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Program

GEMS TWELFTH ANNUAL FALL MEETING

Monday, October 17, 1994
North Raleigh Hilton
Raleigh, NC

Theme for invited talks:

NEW TOPICS IN GENETIC TOXICOLOGY

8:00 - 8:45 **Registration,** Poster Set-up, Coffee and Danish

8:45 - 9:00 **Opening and Introduction:** Dr. Julian Preston, CIIT, President, GEMS, and Dr. Michael D. Waters, EPA, President Elect, GEMS

9:00 - 9:45 **Invited Speaker:** Dr. Rochelle W. Tyl, RTI, "Convergence o Embryology, Developmental Toxicology and Genetic Toxicology"

9:45 - 10:30 **Invited Speaker:** Dr. William R. Kelce, ManTech, Inc. "Endocrine Disrupters: Background and Recent Research"

10:30 - 12:00 **Posters, Exhibits,** Coffee

12:00 - 1:15 **Contributed Papers and Business Meeting**

1:15 - 2:30 **Lunch**

2:30 - 3:15 **Invited Speaker:** Dr. Glenn Merlino, NCI, "Transgenic Mice as Models for the Study of Interactions Between Growth Factors and Chemical Carcinogens"

3:15 - 3:30 **Break**

3:30 - 4:15 **Invited Speaker:** Dr. James A. Swenberg, UNC, "The Dose-Effect Relationship in Mutation and Cancer"

4:15 - 5:15 **Reception**

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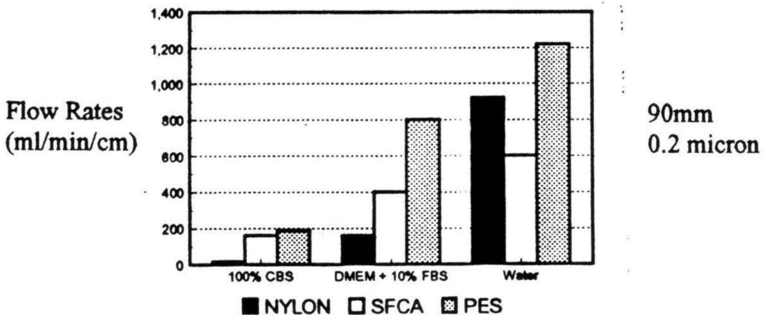
166-0020	500 mL	0.2 um Filter unit PES Sterile	50 mm
167-0020	1000mL	0.2 um Filter unit PES Sterile	90 mm
295-3320	500mL	0.2 um Bottle top fits 33mm bottle	50 mm
295-4520	500mL	0.2 um Bottle top fits 45mm bottle	50 mm



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ABSTRACTS
TALKS

T1 STABLY TRANSFECTED CELL LINES FOR MODELING CHEMOPREVENTIVE FUNCTIONS OF GLUTATHIONE S-TRANSFERASE ISOZYMES: DIFFERENTIAL PROTECTION AGAINST GENOTOXIC VERSUS CYTOTOXIC EFFECTS OF DNA-DAMAGING ELECTROPHILES.

Alan J. Townsend and Ying Li, Bowman Gray School of Medicine, Winston-Salem, N.C. 27157.

The glutathione S-transferase (GST) multigene family is thought to function in protection of sensitive cellular macromolecules against damage by reactive electrophilic species, by conjugation with glutathione, by direct binding, or by reduction of electrophilic species. Various GST isozymes are overexpressed following exposure of cells to Ah receptor ligands, antioxidants, chemopreventive agents, steroid hormones, or anticancer alkylating agents. However, a direct role for GST in prevention of cytotoxicity or genotoxicity remains unproven. We have asked whether cell lines stably transfected with individual GST isozymes are resistant to the DNA damaging (genotoxic) or cell killing (cytotoxic) effects of certain carcinogenic electrophiles. Two end-points were examined following exposure to either the carcinogenic GST substrate 4-nitroquinoline-1-oxide (NQO) or the mutagenic GST substrate 1-Cl-2,4-dinitrobenzene (CDNB): 1) DNA strand scission induced by NQO or CDNB was measured by the alkaline elution assay, which measures relative fragmentation of DNA; and 2) cytotoxicity of NQO or CDNB was measured by the clonogenic survival assay, which measures survival over 6 or more generations required to form colonies ≥50 cells. The results indicated that expression of mu and pi, but not alpha class GST reduced DNA strand scission induced by NQO in transfected MCF-7 cells, in comparison with empty vector-transfected controls. Strand scission induced by NQO was completely prevented in a transfected line (T47Dπ) expressing an exceptionally high level of GSTπ. In contrast, mu and pi class GST expression protected against the cytotoxic effects of NQO only in the T47Dπ line. Similarly, protection by expression of transfected mu and pi class GST was effective against strand scission induced by CDNB in MCF-7 cells, and high level GSTπ expression completely blocked DNA damage in the T47Dπ line. However, GST did not protect at all against the cytotoxic effects of CDNB, even at the high expression level in the T47Dπ cell line. These results indicate that GST expression can confer differential protection in the same cell line against the genotoxic versus cytotoxic effects of a single electrophilic agent. The mechanistic basis for this differential protective function is not known, but may involve conjugation of different metabolites, or compartmentation of the GSTs relative to the most sensitive cellular targets. *(Supported by Grant # 1-ROI-ES-06006 from NIEHS/NIH.)*

