GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

SEVENTH ANNUAL MEETING
PROGRAM AND ABSTRACTS

OCTOBER 19, 1989
Howard Johnson Hotel & Conference Center
Raleigh, North Carolina

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

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Genotoxicity and Environmental Mutagen Society

Dear GEMS Member:

Thank you for participating in the Seventh Annual Meeting of the Genotoxicity and Environmental Mutagen Society. Each Officer and member of the Board of Directors expects that this meeting will be both informative and intellectually stimulating. We look at this meeting not only as an event to give presentations and to get an overview of some of the scientific research being performed in the Triangle, but also as an opportunity to interact with fellow GEMS members, to renew acquaintances, and possibly to develop new collaborations or friendships. We also encourage you to contact the Officers and members of the Board and give them suggestions, criticisms, or recommendations for future meetings and events.

GEMS is pleased to have **Dr. Herbert S. Rosenkranz** from Case Western Reserve University as the Keynote Speaker. He will talk on "Myths, Genotoxicity, and Intelligence (Human or Otherwise)." We also have four other Invited Talks. **Dr. Ray Tice** (ILS) will discuss "The Single-Cell Gel Technique: A New Method for Analyzing DNA Damage and Repair." **Dr. James Roberts** (NIEHS) will discuss "Mechanisms of Mutagenesis During DNA Replication In Vitro." **Dr. Leslie Recio** (CIIT) will talk on "Molecular Mechanisms of Mutagenesis in Human Cells In Vitro and In Vivo." **Dr. Kenneth Tindall** (NIEHS) will discuss "Molecular Analysis of Mutation in Bacteria and Mammalian Cells." In addition, scientists will be competing for the GEMS Travel Award to attend the 21st Annual Meeting of the Environmental Mutagen Society in Albuquerque, NM, in March 1990.

SUSTAINING MEMBERS provide many valuable services to GEMS, and we urge each member to visit their exhibits, talk to them about their services and products, and thank them for supporting the Society. Many of our activities, including a substantial part of this meeting, would not be possible without their financial support.

I thank **Tom Hughes** and his organizing committee for organizing this Annual Meeting; I also thank **Laurie Backer, Louise Ball, David Doolittle, Greg Erexson, Barry Margolin, Vern Steele, and Debra Walsh** for the time and energy that they put into the Society during their terms as Officers or Councilors. I congratulate the new Officers, **David Doolittle, Greg Erexson, and Debra Walsh;** as well as the new Councilors, **Doug Bell, Bill Caspary, Paul Glover, Ray Tennant, and Ron Williams.** I look forward to the continued growth and vitality of the Society. Welcome to the Seventh Annual Meeting of GEMS.

Sincerely,

David

David M. DeMarini
President, GEMS

ACKNOWLEDGMENTS

GEMS gratefully acknowledges the financial support provided by the Sustaining Corporations, and we especially thank **INTEGRATED LABORATORY SYSTEMS** (T.K. Rao) for sponsoring the morning coffee.

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ATLANTIC COAST CONSULTANTS, 2450 Honeysuckle Road, Chapel Hill, NC 27514. Representatives: Allen L. Miller and James Conley (919-929-6212). *Provides tissue culture equipment for biological safety, cryopreservation, and environmental control.*

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PROGRAM
GEMS SEVENTH ANNUAL MEETING
OCTOBER 19, 1989

Howard Johnson Hotel
Raleigh, North Carolina

8:00 - 8:25 **REGISTRATION**

8:25 - 8:30 Welcoming Remarks, D.M. DeMarini, President, GEMS.

8:30 - 8:45 **T1** EVALUATION OF BENZO[a]PYRENE ADDUCTS IN RAT LIVER, LUNG, AND PERIPHERAL BLOOD LYMPHOCYTE DNA. G. Nelson[1], J. Ross[2], R. Gupta[3], K. Earley[3], and S. Nesnow[2]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711; [3]Baylor College of Medicine, Houston, TX 77030.

8:45 - 9:00 **T2** SISTER CHROMATID EXCHANGE AND MICRONUCLEUS ANALYSIS IN RAT PERIPHERAL BLOOD LYMPHOCYTES AFTER IN VIVO EXPOSURE TO BENZO[a]PYRENE. M.F. Bryant[1], G.L. Erexson[1], P. Kwanyuen[1], and A.D. Kligerman[2]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

9:00 - 9:15 **T3** THE INFLUENCE OF MAINSTREAM SMOKE CONDENSATE FROM CIGARETTES THAT BURN OR ONLY HEAT TOBACCO ON INTERCELLULAR COMMUNICATION BETWEEN CULTURED MAMMALIAN CELLS. S.C. McKarns and D.J. Doolittle. R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102.

