GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

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GEMS

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SIXTEENTH ANNUAL MEETING
PROGRAM AND ABSTRACTS

FRIDAY, OCTOBER 2, 1998

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Research Triangle Park, NC

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US EPA
MD 68
RTP, NC 27711
Tel: 541-5036
george.elizabeth@epamail.epa.gov

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NIEHS
PO Box 12233
RTP, NC 27709
Tel: 541-1876
bishop@niehs.nih.gov

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PO Box 13501
RTP, NC 27709
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PO Box 13501
RTP, NC 27709
Tel: 544-4589 x226
fstack@ils-inc.com

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RTP, NC 27711
Tel: 541-3821
cannon1@niehs.nih.gov

Susan Lewis
RTI, Dreyfus Bldg.
PO Box 12194
RTP, NC 27613
Tel: 541-6789
suelewis1@aol.com

Tom Hughes
US EPA
MD 68
RTP, NC 27711
Tel: 541-7644
hughes.thomas@epamail.epa.gov

1996-1999
Amal Abu-Shakra
NCCU
Biology Dept
Durham, NC 27707
Tel: 560-6408
abushak@wpo.nccu.edu

Marc Jackson
ILS, Inc.
POBox 13501
RTP, NC 27709
Tel: 544-4589 x229
mjackson@isl-inc.com

Lori Phillips
ILS, Inc.
POBox 13501
RTP, NC 27709
544-4589 x228
phillipsl_9@hotmail.com

1997-2000
Heinrich Malling
NIEHS
PO Box 12233
RTP, NC 27709
Tel:541-3378
malling@niehs.nih.gov

Julian Preston
CIIT
PO Box 12137
RTP, NC 27709
Tel: 558-1367
preston@ciit.org

Jeff Ross
US EPA
MD 68
RTP NC 27711
Tel: 541-2974
ross.jeffrey@epamail.e

GEMS 16th Annual Fall Meeting

Genetic Susceptibilities Affecting the Response of Children to Toxicants

8:00-8:45	Registration and Coffee
8:45-9:00	Welcome and Introduction Dr. Elizabeth George, President Dr. Jack Bishop, President-Elect
9:00-9:45	**"Neural Tube Closure: An Interface for Environmental and Genetic Action"** Dr. Roger E. Stevenson JC Self Research Institute, Greenwood Genetic Center, Greenwood, SC
9:45 - 10:30	Presented Papers (3)
10:30 - 12:00	Exhibits, Posters and Coffee
12:00 - 1:00	Lunch
1:00 - 1:30	Business Meeting
1:30 - 2:15	Presented Papers (3)
[illegible]15 - 2:30	Break
[illegible]30 - 3:15	**"Anticholinesterase Pesticide Toxicity: Exposure and Physiological Variation in Children"** Dr. Pauline Mendola Epidemiology & Biomarkers Branch, Human Studies Division, US EPA, Chapel Hill, NC Dr. Stephanie Padilla Cellular & Molecular Toxicology Branch, Neurotoxicology Division, US EPA, RTP, NC
3:15 - 4:00	**"Lead Toxicity/ALAD Gene???"** Dr. James Wetmer Experimental Neuropathology, Mt. Sinai School of Medicine, New York, NY
4:00 - 5:00	Reception and Posters

MESSAGE FROM THE PRESIDENT

Members of GEMS,

Thank you for electing me to serve GEMS for the past two years. As you know, one of my goals has been Outreach primarily focused on student participation. I chaired the Outreach Committee last year and Amal Abu-Shakra is the current chairperson. Amal has been very active in recruiting students for membership and meeting attendance. I was pleased with the student and junior scientists' presentations at last year's Fall Meeting. Eight students presented papers which is record for GEMS. My hope is that these presenters and the active student members will convey to their peers how valuable GEMS is by providing them with educational development through participation in the meetings and workshops, and an avenue to interact with recognized scientists. I am grateful to the members of the Outreach Committee (Maria Donner, Lori Phillips, Jeff Ross, Jack Bishop, Ron Cannon) for their active participation. Ron Cannon and Jack Bishop have been instrumental in communicating GEMS' activities to students and faculty at local universities.

I enjoyed organizing the 1997 Spring Meeting, "Use of Biomarkers for Monitoring Human Exposure to Genotoxicants," and received many compliments on the program, which was designed to highlight local university contributions. The 15th Annual Fall meeting, "Cellular and Molecular Responses to Arsenic Exposure," was particularly special because of the overwhelming student participation and attendance by many local nonmembers. Sue Lewis imparted a novel outlook on arsenic and Errol Zeiger's lunchtime presentation will be remembered fondly. Jack Bishop still should be receiving accolades for organizing this year's Spring Meeting, "Epigenetics: A Wider View of Genotoxicity." His timely, multidisciplinary theme, and fabulous speaker line up was substantiated by record attendance.

GEMS is fortunate to have excellent Officers and Councilors and I have enjoyed working closely with these individuals. Our Newsletter has a new look, thanks to the efforts of Marc Jackson and Tom Hughes. Lori Phillips, our Corporate Sponsor Representative, has done a great job this year. I encourage you to support our corporate sponsors whose participation ensures that GEMS' activities will continue. Sue Lewis, our Nominating Committee Chairperson, has lined up an excellent slate of candidates for next year. Jeff Ross' and Frank Stack's efforts have resulted in GEMS exposure on the Internet and enhanced our e-mail capabilities.