T2 EFFECT OF PARTIAL HEPATECTOMY ON THE MUTANT FREQUENCY OF *LACI* IN TRANSGENIC MICE TREATED WITH N-ETHYL-N-NITROSOUREA.

Vanessa J. Wood[1,3], Barbara S. Shane[2,3], Michael L. Cunningham[3], and Kenneth R. Tindall[3], [1]University of North Carolina at Chapel Hill, NC, [2]Louisiana State University, Baton Rouge, LA, and [3]NIEHS, Research Triangle Park, NC.

The Big Blue® transgenic mouse permits the study of the role of pharmacodynamics on mutation frequency (MF) at a chromosomally integrated *lacI* target gene following *in vivo* xenobiotic exposure. We have studied the effects of cellular proliferation on MIF at *lacI* in the liver of male Big Blue® mice. Eight week old mice were injected with either saline [control] or 50 mg/kg N-ethyl-N-nitrosourea (ENU) [treated] for five consecutive days (days 1–5) followed by a 40% partial or sham hepatectomy on day 6. Animals were sacrificed on days 21 and 51, which corresponds to days 15 and 45 following hepatectomy. The observed MF (9.7×10^{-5}) in control mice that were subjected to hepatectomy was not significantly different at the time of hepatectomy (day 6) compared to the MF observed at the time of sacrifice at either 21 (MF = 2.1×10^{-4}) or 51 (MF = 1.0×10^{-4}) days. However, hepatectomy clearly had an effect on the observed MF in the liver following ENU treatment. At sacrifice, the observed MF in the liver of the ENU-treated animals subjected to hepatectomy was 2.1×10^{-3} and 2.5×10^{-3} at 21 and 51 days, respectively. These MF values are approximately one order of magnitude higher than the MF observed in the liver from the same animals taken at the time of hepatectomy on day 6 (MF=2.0×10^{-4}). In addition, the ENU-treated animals subjected to hepatectomy showed a substantially higher MF than was observed in ENU-treated animals without hepatectomy. At 21 days the MF in the mice that received ENU alone was 6.0×10^{-4} while at 51 days the MF was 6.7×10^{-4}. These results suggest that cellular proliferation induced by partial hepatectomy substantially increases the frequency of ENU-induced mutations in the liver.

ABSTRACTS
POSTERS

(* indicates competing posters)

P1 CENTROMERE ANALYSIS OF MICRONUCLEI IN 2-AMINOANTHRAQUINONE-TREATED
***** MOUSE SPLENOCYTES WITH GAMMA-SATELLITE DNA PROBE AND ANTI-KINETOCHORE ANTIBODY.
A. Afshari[1], P. McGregor[1], J. Allen[2], and J. Fuscoe[1]. [1]ILS, RTP, NC 27709, [2]US EPA, RTP, NC 27711.