9:15 - 9:30 **T4** MITOCHONDRIAL MEMBRANE POTENTIAL AS AN INDICATOR OF IN VITRO CYTOTOXICITY. C.A. Rahn, D.W. Bombick, and D.J. Doolittle. R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102.

9:30 - 11:00 **COFFEE, POSTERS, EXHIBITS**

11:00 - 11:15 **T5** ESTIMATION OF INDUCED MUTATION FREQUENCIES IN MAMMALIAN CELLS. D. Daston[1], C. Rudd[2], and W. Caspary[1]. [1]NIEHS, RTP, NC 27709; [2]SRI Int'l., Menlo Park, CA 94025.

11:15 - 11:30 **T6** MOLECULAR ANALYSIS OF HYCANTHONE-INDUCED L5178Y/TK$^{-/-}$ SMALL- AND LARGE-COLONY MOUSE LYMPHOMA CELL MUTANTS. J. Dubins[1], J. Warner[1], R. Krehl[2], C. Rawn[1], P. Glover[2], T. Hughes[1], D. Clive[2], and R. Cobb[1]. [1]RTI, RTP, NC 27709; [2]Wellcome Research Laboratories, RTP, NC 27709.

11:30 - 11:45 **T7** INDUCTION OF DNA STRAND BREAKS BY CROCIDOLITE ASBESTOS IN CULTURED RAT EMBRYO CELLS DEMONSTRATED BY THE NICK-TRANSLATION TECHNIQUE. B.L. Libbus[1], S.A. Illenye[2], and J.E. Craighead[2]. [1]Genetic Research, Inc., P.O. Box 13354, RTP, NC 27709; [2]Dept. of Pathology, U. of Vermont, Burlington, VT 05405.

11:45 - Noon **T8** A MULTI-FACTOR RANKING SCHEME FOR COMPARING THE CARCINOGENIC ACTIVITY OF CHEMICALS. S. Nesnow. U.S. EPA, RTP, NC 27711.

Noon - 1:30 **LUNCH, BUSINESS MEETING, RECOGNITION OF EXHIBITORS AND SPONSORS, AND INSTALLATION OF NEW OFFICERS AND MEMBERS OF THE BOARD OF DIRECTORS**

1:30 - 2:00 **INVITED TALK:** THE SINGLE-CELL GEL TECHNIQUE: A NEW METHOD FOR ANALYZING DNA DAMAGE AND REPAIR. R.R. Tice. ILS, P.O. Box 13501, RTP, NC 27709.

2:00 - 2:30 **INVITED TALK:** MECHANISMS OF MUTAGENESIS DURING DNA REPLICATION IN VITRO. J.D. Roberts. NIEHS, RTP, NC 27709.

2:30 - 3:00 **INVITED TALK:** MOLECULAR MECHANISMS OF MUTAGENESIS IN HUMAN CELLS IN VITRO AND IN VIVO. L. Recio. CIIT, RTP, NC 27709.

3:00 - 3:30 **COFFEE AND EXHIBITS**

3:30 - 4:00 **INVITED TALK:** MOLECULAR ANALYSIS OF MUTATION IN BACTERIA AND MAMMALIAN CELLS. K.R. Tindall. NIEHS, RTP, NC 27709.

4:00 - 5:00	**KEYNOTE ADDRESS:** MYTHS, GENOTOXICITY, AND INTELLIGENCE (HUMAN OR OTHERWISE). H.S. Rosenkranz. Case Western Reserve University, Cleveland, OH 44106.
5:00 - 5:05	**AWARDS**
5:05 - 6:00	**SOCIAL: CHEESE, FRUITS, AND BEVERAGES**
6:00	**ADJOURN**

POSTERS

P1 USE OF THE POLYMERASE CHAIN REACTION TO AMPLIFY A SEGMENT OF THE hisD3052 GENE OF Salmonella typhimurium FOR DNA SEQUENCE ANALYSIS. D.A. Bell[1], J.E. Lee[2], and D.M. DeMarini[3]. [1]National Research Council, U.S. EPA, RTP, NC 27711; [2]Duke University Medical Center, Durham, NC 27710; [3]U.S. EPA, RTP, NC 27711.