I have enjoyed serving as your President and President-Elect. I will miss the lively discussions at the Board meetings but look forward to supporting the Society as an active member.

Sincerely,

Beth George

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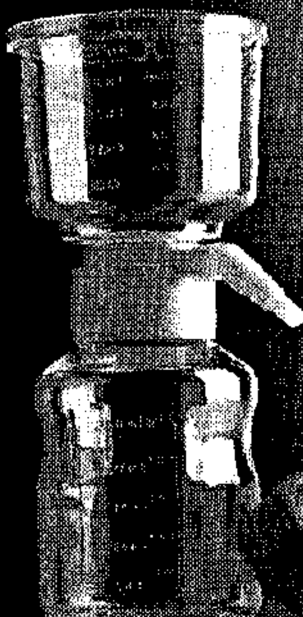

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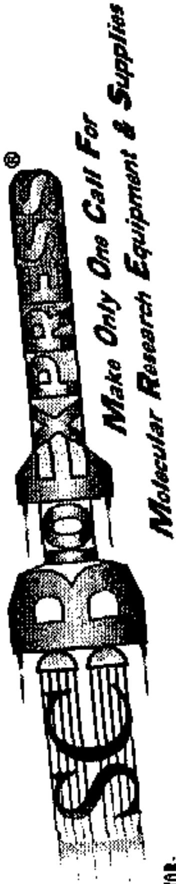
Thank you!

A special thanks to **ISC BioExpress** for sponsoring the registration coffee and pastries.



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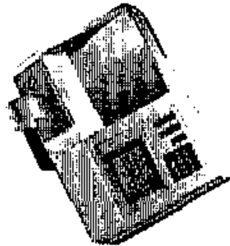

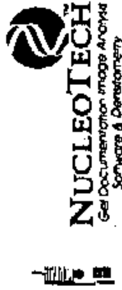

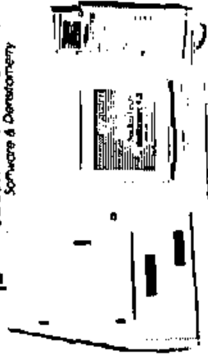

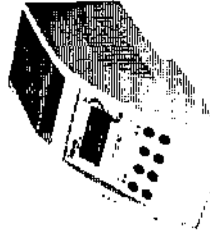

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ORAL PRESENTATIONS

*T1 SUBCELLULAR LOCALIZATION OF THE GLUCOCORTICOID RECEPTOR IN HEPATOCYTES FROM DICHLOROACETIC (DCA) TREATED B6C3F1 MICE AND F344 RATS. S. D. Hester, R. Conway, S. Liu, M. Pimentel, D. Wolf and A. B. DeAngelo, MD68, Environmental Carcinogenesis Division/ National Health Environmental Effects Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

Dichloroacetic acid (DCA) is present in finished drinking water as a by-product of chlorine disinfection process at concentrations from 34 to 160 mg/liter. DCA is also a major metabolite of trichloroethylene and tetrachloroethylene, two widely used industrial solvents. DCA has been identified as a rodent liver carcinogen. Recently, DCA has been shown to alter liver glycogen metabolism in both rodents and fish, resulting in cellular glycogen accumulation. Since adrenal glucocorticoids also exert effects on glycogen metabolism, we examined the impact of DCA-treatment on glucocorticoid receptors (GR) in hepatocytes. Rat and mice were exposed to DCA in drinking water (0.0, 0.5, 1.5 g/l, rats; 0.0, 0.5, 1.0, 2.0, 3.5 g/l, mice) for time points up to 28 days. The animals were euthanized and livers were isolated. Liver homogenates were separated into cytosol and nuclear enriched fractions. Using a monoclonal antibody we determined the presence of GR in intracellular compartments by western blots. We found a dose-dependent increase of both nuclear and cytosolic GR in mouse. Similarly, we observed a dose-dependent increase in nuclear GR ($p < 0.01$) in rat, whereas cytosolic GR remained unchanged. These results suggest that the hepatocarcinogenic effect of DCA may be associated with the translocation of GR in hepatocytes. This abstract does not reflect EPA policy.

*T2 QUANTITATIVE PCR ASSAY DETECTS V(D)J RECOMBINASE-MEDIATED GENE-REARRANGEMENTS: A MOUSE MODEL FOR A POSSIBLE BIOMARKER OF LYMPHOID MALIGNANCY RISK. G. W. Knapp,[1] R. W. Setzer,[2] and J. C. Fuscoe[1]. [1]Environmental Carcinogenesis Division and [2]Research and Administrative Support Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Recent reports show an increase in the rate of lymphoid malignancy among children. Previous studies also indicate that approximately 50% of all lymphoid malignancies contain rearrangements between antigen receptor genes and oncogenes, suggesting that V(D)J recombinase fidelity may be involved in the development of these malignancies. Although normally V(D)J recombinase assembles functional antibody and T cell receptor genes from numerous noncontiguous variable, diversity, and joining segments within one locus, it can occasionally rearrange DNA segments from two distinct loci (trans-rearrangements). In order to examine the fidelity of V(D)J recombination, we have modified a PCR-based assay (Lipkowitz et. al. Cancer Res. 57:4408,1997) to improve the quantitation of aberrant events occurring between two T cell receptor loci (TCRγV-TCRβJ) in the thymus cell population of mice. DNA sequence analysis of the rearrangements revealed hallmarks of V(D)J mediated recombination, including consensus sequence cleavage, end nucleotide nibbling, and non-template derived nucleotide addition. Balb/c, C57BL/6, B6C3F1, and AKR-/- mouse strains were surveyed, and baseline trans-rearrangement frequencies per cell were 4.3×10^{-6}, 5.0×10^{-6}, 6.1×10^{-6}, and 2.5×10^{-6}, respectively. With this new assay we will be able to evaluate various environmental and occupational exposures for their effect on this important mechanism of carcinogenesis. [This abstract does not necessarily reflect EPA policy]