Anthraquinones are widely used in industry and medicine. 2-Aminoanthraquinone (2AAQ) is one of several derivatives known to be carcinogenic in rodents. Mutagenesis test results in mouse lymphoma cells have suggested that 2-AAQ is both aneugenic and clastogenic. We have evaluated 2-AAQ for its potential to induce chromosome loss and/or breakage in cultured mouse splenocytes. Binucleated cells were evaluated for micronucleus (MN) frequencies, and the MN were further probed with two indirect indicators of centromere presence: an anti-kinetochore autoantibody and a DNA probe for the mouse gamma-satellite locus. Over the dose range of 2 to 20 µg/mL 2-AAQ, significant and dose-dependent increases in cells with MN were observed which reached levels > 10 times that of controls. Immunofluorescence and fluorescence in situ hybridization methods of centromere analysis revealed a significant increase of cells with probe-positive as well as probe-negative MN. The relatively higher frequencies of gamma satellite-positive MN as compared with kinetochore-positive MN suggested that 2-AAQ may be cleaving DNA preferentially in the gamma-satellite locus. These results provide strong evidence that the mode of action of 2-AAQ may be as an aneugen by disruption of the kinetochore proteins, as a clastogen with a preferential cleavage site at or near the gamma-satellite locus, or both. Our results also suggest that the use of either of these probes individually may not be an adequate measure of centromere presence. *(This is an abstract of a proposed presentation and does not necessarily represent EPA policy)*

P2 DETECTION OF DIFFERENCES IN ANEUPLOIDY FREQUENCIES IN MALE GERMINAL CELLS AMONG ROBERTSONIAN MOUSE STRAINS
J.E. Baulch, X.R. Lowe, and A.J. Wyrobek. Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA.

Numerical aneuploidies arising in germ cells can have critical health consequences during development and after birth. Efficient animal systems are needed to investigate the mechanisms of induction and reproductive function of aneuploid gametes. This study utilizes fluorescence *in situ* hybridization (FISH) with chromosome-specific DNA probes to compare the aneuploidy frequencies in sperm of males heterozygous for various Robertsonian (Rb) translocations involving chromosome 8. Possible differences between father-derived and mother-derived translocations were also investigated. Both male and female Rb(2.8)2Lub and Rb(8.14)16Rma and male Rb(8.12)22Lub were mated with C57Bl/6Sim to produce male progeny heterozygous for each Rb translocation. Testicular cells were prepared for FISH when offspring reached 8to10 weeks of age. Sperm with 8-8 hyperhaploidy phenotypes were significantly elevated, ~30-fold, exhibiting an average frequency of ~104 per 10,000 as compared to ~3 per 10,000 previously reported for C57Bl/6Sim. Frequencies of 8-8 hyperhaploidy varied among the strains. Rb(2.8)2Lub and Rb(8.14)16Rma showed 8-8 frequencies of 113.5 and 103.5 per 10,000, respectively, while Rb(8.12)22Lub showed a lower 8-8 frequency of 82.4 per 10,000. Significant differences among the three lines of heterozygotes were observed when comparing cases where the Rb translocation is inherited from the father ($p<0.01$). Variation was found based upon the parental source of the abnormality in Rb strains (2.8)2Lub and (8.14)16Rma ($p<0.01$). Frequencies of sperm carrying X-X hyperhaploidy were not significantly elevated in any of the Rb carriers, serving as an internal control for the study. This study utilized a new FISH procedure to confirm that Robertsonian translocation heterozygotes produced highly elevated frequencies of aneuploid gametes which vary among different translocations and detected parent-of-origin effects. *(Work supported by Interagency Agreement with NIEHS Y01-ES-10203-00 and performed under the auspices of the U.S. DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48.)*

P3 CHARACTERIZATION OF THE TEMPORAL PERSISTENCE OF CHROMOSOMAL ABNORMALITIES IN THE SEMEN OF HODGKIN'S DISEASE PATIENTS AFTER TREATMENT WITH NOVP CHEMOTHERAPY USING MULTI-CHROMOSOME FLUORESCENCE *IN SITU* HYBRIDIZATION.

M.J. Cassel[1], W.A. Robbins[1], M.L. Meistrich[2], A.J. Wyrobek[1]. [1]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; [2]Department of Experimental Radiotherapy, University of Texas M.D. Anderson Cancer Center, Houston, Texas.