P2 CELL CYCLE-SPECIFIC CHROMOSOMAL ABERRATIONS IN MOUSE FOLLOWING EXPOSURE TO THE DNA TOPOISOMERASE INHIBITORS CAMPTOTHECIN AND m-AMSA. D.R. Howard[1], L.C. Backer[1], D.M. DeMarini[2], A.D. Kligerman[2], and J.W. Allen[2]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

P3 SELECTION OF AN EXPERIMENTAL PROTOCOL FOR SCREENING TEST AGENTS IN THE MOUSE BONE MARROW MICRONUCLEUS TEST. G.L. Erexson[1], J.L. Huston[1], R.M. Boehm[1], D. Gulati[2], and M.D. Shelby[3]. [1]EHRT, RTP, NC 27709; [2]EHRT, 2514 Regency Road, Lexington, KY 40503; [3]NIEHS, RTP, NC 27709.

P4 EFFECT OF PENTACHLOROPHENOL ON 2,6-DINITROTOLUENE-INDUCED URINE MUTAGENICITY AND INFLUENCE ON INTESTINAL ENZYMES IN THE MALE CD-1 MOUSE. S.E. George[1], R.W. Chadwick[1], M.J. Kohan[1], and J.P. Dekker[2]. [1]U.S. EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

P5 DEVELOPMENT OF AN INDIRECT-ACTING (+S9) DATA BASE IN THE AMES/SALMONELLA ASSAY TO RANK THE MUTAGENICITY OF COMPLEX ENVIRONMENTAL MIXTURES. J.R. Warner[1], T.J. Hughes[1], L.E. Myers[1], V.S. Houk[2], and L.D. Claxton[2]. [1]RTI, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

P6 PLASMA MEMBRANE CHARACTERISTICS AS INDICES OF IN VITRO TOXICITY. D.W. Bombick and D.J. Doolittle. R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102

P7 HETEROZYGOUS EFFECTS OF X-RAY-INDUCED SPECIFIC-LOCUS MUTATIONS IN THE ad-3 REGION OF Neurospora crassa. F.J. de Serres, L.K. Overton, and B.M. Sadler. RTI, RTP, NC 27709.

P8 CASE-SAR ANALYSIS OF PAH CARCINOGENICITY. A. Richard[1], Y. Woo[2], and G. Klopman[3]. [1]U.S. EPA, RTP, NC 27711; [2]U.S. EPA, TS-796, Washington, DC 20460; [3]Dept. of Chemistry, Case Western Reserve University, Cleveland, OH 44106.

P9 EXPRESSION OF MUTATIONS AT THE hgprt LOCUS IN CHINESE HAMSTER OVARY (CHO) CELLS USING SEMISOLID MEDIUM TO SEGREGATE CELLS AFTER MUTATION INDUCTION. P.S. Lee and C.J. Rudd. SRI International, Menlo Park, CA 94025.

P10 AN EVALUATION OF THE METABOLISM OF 1-NITRO[^{14}C]PYRENE BY RABBIT TRACHEAL EPITHELIAL CELLS: KINETIC ANALYSIS. L.C. King[1,2], E. Hodgson[2], and J. Lewtas[1]. [1]U.S. EPA, RTP, NC 27711; [2]Dept. of Toxicology, NCSU, Raleigh, NC 27695.

P11 APPLICATION OF BIOASSAY-DIRECTED CHEMICAL ANALYSIS TO INCINERATOR EFFLUENTS AND OTHER COMBUSTION EMISSIONS. D.M. DeMarini[1], P.M. Lemieux[1], J.A. McSorley[1], W.P. Linak[1], E. Perry[2], and R.W. Williams[2]. [1]U.S. EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

P12 COMPARISON OF DNA ADDUCTS IN MOUSE TISSUES FOLLOWING IN VIVO EXPOSURE TO COMPLEX AIR POLLUTION SOURCES. M.H. George[1], M.A. Jackson[1], J.E. Gallagher[2], and J. Lewtas[2]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

P13 AMBIENT AIR SAMPLING AND SAMPLE PREPARATION FOR MUTAGENICITY BIOASSAY. R.R. Watts[1] and R.W. Williams[2]. [1]U.S. EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

P14 A SOLID-PHASE EXTRACTION (SPE) APPROACH FOR CHEMICAL SEPARATION OF ACIDIC COMPONENTS OF COMPLEX MIXTURES FOR BIOASSAY-DIRECTED CHEMICAL ANALYSIS. D. Bell[1], R. Williams[2], D. Thompson[3], L. Brooks[2], R. Zwiedinger[4], and J. Lewtas[4]. [1]National Research Council, U.S. EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709; [3]NSI, RTP, NC 27709; [4]U.S. EPA, RTP, NC 27711.