An asterisk by the abstract title indicates that a presenter is in competition for the Best Talk or Best Poster Award. The Best Talk will receive an expenses paid trip to the Environmental Mutagen Society Meeting at the Capitol Hilton in Washington, DC, in March 1999. Best Poster recipient is awarded $100.

*T3 DERMAL EXPOSURE TO BENZENE INDUCES CHRONIC MYELOGENOUS LEUKEMIA IN V-HA-RAS TRANSGENIC MICE. M. Saulnier, S. Ward, and J. E. French, National Institute of Environmental Health Sciences, P.O. Box 12233, MD F1-05, Research Triangle Park, NC 27709

There is a critical need to develop an animal model for the leukemia associated with benzene exposure to humans. Human exposure to benzene occurs primarily through inhalation and penetration through the skin. Since mutated ras oncogene is the most frequent abnormality reported in myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML) in humans, we choose to use Tg.AC v-Ha-*ras* transgenic mice to investigate the role of *ras* in the induction of leukemia after benzene exposure. Here, using cytologic and anatomo-pathologic techniques, FACS analysis as well as spleen colony assays, we demonstrate that repeated treatments with 400ml of benzene applied on the shaved skin of the dorsal surface induce chronic myelogenous leukemia in Tg.AC mice. Furthermore, other mice dosed with 800ml of benzene weekly did not develop the disease but had blood and bone marrow changes suggesting persistent bone marrow functional abnormalities. These results lead us to propose a role for the v-Ha-ras transgene in the development of benzene-induced chronic myelogenous leukemia in the Tg.AC mouse. We hypothesize that toxicity on bone marrow caused early activation of *ras* transgene in an uncommited hematopoietic stem cell directed toward the granulocytic lineages after benzene exposure.

*T4 SPONTANEOUS AND METHYL METHANESULFONATE-INDUCED CYTOGENETIC DAMAGE IN MISMATCH REPAIR DEFICIENT AND PROFICIENT HUMAN CANCER CELL LINES. G.L. Erexson[1,2], W.E. Glaab[3], and K.R. Tindall[1]. [1]NIEHS, RTP, NC 27709; [2]College of Veterinary Medicine, NCSU, Raleigh, NC 27606 and [3]Merck Research Laboratories, West Point, PA 19486.

Following exposure to methyl methanesulfonate (MMS) *in vitro*, significant increases in *HPRT* mutations are observed in the mismatch repair (MMR)-deficient cell lines HCT116 (*MLH1* gene defect) and DLD-1 (*MSH6* gene defect) as compared to MMR-proficient derivatives of these same cell lines. However, little is known about either spontaneous or alkylation-induced cytogenetic damage in MMR-deficient and MMR-competent cell lines. The present study was designed to investigate possible differences in both spontaneous and MMS-induced sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) in the MMR-deficient human colon cancer cell lines, HCT116 and DLD-1, as well as in complementary MMR-competent cell lines derived by chromosome transfer, HCT116 + chromosome 3 and DLD-1 + chromosome 2. Cells were exposed to 0, 0.5 or 1.5 mM MMS for 1 hour at 37°C in 5% CO_2. The MMS was removed by washing the cells and bromodeoxyuridine (20 μM) was added for 48 hours prior to cell harvest in order to define first- and second-division metaphase cells for CA and SCE analyses, respectively. Similar spontaneous (baseline) and MMS-induced SCE frequencies were observed in both the MMR-deficient and MMR-proficient cell lines; however, both the SCE frequencies were slightly higher in the DLD-1 and DLD-1 + chromosome 2 cell lines. CA analyses yielded significantly higher frequencies of chromosomal-type aberrations in the HCT116 (MMR-deficient) cell line as compared to the HCT116 + chromosome 3 (MMR-proficient) cell line, although chromatid-type aberrations were not significantly different in these two cell lines. In the DLD-1 and DLD-1 + chromosome 2 cell lines, however, higher incidences of both chromatid-type and chromosomal-type aberrations were observed. These data suggest that the MMR-response is not involved in the formation of SCEs and that the MMR response involving either MSH6 or MLH1 can lead to a significant increase in CAs in human cells.