Three-chromosome fluorescence *in situ* hybridization (FISH) was applied to sperm of men with Hodgkin's disease to measure the persistence of chromosomally abnormal sperm within the time interval of 3 to 33 months after the end of treatment. NOVP chemotherapy includes the agents novantrone, oncovin, vinblastine, and prednisone, two of which are spindle poisons expected to induce aneuploidy. Semen samples were evaluated for the frequencies of fluorescence phenotypes representing hyperhaploidy, hypohaploidy, and genomic duplications using DNA probes specific for repetitive sequences on chromosomes X,Y, and 8. Using this procedure, NOVP was previously shown to induce chromosomally abnormal sperm in treated patients (Robbins et al). In a longitudinal assessment of 17 semen samples from 3 men, frequencies of abnormal sperm appeared to return to pre-treatment levels at ~6 months after the end of treatment and remained at these levels up to 33 months after the end of treatment. However, pre-treatment frequencies of chromosomally abnormal cells in Hodgkin's patients were elevated above those found in normal healthy men (Robbins et al). *(Work performed under the auspices of the U.S. DOE by Lawrence Livermore National Laboratory under contract no. W-7405-Eng-48)*

P4 FREQUENCY AND DNA SEQUENCE ANALYSIS OF A T(14;18) TRANSLOCATION FOUND IN LYMPHOCYTES FROM NORMAL INDIVIDUALS.

Deborah D. Collard Martha M. Moore[2], James C. Fuscoe[1]. [1]Integrated Laboratory Systems, POB 13501, RTP, NC 27709; [2]Genetic Toxicology Division, US EPA, RTP, NC 27711.

A t(14;18) chromosomal translocation has been found in approximately 85% of follicular lymphomas by both cytogenetic and molecular analyses. This rearrangement deregulates expression of the *bcl-2* oncogene by juxtaposition into the *Ig* heavy chain locus and is probably mediated by illegitimate V(D)J recombination. We describe a quantitative nested PCR method for detecting this event in lymphocytes of healthy individuals. Briefly, genomic DNA is purified from peripheral blood lymphocytes, and 2.5 µg (representing 4×10^5 cells) are amplified with deletion-specific primers under conditions in which a single copy, if present, will give a detectable PCR product. Multiple replicates are analzed for each individual, and Poisson statistics are then used to estimate the translocation mutant frequency. We have examined lymphocyte DNA from 6 normal individuals by this assay and found the frequency of cells with t(14;18) to range from < 0.8 to 12×10^{-7}. The molecular nature of the translocations has been investigated by determining the DNA sequence at the translocation junctions. In one individual, multiple isolates of the same translocation event were recovered, indicating that the cell with the original translocation had undergone clonal expansion. Since this translocation appears to be one step in the progression of a normal cell to a cancer cell, we are investigating the utility of this assay as a biomarker of exposure to environmental carcinogens. *(This is an abstract of a proposed presentation and does not necessarily represent EPA policy.)*

P5 ***IN VIVO*** **MUTANT FREQUENCY IN LIVER DNA OF *LACI* TRANSGENIC MICE FOLLOWING**
* **CHRONIC EXPOSURE TO THE CARCINOGEN-NONCARCINOGEN PAIR 2,4- AND 2,6-DIAMINOTOLUENE.**

Jeffrey J. Hayward, Barbara S. Shane#, Kenneth R. Tindall, H.B. Matthews, and Michael L. Cunningham. , NIEHS, RTP, NC and #LSU, Baton Rouge, LA.

Results of research on chemicals that are mutagenic *in vitro* but do not produce carcinogenesis *in vivo* can be used with data from short-term tests for the prediction of carcinogenicity and for the investigation of basic mechanisms of carcinogenesis. The carcinogen–noncarcinogen pair 2,4- and 2,6-diaminotoluene (DAT) are equally mutagenic in the Ames/Salmonella assay in the presence of S9. Both are readily absorbed, metabolized and excreted in rodents. In NTP rodent bioassays, 2,4-DAT is a potent hepatocarcinogen, whereas 2,6-DAT is not tumorigenic in any tissue in either rats or mice, even when administered at a higher dose than 2,4-DAT. The present study was designed to determine whether the Big Blue® transgenic mouse mutagenesis assay could be used to detect a difference in the mutagenic activity of these two DAT isomers *in vivo*. The Big Blue® mouse allows for the recovery of mutations induced *in vivo* at a stable chromosomally integrated *lacI* gene. Mice were fed 2,4- or 2,6-DAT in the diet at 0 or 1000 ppm. Groups of 5 mice were sacrificed at 30 and 90 days of continuous dietary exposure. A positive control group of mice was treated with five daily ip injections of dimethylnitrosamine (DMN) at 6 mg/kg/day and sacrificed 15 days later. Results showed the mutant frequency was essentially identical for the control and DAT treated groups at 30 days. However, the mutant frequency for animals treated with the hepatocarcinogen 2,4-DAT ($12.10 \pm 1.43 \times 10^{-5}$) was significantly higher ($p<0.01$) than for both age-matched control animals ($5.65 \pm 2.93 \times 10^{-5}$) and 2,6-DAT treated animals ($5.56 \pm 2.35 \times 10^{-5}$) at 90 days. DMN increased the mutant frequency to $38.4 \pm 4.8 \times 10^{-5}$ ($p<0.001$). These results indicate that the duration of exposure may be a significant variable for detecting an increased mutant frequency for moderately mutagenic but highly carcinogenic compounds such as 2,4-DAT.