P15 RELATIONSHIP BETWEEN INDOOR AND OUTDOOR MUTAGENICITY IN A WOODSMOKE-IMPACTED AIRSHED. S. Warren[1], R. Zweidinger[2], L. Claxton[2], J. Dorsey[2], R. Highsmith[2], L. Cupitt[2], and J. Lewtas[2]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

P16 THE COMPARISON OF MUTAGEN-INDUCED THYMIDINE KINASE (TK) MUTANT FREQUENCIES IN HUMAN AND MOUSE LYMPHOMA TESTING CELLS. M.M. Moore[1], K. Karrington-Brock[2], L. Parker[2]. [1]U.S. EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

P17 CORRELATION BETWEEN CHROMOSOME ABERRATION FREQUENCY AND SMALL-COLONY TK-DEFICIENT MUTANT FREQUENCY IN L5178Y/TK$^{+/-}$ -3.7.2C CELLS. C.L. Doerr[1] and M.M. Moore[2]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

P18 MUTATIONAL SPECIFICITY OF 2-CYANOETHYLENEOXIDE IN HUMAN LYMPHOBLASTOID CELLS. [1]L. Recio, [1]D. Simpson, [1]J. Cochrane, [2]H. Liber, and [3]T.R. Skopek. [1]CIIT, RTP, NC 27709; [2]Harvard School of Public Health, Boston, MA 02115; [3]Glaxo, RTP, NC 27709.

P19 RAT PLEURAL MESOTHELIAL CELLS: CHARACTERIZATION OF A MODEL FOR FIBER-INDUCED EFFECTS. E. Bermudez, J. Everitt, and C. Walker. CIIT, RTP, NC 27709.

P20 EVALUATION OF FURAN-INDUCED GENOTOXICITY AND CELL PROLIFERATION IN RAT AND MOUSE HEPATOCYTES. D.M. Wilson, T.L. Goldsworthy, and B.E. Butterworth. CIIT, RTP, NC 27709.

P21 CYTOTOXICITY OF HAZARDOUS INDUSTRIAL WASTES IN A RAT TRACHEAL EPITHELIAL CELL LINE. D.K. Beeman, D.M. DeMarini, and M.J. Mass. U.S. EPA, RTP, NC 27711.

P22 EFFECT OF THE ANTICARCINOGENIC BOWMAN-BIRK PROTEASE INHIBITOR ON ONCOGENE EXPRESSION AND EPITHELIAL CELL PROLIFERATION IN IRRADIATED RAT COLON. W.H. St. Clair. Dept. of Radiology, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

P23 MAMMALIAN CELL MUTAGENESIS: A MAJOR ROLE FOR NON-DNA TARGETS. D. Clive. Wellcome Research Laboratories, RTP, NC 27709.

P24 MOLECULAR SPECTRA OF L5178Y/TK$^{+/-}$ MUTANTS INDUCED BY DIVERSE MUTAGENS. P. Glover, R. Krehl, and D. Clive. Wellcome Research Laboratories, RTP, NC 27709.

P25 SMOKE EXPOSURE, AGE, SEX, RACE, AND POTENTIATION AS VARIABLES AFFECTING SISTER CHROMATID EXCHANGE INDUCTION IN HUMANS. D.A. Tulis, J.K. Smollinger, and W.H. McKenzie. Genetics Dept., NCSU, Raleigh, NC 27695.

ABSTRACTS
TALKS

T1

EVALUATION OF BENZO[a]PYRENE ADDUCTS IN RAT LIVER, LUNG AND PERIPHERAL BLOOD LYMPHOCYTE DNA. G. Nelson[1], J. Ross[2], R. Gupta[3], K. Earley[3], and S. Nesnow[2]. [1]EHRT, Inc., P.O. Box 12199, Research Triangle Park, NC 27709, [2]U.S. E.P.A., Research Triangle Park, NC 27711, and [3]Baylor College of Medicine, Houston, TX 77030.