***T5 ALBUMIN AND HEMOGLOBIN ADDUCTS OF BENZENE OXIDE AND 1,4-BENZOQUINONE IN BENZENE-EXPOSED WORKERS.** K. Yeowell-O'Connell[1], N. Rothman[2], M.T. Smith[3], R.B. Hayes[2], G. Li[4], S. Waidyanatha[1], Mustafa Dosemeci[2], L. Zhan S. Yin[4], N. Titenko-Holland[3], and S.M. Rappaport[1] 1. Dept Environ Science & Eng, Scho Public Health, UNC, Chapel Hill NC 27599-7400 2. Div Cancer Epidemiol & Genetics, N Bethesda, MD 20892 3. School of Public Health, UC, Berkeley, CA 94720 4. Chinese Academy Preventive Medicine, Beijing, China

The benzene metabolites, benzene oxide (BO) and 1,4-benzoquinone (1,4-BQ, which arises from hydroquinone) react with cysteinyl residues in albumin (Alb) and hemoglobin (Hb) to form protein adducts (denoted BO-Alb, 1,4-BQ-Alb, BO-Hb, and 1,4-BQ-Hb, respectively), which are expected to represent specific biomarkers of exposure to benzene. These four adducts were detected by GC-mass spectrometry (negative ion chemical ionization) following reaction of the protein with trifluoroacetic anhydride and methanesulfonic acid. We analyzed the Hb adducts in 44 benzene-exposed workers and 44 unexposed controls, and the Alb adducts in a subsample of 20 exposed workers and 20 controls in Shanghai, China as part of a larger cross-sectional study of benzene biomarkers. Preliminary analyses showed that mean adduct levels (in nmol adduct /g protein) were statistically higher (2-tailed t-test) in the exposed workers for BO-Alb, 1,4-BQ-Alb, and BO-Hb, but not 1,4-BQ-Hb (BO-Alb: 0.14 (controls) vs 1.7 (exposed), $p = 0.0001$; 1,4-BQ-Alb: 2.5 (controls) vs 8.4 (exposed), $p = 0.0003$; BO-Hb: 0.38 (controls) vs 0.99 (exposed), $p = 0.0001$; 1,4-BQ-Hb: 1.2 (controls) vs 1.2 (exposed), $p = 0.65$). Of particular interest was the finding of significant levels of both BO and 1,4-BQ adducts in theoretically unexposed individuals. When individual workers' adduct levels were regressed upon their corresponding urinary metabolite levels, significant correlations were also observed for all but 1,4-BQ-Hb (BO-Alb vs phenol: $r = 0.80$, $p = 0.0001$; 1,4-BQ-Alb vs hydroquinone: $r = 0.82$, $p = 0.0001$; BO-Hb vs phenol: $r = 0.81$, $p = 0.0001$; 1,4-BQ-Hb vs hydroquinone: $r = -0.14$, $p = 0.32$). These results are the first to show that BO-Alb, 1,4-BQ-Alb and BO-Hb are correlated with markers of benzene exposure in humans. The discrepancy between Hb and Alb adducts of 1,4-BQ is currently under investigation (supported in part by NIH grant P42ES05948).

***T6 A COMPARISON BETWEEN STYRENE OXIDE AND BENZO[*a*]PYRENE-4,5-OXIDE AS SUBSTRATES FOR HUMAN HEPATIC MICROSOMAL EPOXIDE HYDROLASE.** I. G. Popa[1], C. Hassett[2], C. J. Omiecinski[2] and S. M. Rappaport[1], [1]School of Public Health, UNC Chapel Hill, CB 7400, NC 27599, [2]Department of Environmental Health, University of Washington, Seattle, WA 98105

Epoxides represent an important class of xenobiotics, which exert cytotoxic, mutage teratogenic and carcinogenic effects in mammalian systems. Epoxide hydrolases (E catalyze the hydration of epoxides to corresponding less reactive diols. Because of o interest in possible genotoxic effects of styrene oxide (SO) among workers in the reinforced plastics industry (Rappaport *et al.*, 1996), we wished to evaluate the influence of microsomal EH (mEH) upon levels of SO in human blood. This motivated us to develop a sensitive assay for mEH, using SO as the substrate, and afforded the opportunity to compare activity levels from this assay with those obtained previously, using benzo[*a*]pyrene-4,5-oxide (BPO), as the substrate in 40 specimens of human liver (Hassett *et al.*, 1997). Since investigations of mEH are rare in humans, this study sheds light upon the substrate specificity of this important detoxication system. The precision of the SO assay developed (CV = 17%) was comparable to other reported assays (CVs between 10 and 20%, using substrates other than SO). The Spearman correlation coefficients indicated that only 30-40% of the variance of SO-measured mEH activity was explained by the BPO-measured activity. Values of the Kappa statistic were less than 0.4 for comparison between the two substrates. The results obtained suggest that mEH exhibits rather large specificity between SO and BPO in human liver samples. Thus, studies employing phenotype assays with substrates other than the epoxides of interest should consider the impact of such specificity.

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POSTERS

***P1 TRANSPLACENTAL EFFECTS OF DIETARY AND ENVIRONMENTAL CARCINOGE[N]S.**

K. Gressani,[1] L. Rollins,[1] S. Leone-Kabler,[1] A. Malkinson[2] and M.S. Miller.[1] Comprehensive Cancer Center of Wake Forest University, Winston-Salem, NC [1] and University of Colorado, Denver, CO.[2]