P6 IDENTIFICATION OF CHLOROFORM-INDUCED CHANGES IN HEPATIC GENE mRNA
* **LEVELS USING DIFFERENTIAL DISPLAY.**

A. E. Kegelmeyer, G. J. Horesovsky, C. S. Sprankle, J. L. Larson, and B. E. Butterworth. CIIT, Research Triangle Park, NC 27709.

Chloroform induces hepatocellular carcinomas in female B6C3F$_1$ mice when given by gavage at 238 and 477 mg/kg/day, but not when similar daily amounts are given in the drinking water. Chloroform is not genotoxic and appears to produce tumors secondary to events associated with induced cytolethality, necrosis and regenerative cell proliferation. Changes in hepatic gene mRNA levels following chloroform administration were identified using differential display. Liver RNA was isolated from female mice administered chloroform by gavage at doses of 0, 3, 238, and 477 mg/kg/day or in the drinking water at 0 and 1800 ppm for 4 days or 3 weeks. Differential display was performed using one of twelve 3'-terminal T_{11}VN primers and a single internal primer to produce a representative subset of the total mRNA population in female mouse livers. 387 individual bands were identified from the 12 gels, nine of which (2.3%) appeared to be differentially expressed when compared to controls. Three bands were reduced in density in animals treated with either 238 or 477 mg/kg/day chloroform, one of which had strong homology to mouse non-muscle myosin light chain. The other six bands were increased in density in animals treated with 477 mg/kg/day for 3 weeks and have been identified as follows: 1) mouse fibronectin; 2) a gene with significant homology to the *Homo sapiens* TL7 gene isolated originally from the LNCap human prostate carcinoma cell line; and 3) the mouse TIS21 gene, which is a primary response gene induced by growth factors and tumor promoters. Four of the nine bands had no significant homologies to known sequences and may represent novel genes. No differences in mRNA levels were observed in 3 mg/kg/day gavage or 1800 ppm drinking water livers. Confirmation of the differential expression of these clones is ongoing. These results indicate that the differential display technique can be a useful tool for isolating and identifying genes potentially involved in tumor formation, necrosis and cell proliferation. Studies of changes in gene expression may lead to a greater understanding of the molecular mechanisms involved in chloroform-induced cancer. Measurements of tumor formation, cytotoxicity, cell proliferation and changes in the genes noted above help complete the profile of biological activity of chloroform. Knowledge of dose- and time-response curves for such critical events may be of value in risk assessments.

P7 MOLECULAR DETECTION OF CHROMOSOMAL ABNORMALITIES IN GERM AND SOMATIC CELLS OF AGED MALE MICE. X. Lowe[1], J. Baulch[1], L. Quintana[1], B. Collins[2], J. Allen[2], M. Ramsey[1], J. Breneman[1], J. Tucker[1], N. Holland[3], A. Wyrobek[1]; [1]Biology and Biotechnology Research Proggram, Lawrence Livermore National Laboratory, Livermore CA. [2]US EPA, Research Triangle Park, NC, [3]School Public Health, University of California, Berkeley, CA.