Benzo[a]pyrene (BaP) adducts in the lungs, livers and peripheral blood lymphocytes (PBLs) of male CD rats were examined at various times following a single i.p. dose of 100 mg BaP/kg body weight. DNA was isolated from each tissue and BaP-DNA adducts were analyzed by ^{32}P postlabeling with nuclease P1 enhancement. BaP-DNA adducts were detected in each tissue at times between 1 and 56 days. The maximum level of total BaP-DNA adducts was observed at about 4 days after i.p. injection. A major adduct found in all three tissues was the previously identified BPDEI-dGuo adduct. Liver DNA exhibited the highest total adduct level followed by lung DNA and PBL DNA. However, the BPDEI-dGuo adduct level was similar in liver DNA and PBL DNA and a factor of 2 greater in lung DNA. The presence of additional major adducts in the liver DNA and lung DNA gave rise to the observed differences in total adduct level. To determine the metabolic pathways leading to the formation of the additional major adducts, various BaP metabolites were injected i.p. at approximately 100 mg/kg body weight. Examination of the additional adduct in liver DNA indicates that the adduct is apparently derived from further metabolism of BaP-7,8-dihydrodiol. In lung DNA, the additional adduct appears to be formed from a metabolic pathway which includes the formation of 9-hydroxy-BaP.

This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.

T2

SISTER CHROMATID EXCHANGE AND MICRONUCLEUS ANALYSIS IN RAT PERIPHERAL BLOOD LYMPHOCYTES AFTER IN VIVO EXPOSURE TO BENZO(A)PYRENE. M.F. Bryant[a], G.L. Erexson[a], P. Kwanyuen[a], and A.D. Kligerman[b], [a]EHRT, Inc., RTP, NC 27709, and [b]U.S. EPA, RTP, NC 27711.

In an effort to determine the persistence of lesions leading to sister chromatid exchanges (SCE) and micronuclei (MN) in peripheral blood lymphocytes (PBLs) following exposure to a polycyclic aromatic hydrocarbon known to exist at hazardous waste cleanup sites, experiments were conducted using male Sprague-Dawley rats injected with a single i.p. injection of either 0, 100, or 250 mg benzo(a)pyrene (BP)/kg. Peripheral blood was removed by cardiac puncture from each rat at 1, 2, 5, 7, 14, and 21 days after injection. Isolated PBLs from three animals/dose/harvest time were cultured according to previously published methods for analysis of SCEs in second-division metaphase cells and MN in cytochalasin B-blocked binucleated lymphocytes. Both the SCE and MN frequencies remained elevated for 21 days post-injection. These results suggest that rat PBLs or their precursor cells containing SCE- and MN-inducing lesions remain viable for at least three weeks. Due to high spontaneous SCE frequencies in isolated PBLs and variability among replicate animals, a 56 day study was undertaken in which whole blood (WB) from rats treated with a single i.p. injection of 100 mg BP/kg was used for SCE analysis. This study showed that although the mean SCE frequency from the treated aminals was not significantly higher than the concurrent controls at 56 days post-injection, a significant number of cells with high SCE frequencies was present.
(This abstract does not necessarily reflect US EPA policy.)

T3

THE INFLUENCE OF MAINSTREAM SMOKE CONDENSATE FROM CIGARETTES WHICH BURN OR ONLY HEAT TOBACCO ON INTERCELLULAR COMMUNICATION BETWEEN CULTURED MAMMALIAN CELLS

S. C. McKarns and D. J. Doolittle, R. J. Reynolds Tobacco Company, Winston-Salem, NC 27102

Assessment of intercellular communication is useful for measuring the effects of chemical treatment on mammalian cell membranes. The objective of this study was to quantify and compare the activity of mainstream smoke condensate from tobacco-heating and tobacco-burning cigarettes in the in vitro intercellular communication assay. Gap-junctional intercellular communication (GJIC) was determined by quantifying fluorescence redistribution after photobleaching following a one hour exposure to noncytotoxic concentrations (0 - 250 ug/ml) of mainstream cigarette smoke condensate (CSC) in G1 synchronized cell cultures. GJIC was measured in three cultured mammalian cell types (rat hepatic epithelial, rat leydig, and human skin fibroblasts). Cytotoxicity of CSC was determined by two independent assays, Lucifer yellow uptake and lactate dehydrogenase leakage, which measure plasma membrane integrity. In each of the cell types tested, CSC from tobacco-heating cigarettes did not inhibit GJIC under conditions where CSC of tobacco-burning cigarettes significantly inhibited GJIC. These results suggest that mainstream smoke condensate of cigarettes which heat tobacco is less biologically active than mainstream smoke condensate of cigarettes that burn tobacco as measured by this assay.