Heterocyclic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) are two classes of dietary and environmental carcinogens. The goal of this study was to determine the carcinogenicity of these compounds and to elucidate the mechanisms by which these chemicals mediate initiation and promotion of tumorigenesis in transplacentally-treated C57BL/6 and BALB/c mice. Treatment of pregnant C57BL/6 mice with the model PAH, 3-methylcholanthrene (MC), resulted in the development of macroscopically visible lung tumors in the offspring. Likewise, BALB/c mice developed lung tumors following *in utero* exposure to MC in the presence or absence of the putative lung tumor promoter butylated hydroxytoluene (BHT). Transplacental exposure to the prototypical HA, 2-amino-3-methylimididazo[4,5-*f*]quinoline (IQ), did not result in the formation of neoplasms in C57BL/6 mice. Lysates from paraffin-embedded lung tumor tissue were screened for the presence of point mutations at the Ki-*ras*, *p16*, and *p53* gene loci via allele-specific oligonucleotide hybridization and SSCP analyses. Ki-*ras* point mutations were found in 79% (37/47) of the lung tumors of C57BL/6 mice, and 47% (17/36) of the lung tumors of BALB/c mice. Initial findings suggest that both strains exhibited similar mutational spectra at this gene locus. SSCP analysis of the tumor suppressor gene, *p16*, indicated the presence of point mutations in 7% (2/28) of the lung tumors in C57BL/6 mice tumors. No mutations were found in exons 5, 6, 7 or 8 of the *p53* gene. Mutational analysis of the *p16* gene from BALB/c mice is currently in progress. These results suggest that mutagenic damage to Ki-*ras* may be a critical early event mediating lung tumorigenesis in transplacentally-treated C57BL/6 and BALB/c mice. It appears that HAs may not pose a risk for initiation of cancer during gestation. (Supported by NIH grants ESO6501 and ESO08252)

***P2 ABSENCE OF MUTATIONS IN Ki-, Ha- and N-*ras* AND *p53* GENES IN TRANSPLACENTALLY INDUCED HAMSTER PANCREATIC ADENOCARCINOMAS.**

Liyan Zhang[1], Hildegard M. Shuller[2] and Mark S. Miller[1]. [1]Comprehensive Cancer Center, Wake Forest University School of Medicine; Winston-Salem, NC; [2]University of Tennessee College of Veterinary Medicine, Knoxville, TN

Clinical observations have shown that maternal exposure to cigarette smoke is a risk fac[tor] for the development of cancer in children and young adults. The nicotine-derived nitrosami[ne] NNK is one of the most potent and most abundant tobacco-specific carcinogens. It is we[ll] established that chronic consumption of alcoholic beverages enhances the carcinogenic risk in smokers. Previous studies with a hamster model have shown that ethanol changed the organ site of tumor development from lung to pancreas following *in utero* exposure to NNK, with 60 % of the off spring developing cancer of the exocrine pancreas. Pancreatic carcinoma is the fourth leading cause of cancer deaths among males in the U. S., yet the molecular events involved in pancreatic carcinogenesis has not been fully elucidated. It has been reported that frequent mutations in the Ki-*ras* and *p53* genes were found in human and hamster exocrine pancreatic cancers. This study has further focused on the molecular bases by which NNK and alcohol cause trans-placental pancreatic cancers in the hamster model. Using PCR-SSCP and direct sequencing techniques, we examined mutations in exons 1 and 2 of the Ki-, Ha- and N-*ras* oncogenes and in exons 5-8 of the *p53* tumor suppressor gene in pancreatic adeno-carcinomas that were induced following transplacental exposure to NNK and alcohol. No mutations were found in any of the ras oncogenes or the *p53* tumor suppressor gene. Thus, the results show that different molecular alterations may be involved in transplacental pancreatic carcinogenesis, and further efforts are needed to delineate the potential mechanisms ofcarcinogenesis in order to develop targetspecific therapeutics for this dismal disease.

*P3 A COMPARATIVE ANALYSIS OF DNA ADDUCT FORMATION BY ALACHLOR METABOLITES *IN VITRO* AND *IN VIVO* IN RAT LIVER AND OLFACTORY TISSUES BY ^{32}P-POSTLABELING, D. A. McLaughlin, S. A. Meyer, and R.L. Rose, Department of xicology, NCSU, Raleigh, NC 27695.

lachlor (2-chloro-2'6'-diethyl-N-(methoxymethyl) acetanilide) is a restricted use herbicide associated with nasal carcinogenicity and hepatotoxicity in rats; however, the mechanism responsible for its biological effects remains unclear. The complex metabolism of alachlor to reactive metabolites capable of binding DNA has been implicated in its carcinogenicity. This study was undertaken to investigate the formation of DNA adducts upon bioactivation of alachlor and 2,6-diethylaniline (DEA), a major metabolite of alachlor, *in vitro* with calf thymus DNA in the presence of liver and olfactory microsomal activation systems from male Long-Evans rats. Results were compared with adducts formed by alachlor *in vivo* in DNA from liver tissues of rats exposed ip to 126 mg/kg/day of alachlor for 1, 4, or 28 days. DNA adduct analysis was performed using ^{32}P-postlabeling with adduct enrichment by either nuclease P1 or butanol extraction and adduct resolution by TLC. Comparison of adduct migration patterns resulting from alachlor bioactivation in olfactory and hepatic *in vitro* activation systems identified a metabolism dependent adduct specific for each tissue. Metabolism of DEA using the same activation systems revealed co-migration of three adducts in addition to one adduct unique to liver metabolism. *In vivo* adduct formation in DNA from liver tissues revealed several adducts for all time points. One adduct common to all *in vivo* treatments co-migrated with the adduct formed following *in vitro* liver metabolism. These data indicate that bioactivation of both alachlor and DEA result in formation of reactive metabolites capable of covalently binding DNA as evidenced by the formation of multiple DNA adducts in both *in vitro* systems and *in vivo* treatments. Finally, evidence is provided indicating bioactivation of alachlor to tissue specific adduct-forming metabolites consistent with different target tissue effects. .