Three cytogenetic methods were applied to eight B6C3F1 male mice aged 22.5 to 30.5 mo to determine if advanced age was associated with an elevated risk of producing chromosomally defective germinal and somatic cells: sperm aneuploidy analysis by multi-color fluorescence *in situ* hybridization for three chromosomes, spermatid micronucleus analysis with anti-kinetochore antibodies, and translocation analysis of somatic metaphases by "painting" for two chromosomes. Eight mice aged 2.4 mo served as controls. Sperm aneuploidy was measured by multi-color fluorescence *in situ* co-hybridization with DNA probes specific for chromosomes X, Y and 8, scoring 10,000 cells per animal. The aged group showed significant 1.5 - 2.0 fold increases in the hyperhaploidy phenotypes X-X-8, Y-Y-8, 8-8-Y, and 8-8-X with the greater effects appearing in animals aged >29 mo. The aged group also showed significantly increased frequencies of micronucleated spermatids (2.0 vs 0.4 per 1000; all were kinetochore negative). Analysis of metaphase chromosomes from blood by "painting" of chromosomes 2 and 8 yielded 4 translocations per 858 cell-equivalents in the aged group which was a non-significant elevation over 0/202 in controls. Although interpretation must be cautious due to the small number of animals analyzed, these findings suggest that advanced paternal age may be a risk factor for chromosomal abnormalities of reproductive and somatic importance. *(Work was performed under the auspices of the US DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48, with support from NIEHS Y01-ES-10203-00, and does not necessarily represent US EPA policy.)*

P8 DNA ADDUCTS FORMED BY THE PESTICIDE ALACHLOR AND ITS METABOLITES. G. Nelson[1] and J. Ross[2], [1]ILS, PO Box 13501, RTP, NC 27709; [2]EPA, MD 68, RTP, NC 27711.

Alachlor (2-chloro-N-methoxymethyl-N-(2',6'-diethylphenyl)-acetamide, (CAS 15972-60-8) is a preemergent herbicide used primarily on corn and soybeans. Alachlor has been demonstrated to induce cytogenetic damage in human lymphocytes *in vitro* and mouse bone marrow *in vivo,* and induces tumors in both mice and rats. Alachlor is metabolized to 2-chloro-N-(2,6-diethylphenyl)acetamide (CDEPA). We have examined the formation of covalent DNA adducts induced by alachlor and CDEPA using 32P-postlabeling analysis. Alachlor reacts *in vitro* with DNA to produce three major adducts detectable by postlabeling. Reaction of alachlor with poly dG and poly dT also yields adducts with similar mobilities. CDEPA also reacts directly with DNA *in vitro,* yielding at least two adducts. CDEPA also forms adducts with poly dT and poly dG. Alachlor reacts with 3'-dTMP to produce adducts with mobilities similar to those formed in the reaction with poly dG. Characterization by NMR and mass spectroscopy has identified the major product to be N-3-(N-methoxymethyl-N-(2,6-diethylphenyl)acetamide)thymidine-3'-monophosphate. Reaction of CDEPA with 3'-dGMP yields one major adduct, N-1-(N-(2,6-diethylphenyl)acetamide)-2'-deoxyguanosine-3'-monophosphate, which does not appear to be formed in the reaction of CDEPA with DNA or poly dG.

P9 ANEUPLOIDY IN SPERM OF HODGKIN'S DISEASE PATIENTS RECEIVING NOVP CHEMOTHERAPY.
W. A. Robbins[1,2,3] M. L. Meistrich[4], M. J. Cassel[1], A. J. Wyrobek[1]. [1] Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, CA. [2] Department of Epidemiology, University of California, Berkeley; [3] NIEHS, Research Triangle Park, [4]University of Texas, M.D. Anderson Cancer Center.

Induction of genetic damage in germ cells of young patients receiving chemo- or radiotherapy for cancers with probable cure, such as Hodgkin's disease, is cause for concern. These young patients may someday desire children, and germ cell alterations presenting as numerical chromosomal abnormalities in sperm may place their future offspring at risk. To address this concern, we measured aneuploidy in sperm from eight young Hodgkin's disease patients: four pre-treatment, four during treatment, and three over a 45 month period following treatment with NOVP (Novantrone, Oncovin, Vinblastine and Prednisone). Patients ranged in stage of disease from IA to IIEB and none had received prior radiation or chemotherapy. Using multi-chromosome sperm FISH with repetitive sequence probes specific for chromosomes X, Y, and 8, we found a significant 2- to 4-fold increase in particular numerical chromosomal abnormalities during treatment which were limited in persistence post-treatment. Additionally, pre-treatment Hodgkin's disease patients showed elevations in some numerical chromosomal abnormalities when compared to a healthy reference group. In several men, the fraction of aneuploid sperm did not return to healthy reference group levels, even after completion of therapy. These results show that elevated sperm aneuploidy occurs in germ cells of young cancer patients during chemotherapy and suggest caution to prevent conceptions during this period. The elevated sperm aneuploidy appears transient, but in some cases never returns to healthy reference group levels. *(This work performed under the auspices of the US DOE, by Lawrence Livermore National Laboratory under contract no. W-7405-Eng-48.)*