T4

MITOCHONDRIAL MEMBRANE POTENTIAL AS AN INDICATOR OF IN VITRO CYTOTOXICITY

C.A.Rahn, D.W. Bombick, and D.J. Doolittle,
R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102

Mechanistically based short-term in vitro tests to evaluate the relative cytotoxicity of chemicals are not widely used. In the present study, mitochondrial membrane potential, an integral component of cellular energy homeostasis and normal cellular function, was examined for potential use in an in vitro assay to evaluate chemically induced cytotoxicity. Rhodamine 123, a cationic fluorescent dye whose mitochondrial fluorescence intensity decreases in response to dissipation of mitochondrial transmembrane potential, was used to evaluate disturbances in mitochondrial membrane potential. Cultured rat liver epithelial cells or human skin fibroblasts treated with the oxidative phosphorylation uncoupler 2,4-dinitrophenol (DNP) or the cytochrome oxidase inhibitor sodium azide were used to characterize the system. In addition, acetaldehyde was used to demonstrate the specificity of this assay system. Studies also were conducted to design an optimum treatment protocol and to ascertain the effects of cell cycle and cell culture density on mitochondrial membrane potential. Mitochondrial membrane potential was not affected by either the stage of cell cycle or by increased cell culture density. DNP and sodium azide significantly ($p < 0.01$) reduced the mitochondrial membrane potential compared to untreated cultures, while acetaldehyde had no effect ($p > 0.05$). This assay system should provide a valuable tool for the evaluation of chemical treatments on mitochondrial membrane potential, as well as a mechanistically based indicator of cytotoxicity which does not require the use of animals.

T5

ESTIMATION OF INDUCED MUTATION FREQUENCIES IN MAMMALIAN CELLS. Diane Daston[1], Colette Rudd[2], William Caspary[1],[1]NIEHS/NIH, RTP, NC, and [2]SRI Int'l, Menlo Park, CA.

Procedures used to assess the mutagenic response of mammalian cells to chemicals mix mutants during the expression phase either because the cells are in suspension or are trypsinized and pooled. Under these circumstances if a population of mutants grows more slowly than the wild-type cells, the ratio of mutants to wild-type cells will decrease during the expression period. We developed a procedure which segregates and immobilizes cells during expression and which estimates the mutation frequency, the number of new mutations produced per million cells plated. Segregating colonies during expression insures the independence of the mutant colonies. In this procedure, mouse lymphoma cells heterozygous at the thymidine kinase locus were plated in semisolid medium immediately after treatment and allowed to form microcolonies. The selective agent was added as an overlay at specified times permitting only $tk^{-/-}$ cells to survive. Using this procedure the mutation rate at the tk locus ($tk^{+/-} \rightarrow tk^{-/-}$) was calculated to be 37 mutations per million cell divisions, or 50-fold greater than the mutation rate obtained when the cells were allowed to express the mutant phenotype in suspension. The expression curve for the spontaneous mutations began to increase exponentially approximately 30 hrs after plating in semisolid medium. Mutations were induced in chemically treated cell cultures. The numbers of TFT^r colonies at various times after treatment indicated that the chemically treated cell cultures had higher mutation frequencies than the solvent controls. For chemically treated cultures, the numbers of induced mutant colonies began to increase around 30 hrs, then reached a plateau at a later time that was dependent on the chemical. The largest ratio of induced to spontaneous mutation frequencies occurred at around 40 hours for most chemicals. Because the *in situ* assay captures all mutations as individual colonies, the induced mutation frequency should accurately reflect the mutagenic events that occurred.