P4 CHARACTERIZATION OF THE SINGLE CELL GEL (SCG) ASSAY: EFFECT OF SLIDE POSITION DURING ELECTROPHORESIS VERSUS MULTIPLE ELECTROPHORETIC RUNS ON DNA MIGRATION DATA QUALITY, M. Vasquez[1], R.R. Tice[1], G.J.Hook[2], and D. McRee[2]. [1]Integrated Laboratory Systems, Research Triangle Park, NC, [2]Wireless Technology Research, L.L.C, Washington, DC.

The Single Cell Gel (SCG) technique is a sensitive assay for detecting DNA strand breaks, alkali-labile sites, incomplete excision repair sites, and DNA crosslinking at the level of the individual eukaryote cell under virtually any experimental condition. However, there is concern that variation in slide position during electrophoresis and/or small variations in electrophoretic nditions between electrophoretic runs may have a profound impact on the quality of the sulting DNA migration data. To address this issue, we evaluated the effect of slide position during electrophoresis and across different electrophoretic runs on the extent of DNA migration for ddymphocytes. After processing for SCG analysis, replicate slides were placed in the four extreme corners of a recirculating gel electrophoresis box, where they underwent 60 minutes of unwinding and 40 minutes of electrophoresis at 25 volts and 300 mA under alkaline pH>13 conditions. This procedure was repeated twice more with replicate slides using identical experimental conditions. This same procedure was conducted three more times under electrophoretic conditions which resulted in increasing amounts of DNA migration. Following electrophoresis, the alkali in the gels was neutralized, the DNA was fixed in 100% cold ethanol, and the gels were allowed to air dry. After staining with SYBR Green, the extent of DNA migration, assessed as the percentage of migrated DNA, the length of DNA migration, and tail moment (% migrated DNA x tail length), was analyzed using the Komet 3.1 image analysis system. The resulting data generally indicate more variability between electrophoretic runs than among slides within the same run. These results should be considered when determining the optimal design for collecting conducting SCG studies. Supported by Wireless Technology Research, L. L. C.

***P5 GENOTOXICITY OF ANALOG AND TIME DIVISION MULTIPLE ACCESS (TDMA) 837 MHZ RADIOFREQUENCY RADIATION (RFR) IN HUMAN BLOOD CELLS EVALUAT USING THE SINGLE CELL GEL (SCG) ELECTROPHORESIS ASSAY AND THE YTOCHALASIN B MICRONUCLEUS (CB-MN) ASSAY.**

J.J. Clancy[1], M. Vasquez[1], E.M. Donner[1], R.R. Tice[1], G.J. Hook[2] and D. McRee[2]. [1]Integrated Laboratory Systems, Research Triangle Park, NC, [2]Wireless Technology Research, L.L.C, Washington, DC.

Exposure to RFR from the use of cellular telephones has increased dramatically during the last decade. Based on results obtained using the SCG assay, Lai and Singh (*Bioelectromagnetics* 16: 207, 1996) concluded that exposure to 2450 MHz RFR resulted in increased levels of DNA damage in brain cells of rats. As part of a *in vitro/in vivo* program to evaluate the biological effects of RFR at cellular telephone frequencies (800-900 MHz), the SCG assay was used to examine for the induction of DNA damage by 837 MHz RFR (generated by both Analog and TDMA technologies) *in vitro* in human leukocytes. Concomitantly, the ability of 837 MHz RFR to induce MN in CB-induced binucleate lymphocytes stimulated post exposure with phytohaemagglutinin (PHA) was evaluated. Cells in whole blood were exposed for 3 hours (37±1°C) to 837 MHz continuous wave (voice modulated) fields at Specific Absorption Rates (SAR) levels of 10, 5, 2.5, and 1 W/kg in a transverse electromagnetic (TEM) cell optimized for *in vitro* studies. After exposure, for SCG analysis, gels were prepared, the cells were lysed, the DNA treated with proteinase K, and the released DNA exposed to alkali (pH >13) and then electrophoresed at 25 V, 300 mA. To conduct the CB-MN assay, exposed blood was cultured at 37±1°C for 72 hrs in complete RPMI1640 medium with PHA; CB was added at 44 hrs of culture. The study included a primary test as described and an independent replicate test involving a 24-hr exposure to 10 W/kg. Appropriate sham and positive controls were included. Under the experimental conditions used, neither Analog nor TDMA RFR at 837 MHz induced a significant increase in DNA damage or in micronucleated binucleate cells. Research supported by Wireless Technology Research, L.L.C.

***P6 IDENTIFICATION AND CHARACTERIZATION OF NON-RESPONDING Tg.AC MICE BY A NON-ISOTOPIC DNA RFLP ANALYSIS.**

D.C. Kantz[1] and R.E. Cannon[2], [1]Integrated Laboratory Sciences and [2]LECM, NIEHS, Research Triangle Park, NC.