P10 ASSAY OF mRNA LEVELS OF GROWTH CONTROL GENES IN LIVERS OF FEMALE * MICE AND KIDNEYS OF MALE RATS AFTER A SINGLE DOSE OF CHLOROFORM.
C.S. Sprankle, J.L. Larson, S.M. Goldsworthy, D.C. Wolf, and B.E. Butterworth, CIIT, P.O. Box 12137, RTP, NC, 27709.

Chloroform is a trace contaminant of drinking water. Daily chloroform administration by gavage in corn oil increased the incidence of liver tumors in both sexes of B6C3F$_1$ mice and the incidence of kidney tumors in male Osborne-Mendel rats. Chloroform is not genotoxic. The carcinogenicity of chloroform is thought to involve cytotoxicity and the resulting regenerative cell proliferation in the target organs. Increases in expression of growth control genes accompanying the regenerative process may play a part in the development of chloroform-induced tumors. Northern blot hybridization was used to examine the mRNA levels for the *myc*, *fos*, Ha-*ras*, *met*, and HGF genes in livers of female B6C3F$_1$ mice and kidneys of male F-344 rats from 12 hrs to 8 days following a single, cytotoxic gavage dose of chloroform in corn oil (350 mg/kg for mice, 180 mg/kg for rats). Analysis of mRNA from livers of female mice showed large transient increases in the levels of *myc* and *fos*, peaking at 12–24 hrs after treatment. The mRNA levels for Ha-*ras*, *met*, and the HGF gene remained near those seen in control livers. In the male rat kidney, mRNA levels for *myc* increased, peaking at 12 hrs after treatment and returning to control levels at 2 days. Levels for all other genes examined remained near those seen in kidneys of control animals. These mRNA levels exhibited in female mouse liver and male rat kidney after a single, cytotoxic dose of chloroform are consistent with those observed after other cytotoxic treatments. Examination of tissues involved in longer treatment periods will be required to determine whether alteration of the *myc* and *fos* genes plays any role in the development of chloroform-induced tumors.

P11 EXAMINATION OF THE ADDITIVITY ASSUMPTION USING THE SPIRAL AND STANDARD SALMONELLA ASSAYS TO EVALUATE BINARY COMBINATIONS OF MUTAGENS.
*
M.S. Taylor[1], R.W. Setzer[2], and D.M. DeMarini[2]. [1]Dept. of Environmental Science and Engineering, UNC, Chapel Hill, NC 27599 and [2]US EPA, HERL, RTP, NC 27711.

Binary combinations of pure chemicals and complex mixtures were evaluated for their ability to produce additive mutagenicity responses in the spiral and standard Salmonella mutagenicity assays. Single chemicals were selected that were representative of the primary chemical class responsible for much of the mutagenic activity of each complex mixture. The following agents were evaluated in the absence of S9: 1-nitropyrene, diesel exhaust extract, and the chlorinated drinking water mutagen 3-chloro-4-dichloromethyl-5-hydroxy-2-[5]-furanone (MX) . In the presence of S9, the following agents were evaluated: 4-aminobiphenyl, benzo(a)pyrene, and an organic extract from the particulate emissions resulting from the combustion of polyethylene in a rotary kiln incinerator. Binary combinations of the agents within each S9 group were tested. In general, the combinations of mutagens produced additive responses at low doses in both assays. However, at high doses, the results generally departed from additivity, especially for combinations of indirect-acting mutagens. A requirement for exogenous metabolic activation imposes conditions, such as different S9 optima for each mutagen, that complicate the application of the additivity assumption to binary combinations of indirect-acting mutagens. Although the two bioassays generally gave similar results for each binary combination of mutagens, the spiral assay permitted a gradient of mutagen and/or S9 concentrations to be evaluated, which may be advantageous when studying chemical interactions. *(Abstract does not necessarily reflect EPA policy.)*

P12 DNA ADDUCTS AS BIOMARKERS OF EXPOSURE IN AORTA CELLS FROM ATHEROSCLEROTIC PATIENTS.
D. Walsh[1], L. Phillips[1], M. Schmitt[1], J. Scott[1], A. Izzotti[2], S. DeFlora[2], and J. Lewtas[3]. [1]Integrated Laboratory Systems, RTP, NC, [2]University of Genoa, Genoa, Italy, [3]US EPA, RTP, NC.

