold database. Input is being sought on the course of future development and application of the methodology. (This is an abstract of a proposed presentation and does not necessarily represent EPA policy).

DICHLOROACETIC ACID IS MUTAGENIC IN BIG BLUE TRANSGENIC $B6C3F_1$ MOUSE LIVER, Sharon A. Leavitt and Jeffrey A. Ross, Biochemistry and Pathobiology Branch (MD-68), Environmental Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, U.S. EPA, RTP, NC 27711

The water disinfection byproduct dichloroacetic acid (DCA) was administered in drinking water at 0, 1, or 3.5 g/l to male transgenic $B6C3F_1$ mice harboring the *E. coli lacI* gene. Groups of animals were sacrificed at 4, 10, and 60 weeks after exposure and livers were removed. Genomic DNA was isolated from whole liver homogenates, and the *lacI* gene was recovered by *in vitro* packaging into lambda phage. Mutations in the *lacI* gene were detected by infecting *E. coli* host cells with phage and assaying for ß-galactosidase activity. Animals sacrificed at four and 10 weeks after treatment showed no significant increase in mutation frequency over the concurrent controls at either dose, while mice treated with 1 and 3.5 g/l DCA for sixty weeks had 30% (p=0.046) and 133% (p=0.002) increases in mutation frequency over concurrent control animals, respectively. Sequence analysis of the induced mutants shows a striking increase in the proportion of mutations occurring at A:T base pairs. The treatment conditions employed in this study are those previously demonstrating DCA hepatocarcinogenicity in male $B6C3F_1$ mice. Our findings support a genotoxic component for DCA carcinogenesis. This abstract does not necessarily reflect EPA policy.

14th meeting

ABSTRACTS
TALKS

The use of Transgen

(* Indicates competing Talks)

T1 * MICRONUCLEI INDUCED IN ROUND SPERMATIDS OF MICE AFTER STEM-CELL TREATMENT WITH CHLORAL HYDRATE: EVALUATIONS WITH CENTROMERIC DNA PROBES AND KINETOCHORE ANTIBODIES.

B.W. Collins[1], E.V. Nutley[2], A.C. Tcheong[2], J.W. Allen[1], M. Ma[2], X.R. Lowe[2], J.B. Bishop[3], D.H. Moore, II[2], and A.J. Wyrobek[2]. [1]Environmental Carcinogenesis Division, U.S. EPA, Research Triangle Park, NC; [2]Biology and Biotechnology Program , Lawrence Livermore National Laboratory, Livermore, CA; [3]NIEHS, Research Triangle Park, NC.

The chromosomal effects of chloral hydrate (CH) on germ cells of male mice were investigated using two methods to detect and characterize spermatid micronuclei (SMN): (a) anti-kinetochore immunofluorescence (SMN-CREST) and (b) multicolor fluorescence in situ hybridization with DNA probes for centromeric DNA and repetitive sequences on chromosome X (SMN-FISH). B6C3F1 mice received single intraperitoneal (i.p.) injections of 82.7, 165.4, or 413.5 mg/kg, and round spermatids were sampled at three time intervals representing cells treated in late meiosis, or as spermatogonial stem cells. No increases in the frequencies of SMN were detected for cells treated during meiosis using either SMN-CREST OR SMN-FISH methods. After spermatogonial stem-cell treatment, however, elevated frequencies of SMN were detected by both methods. With SMN-FISH, dose trends were observed both in the frequencies of spermatids containing micronuclei and in the frequency of spermatids carrying centormeric label. These finds corroborate a recent report that CH treatment of spermatogenic stem cells induced SMN [Mutation Research 323:81088(1994)]. Furthermore, our findings suggest that chromosomal malsegregation or loss may occur in spermatids long after CH treatment of stem cells. [This abstract does not necessarily reflect U.S. EPA policy].

T2 * CHARACTERIZATION OF DNA ADDUCTS DERIVED FROM THE ENVIRONMENTAL MUTAGEN CYCLOPENTA[cd]PYRENE,

J.J. Hayward, R. Sangaiah, A. Gold and L.M. Ball, Dept. Environmental Sciences and Engineering, UNC-CH, Chapel Hill, NC 27599-7400.

The environmental contaminant cyclopenta[cd]pyrene (CPP), a member of the class of cyclopentafused polycyclic aromatic hydrocarbons, exhibits potent mutagenicity in vitro. It is also an unusually potent carcinogen in the Strain A mouse lung assay, with induced lung tumors exhibiting an unique GGT —> CGT mutation in codon 12 of the Ki-ras oncogene. CPP is believed to undergo oxidative metabolism by the cytochrome P450 family of enzymes to produce the ultimate mutagenic species, CPP-3,4-oxide. We have examined the chemical nature of the reaction between CPP-3,4-oxide with calf thymus DNA in vitro. By optimizing conditions for formation and isolation of modified nucleosides, we were able to collect quantities of DNA adducts suitable for spectroscopic characterization. Structural information obtained by 1H-NMR spectroscopy and electrospray mass spectrometry allowed us to identify four major CPP adducts, including three deoxyguanosine-derived adducts and one deoxyadenosine-derived adduct. Under our experimental conditions we observed a clear preference for cis rather than trans opening of the cyclopenta[cd]pyrene-3,4-epoxide ring by the exocyclic N2 of deoxyguanosine, and a prominent role for adduction at the exocyclic N6 of deoxyadenosine. Other studies of CPP adducts carried out with the 32P-postlabeling method have detected a prevalence of deoxyguanosine adducts. Such chemical characterization should aid in determining the absolute structure of DNA adducts formed by CPP-3,4-oxide in vivo, and might help explain the unique mutational spectrum produced in codon 12 of the Ki-ras oncogene. Supported by NIH R01-ES06692 and PHS A03-AH01176.

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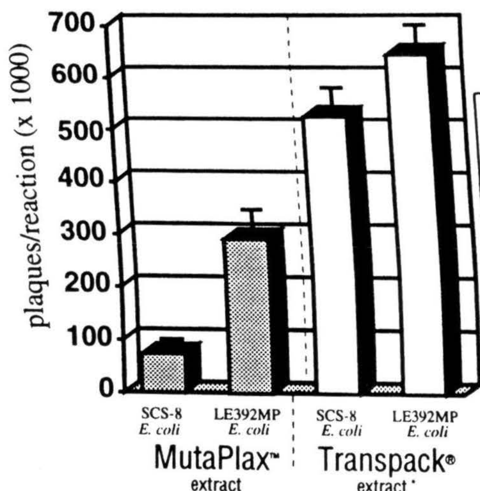

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