The Tg.AC mouse model is under evaluation by the National Toxicology Program (NTP) as a possible adjunct or alternative to the conventional two-year bioassay. The short term 26-week bioassay relies on the empirical observation that Tg.AC transgenic mice produce sk papillomas when topically treated with chemical carcinogens. Tg.AC studies indicate that t tumorigenic response to chemical carcinogens is dependent on the activation and sustained expression of the oncogenic v-Ha-ras transgene. However, the presence of Tg.AC mice incapable of responding to a known inducer of skin papillomas (i.e., nonresponders) in a number of recent Tg.AC tumor studies has created the need for a sensitive and specific assay to identify these mice. DNA blot experiments have linked a 2000 base pair Bam HI fragment containing two inverted copies of the zetaglobin promoter to the responder phenotype; nonresponding mice are thought to be the result of deletions in the palindrome region during replication. Recently, Frank Sistare (FDA) has developed a ^{32}P-based Southern blot assay which allows for the identification of nonresponders based on a band shift in the 2000 base pair Bam HI fragment of the promoter region. To increase the efficiency of detecting nonresponders, we have developed an analogous non isotopic DNA Blot procedure which can be used identify responder/non responder Tg.AC mice. This approach obviates the need for radioisotopes and decreases the time needed to conduct each assay from 9 days to 7 days. Research supported by NIEHS under contract N01-ES-65399.

***P7** GENOTOXICITY OF ANALOG AND TIME DIVISION MULTIPLE ACCESS (TDMA) 837 MHZ RADIOFREQUENCY RADIATION (RFR) IN A BATTERY OF *IN VITRO* BACTERIAL D MAMMALIAN CELL ASSAYS. L.A. Phillips[1], D.M. Blackwell[1], J.J. Clancy[1], L.S. Clark,[1] I. Donner[1], R.R. Tice[1], G.J. Hook[2] and D. McRee[2]. [1]Integrated Laboratory Systems, RTP, NC, [2]Wireless Technology Research, L.L.C, Washington, DC.

Exposure to RFR from the use of cellular telephones has increased dramatically during the last decade. Recent studies have reported genotoxic and tumorigenic effects at exposure levels purported to be athermal. As part of a program to evaluate the biological effects of RFR at cellular telephone frequencies (800-900 MHz), the genotoxicity of 837 MHz (Analog and TDMA) was assessed using a battery of standard *in vitro* assays (mutations in *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1538/*Escherichia coli* strain WP2*uvr*A; mutations in mouse lymphoma L5178Y $TK^{+/-}$ cells; chromosomal aberrations in proliferating mitogen-stimulated human blood lymphocytes), all without metabolic activation. Cells were exposed at 37±1°C for 3 hrs to 837 MHz continuous wave (voice modulated) fields at Specific Absorption Rates (SAR) levels of 10, 5, 2.5, and 1 W/kg in a transverse electromagnetic (TEM) cell optimized for *in vitro* studies. Dielectric properties for each media/cell combination were measured in a slotted line at 837 MHZ and 37°C. Theoretical SAR distributions were developed using the X-FDTD method. The average SAR for each media/cell combination was calculated from temperatures measured with Luxtron probes. Each genotoxicity test consisted of a primary test and an independent replicate test (both with appropriate sham and positive controls) using the same experimental protocol except for the chromosomal aberration test, where the repeat test involved a 21-hour exposure to 10 W/kg along with appropriate sham and positive controls. Under the experimental conditions used, neither Analog or TDMA RFR at 837 MHz induced a significant mutagenic or clastogenic response. Research supported by Wireless Technology Research, L.L.C.

P8 THE EFFECT OF DOSING REGIMEN ON MUTANT FREQUENCIES INDUCED BY DIBENZO[*a,l*]PYRENE AND BENZO[*a*]PYRENE IN B6C3F1 BIG BLUE TRANSGENIC MOUSE LUNG S.A. Leavitt and J.A. Ross, US EPA, MD 68, RTP, NC 27711

Dibenzo[*a,l*]pyrene (DB[*a,l*]P) and benzo[*a*]pyrene (B[*a*]P) are polycyclic aromatic hydrocarbons found in cigarette smoke condensate, coal combustion by-products, and in other environmental pollutants. In this study, we investigated the effect of variations in dosing regimen of DB[*a,l*]P and B[*a*]P on *in vivo* mutagenicity using the Big Blue® transgenic mouse. Animals were dosed i.p. with either 6 mg/kg DB[*a,l*]P once or 1.2 mg/kg DB[*a,l*]P on five consecutive days. Animals treated with B[*a*]P were dosed i.p. either once with 200 mg/kg or h 40 mg/kg on five consecutive days. Animals were sacrificed after 31 days and lungs re removed. DNA extracted from the lungs was assayed for the presence of mutations in the *lacI* gene. After 4 weeks of treatment, the mutant frequency for the control animals was 3.4×10^{-6}. The mutant frequencies obtained from the DB[*a,l*]P-treated animals were 8.1×10^{-6} for the single injection group and 9.5×10^{-6} for the multiple injection group. The mutant frequencies obtained from the B[*a*]P-treated animals were 47.2×10^{-6} for the single injection group and 51.8×10^{-6} for the multiple injection group. The present study demonstrates that both DB[*a,l*]P and B[*a*]P are mutagenic in the Big Blue® transgenic mouse system. This study shows that there is no significant difference in the mutant frequency from either the single or multiple dosing protocols (for DB[*a,l*]P, p=0.27; for B[*a*]P, p=0.68). This is an abstract of a proposed presentation and does not reflect EPA policy.

P9 SCREENING OF PERIPHERAL BLOOD LYMPHOCYTES FOR ILLEGITIMATE V(
RECOMBINATION EVENTS AS POTENTIAL BIOMARKERS, *J Scheerer[1], G Knapp[2], L
W Bigbee[3], and J Fuscoe[2], [1]UNC, Chapel Hill, NC 27599, [2]U.S.EPA, MD-68, RTP, NC 27711,
and [3]U Pittsburgh/U Pittsburgh Cancer Institute, Pittsburgh, PA 15238.