Various cardiopulmonary tissues are being explored for use in autopsy and clinical intervention studies of DNA adducts as biomarkers of exposure. Based on evidence that cigarette smoke and benzo(a)pyrene (BaP) induce exposure related DNA adducts in the heart, we have examined the relationship between DNA adducts and tobacco exposure in smooth muscle cells of the abdominal aorta from 40 atherosclerotic patients. The patients include 18 current smokers and 22 ex-smokers. DNA was extracted from the isolated smooth muscle cells and adducts were analyzed by ^{32}P postlabeling after enhancement by nuclease PI or butanol extraction. In the samples analyzed, the relative adduct level (RAL) for the total butanol extracted adducts in current smokers varied from 5.9 to 21.3 x 10^8 and ex-smokers from 7.0 to 18.1 x 10^8. These relatively high levels of DNA adducts are consistent with those measured in autopsy heart tissue of both smokers (15.5 x 10^8) and nonsmokers (3.01 X 10^8) . This is part of a larger study to examine the relationship between DNA adducts and induction of atherosclerotic plaques. *(This is a proposed abstract and does not reflect US EPA policy.)*

P13 COMPARISON OF AMBIENT AIR PAH, NICOTINE, AND MUTAGEN EXPOSURE IN TOKYO, JAPAN AND RTP, NC USING PERSONAL EXPOSURE MONITORING.

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A study to evaluate a new personal exposure monitor (PEM) and analytical procedures for PAHs, nicotine, and mutagens was conducted in Tokyo, Japan and Research Triangle Park, NC. Both smoking and nonsmoking individuals, 7 at each location, were selected for the study. The PEM sampled coarse and fine particulate matter (PM_c and $PM_{2.5}$) as well as vapor phase nicotine, and HPLC time-programmed fluorescence detection of PAHs. Mutagenicity of particle associated organics was determined using an ultramicro forward mutation assay (8-azaguanine resistance in Salmonella TM677). A wide range of PAH and mutagen levels was observed between the two groups. Greater that 90% of the PAH and mutagens detected were found to be associated with fine particulate matter. The Tokyo participants were exposed to an average of 3.7 ng/m3 BaP, which was 10-fold higher than the concentration observed for the RTP group. Mutagen exposure for the Tokyo group averaged 6 times higher than those observed in RTP. Cigarette smoking, lifestyle factors, and other pollutant sources might have resulted in higher PAH exposures for Tokyo participants as compared to the RTP group. Evaluation of the sample collection and analysis procedures revealed that low volume air samples (~2.5 m^3) could be successfully used in an urban environment to determine personal air exposures to PAH, mutagens and nicotine. *(This is a proposed abstract and does not reflect US EPA policy.)*

P14 COLOCALIZATION OF ANDROGEN RECEPTOR AND THYROTROPIN RELEASING HORMONE PRO HORMONE IN RAT BRAIN.

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Evidence from our laboratory and others has indicated a direct action of androgen on thyrotropes in rat pituitary while androgen action of TRH neurons is not known. This study was conducted to determine whether thyrotropin releasing hormone prohormone(pro-TRH) neurons contain androgen receptor (AR). Adult male rats treated with testosterone propionate sc also received colchicine. Twenty hr later the rats were perfused with Zamboni's fixative. Brains were frozen and 10 µm serial sections were processed for dual immunostaining. Sections were immunostained with antipeptide antibody AR32 using DAB and with anti pro-TRH antibody using 4-chloro-1 naphthol, AR was localized in several nuclear groups of the brain where immunoreactive TRH or Pro-TRH cell exist. These include the preoptic region, bed nucleus of the strial terminalis, periventricular nucleus, parvocellular portion of the paraventricular nucleus, dorsomedial nucleus and basolateral hypothalamus. Nuclear localization of AR was observed in a subpopulation of immunoreactive pro-TRH cells in preoptic nucleus, bed nucleus of the strial terminalis, periventricular hypothalamic nucleus, and basolateral hypothalamus. The results suggest that androgen may have direct effects on certain TRH neurons. *(Supported by NIH Grant NS17479).*

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