We are using a PCR-based assay to screen peripheral blood lymphocytes for mutations which result from illegitimate V(D)J recombination. V(D)J recombination is most active during lymphocyte differentiation for formation of functional T-cell receptor genes and immunoglobulin genes. Illegitimate V(D)J recombination can occur at loci with V(D)J consensus-like signal sequences and structure. This appears to be the mechanism for a number of different translocations which activate oncogenes (such as BCL2 translocating to the IgH locus = t(14;18) in Non-Hodgkin's Lymphoma) and for a deletion event within the HPRT gene. Previous studies using t-cell cloning have found that newborns have a much lower frequency of HPRT mutant T-cells than adults, but the proportion of V(D)J recom-binase-mediated mutations is 20 times greater in newborns. This suggests that fetal development may be a susceptible life stage for this type of mutational event, likely as a result of the high V(D)J recombinase activity during development. Quantitation of this class of mutants serves as a biomarker for the level of illegitimate V(D)J recombination occurring in peripheral blood lymphocytes of humans. The PCR-based assay used in these studies is designed to simultaneously quantify BCL2;IgH translocations and deletion mutations in HPRT. Screening of an initial set of 21 newborns (cord blood lymphocytes) detected HPRT deletion mutations at a frequency of about 1×10^{-8}. This is significantly lower than values derived from t-cell cloning assays. To account for this difference, we examined the effect of treatment of lymphocytes with phytohemagglutinin (PHA), as in t-cell cloning assays, prior to DNA isolation. The frequency of HPRT deletion events increased 3-fold after PHA-treatment. t(14;18) events were detected at a frequency of 7.5×10^{-9} in the PHA-treated samples. In contrast, control and PHA-stimulated lymphocytes from 2 adults had no detectable HPRT deletion events, but t(14;18) events were found at a frequency of 1.2×10^{-7}, which is consistent with previous reports of increased incidence of translocations with age. Funding for JS is from the Curriculum in Toxicology, UNC. (Abstract does not reflect US EPA policy).

P10 EFFECT OF HALOACETIC ACIDS ON THE ENZYME ACTIVITIES OF THE RAT CECAL MICROBIOTA. G Nelson, L Brooks, K Bailey, and S George. US EPA, RTP, NC.

Drinking water disinfection by-products (DBPs) have been associated with cancer laboratory rodents and humans. The intestinal microbiota are capable of transforming so procarcinogens to their carcinogenic forms. Our purpose is to determine if the intesti microbiota contribute to the metabolism and bioactivation of haloacetic acids to genotoxicant compounds. Enzyme activities are reported for rat cecal microbiota and selected rat intestinal isolates with each of six haloacetic acids. Mono-, di-, and trichloro-acetic acid and mono-, di-, and tribromoacetic acid were added individually to 10 ml tubes of media at a concentration of 1 mg/ml. Tubes were then inoculated with the cecal micro-biota or an intestinal isolate and incubated anaerobically for 15 hr. Aliquots were incubated with the enzyme substrates for 1 hr and the activities were determined. The seven enzymes assayed were nitroreductase, dechlorinase, dehydrochlorinase, azo-reductase, β-glucosidase, β-galactosidase, and β-glucuronidase. Cultures of the intestinal isolates *Clostridium bifermentens* and *Lactobacillus johnsonii* had no detectable enzyme activity. Azoreductase and dehydrochlorinase were not detected in any of the cultures. Significant increases in β-glucosidase and β-glucuronidase activities were found for DBA-treated cultures of rat cecal flora. Significant decreases in β-galactosidase activity were seen in most treatment groups for the intestinal isolates *B. distasonis* and *B. uniformis*, but not for the rat cecal flora. Therefore, even though these isolates are predominant in the cecum, they probably are not the major contributors to the overall enzyme response of the rat cecal flora. *In vitro* treatment with haloacetic acids alters rat cecal flora enzyme activity, suggesting that the potential exists for these compounds to alter compound genotoxicity. [Does not reflect EPA policy]

P11 IDENTIFICATION AND CHARACTERIZATION OF NON-RESPONDING Tg.AC MICE.

Cannon, R.W., *Kantz, D.C., and Tennant, R.W. Laboratory of Environmental Carcinogenesis and Mutagenesis, NIEHS, Research Triangle Park, NC, USA; *Integrated Laboratory Sciences, Research Triangle Park, NC, USA.

The v-Ha-ras transgenic Tg.AC mouse model is under consideration by the National Toxicology Program NTP as a possible alternative to the conventional two-year bioassay. The short term 26 week bioassay relies on the empirical observation that Tg.AC transgenic mice produce skin papillomas when topically treated with chemical carcinogens but fail to do so when treated with noncarcinogens. Tg.AC studies indicate that the tumorigenic response to chemical carcinogens is dependent on the activation and sustained expression of the oncogenic v-Ha-ras transgene. Tumor data from recent experiments indicate a higher than expected incidence of nonresponding Tg.AC mice (> 1%). DNA blot experiments have linked a 2000 base pair Bam HI fragment containing the zetaglobin promoter with responder Tg.AC mice. Genotypic analyses in conjunction with test mating (outcrossing) to FVB/N mice were performed to characterize and ensure complete elimination of the nonresponder alleles from newly established Tg.AC founder breeders. The possible instability of the v-Ha-ras transgene and the implications for the utility of the Tg.AC mouse model will be presented.

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