T6

MOLECULAR ANALYSIS OF HYCANTHONE-INDUCED L5178Y $tk^{-/-}$ SMALL- AND LARGE-COLONY MOUSE LYMPHOMA CELL MUTANTS. J. Dubins[a], J. Warner[a], R. Krehl[b], C. Rawn[a], P. Glover[b], T. Hughes[a], D. Clive[b], and R. Cobb[a], [a]Research Triangle Institute, Research Triangle Park, NC 27709, and [b]Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Mouse lymphoma cells (L5178Y $tk^{+/-}$-3.7.2C cell line) were exposed to three different doses (1, 5, and 10 µg/ml) of hycanthone methanesulfonate and, following two days expression, cloned for the determination of $tk^{-/-}$ mutant frequency. Colonies of exposed and untreated control cells, resistant to triflourothymidine (TFT), were isolated for confirmation of phenotype by growing in culture medium alone and culture medium supplemented with either TFT or THMG (thymidine + hypoxanthine + methotrexate + glycine which permits growth of $tk^{+/-}$ but not $tk^{-/-}$ cells). These mutants fell into two classes: fast growing (large colony formers) and slow growing (small colony formers). Southern blot analysis of *Nco*-1-digested DNA from small and large TFT-resistant colonies yield two distinct restriction fragment banding patterns when hybridized to the thymidine kinase cDNA clone, pMTK4. The first pattern was identical to that of the $tk^{+/-}$ controls, while the second pattern differed only in the absence of the 6.4 kb fragment, indicative of the loss of the active *tk* allele (Applegate and Hozier, Banbury #28, p. 213, 1987). The present study was initiated to examine the dose effect of hycanthone at the DNA level in mouse lymphoma cells based on the *Nco*-1 restriction fragment patterns.

T7

INDUCTION OF DNA STRAND BREAKS BY CROCIDOLITE ASBESTOS IN CULTURED RAT EMBRYO CELLS DEMONSTRATED BY THE NICK TRANSLATION TECHNIQUE. B.L. Libbus[a], S.A. Illenye[b] and J.E. Craighead[b] . [a]Genetic Research, Inc., P.O. Box 13354, RTP, NC 27709 and University of Vermont, Department of Pathology, Burlington, VT 05405.

Asbestos, a proven carcinogen, induces numerical and structural chromosome aberrations. Repeated attempts, however, failed to document the induction by fibers of mutations or DNA strand damage in exposed cells. We hypothesized that, in light of its clastogenic effects, one mechanism by which asbestos induces cell transformation and tumorigenesis may involve DNA strand scission. Cultured rat embryo cells were exposed to low concentrations of UICC crocidolite, ranging from 0.05 to 2.0 ug/cm^2, and examined at intervals ranging from 2 to 48 h. DNA damage was assessed in fixed or permeabilized cells that were nick translated and processed for autoradiography or scintillation counting. In the nick translation reaction exogenous DNA polymerase I "translates" nicks in the DNA by digesting the nicked strand and synthesizing a complementary strand. Cells exposed to 0.05 ug/cm^2 crocidolite exhibited a higher grain density than control cells following nick translation and autoradiography, indicating an increased incidence of DNA strand breaks. This effect was evident as early as 2 h after exposure to the fibers. The incidence of DNA damage, measured by scintillation counting, was dose-related. On the other hand, cells exposed to ball-milled crocidolite or to riebeckite, a non-fibrous analog of crocidolite, did not exhibit DNA damage. This is in agreement with previous observations on the importance of fiber morphology in asbestos carcinogenesis. The observed DNA damage, however, may not necessarily require direct interaction between fibers and cellular DNA. These observations suggest that the classification of asbestos as a nongenotoxic carcinogen need to be reconsidered.

T8

A MULTI-FACTOR RANKING SCHEME FOR COMPARING THE CARCINOGENIC ACTIVITY OF CHEMICALS, S. Nesnow, Carcinogenesis and Metabolism Branch, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

This activity scheme uses as its base, dose potency measured as TD_{50}. The TD_{50} is converted into a decile scale and adjusted by weighting factors that describe other parameters of carcinogenic activity. These factors include positive or negative weightings for the induction of tumors at tissues or organs associated with high historical control tumor incidences; the induction of tumors at multiple sites; in both sexes of the species; and in more than one species. In order to construct a measure to express the inactivity of chemicals as inducers of cancer, a measure analogous to the TD_{50} has been developed: the highest average daily dose or HADD. The HADD is the highest average daily dose in mg chemical/kg body weight administered in a chronic cancer study, and that did not induce a statistically significant increase in tumors. HADD values were converted to log decile units and adjusted by weighting factors relating to inactivity in both sexes of a species, and the inactivity in more than one species. Three activity ranking schemes were developed using a 142-chemical data set from NTP studies: the Carcinogen Activity-F344 Rat, an activity scheme based on cancer data obtained with the F344 rat; the Carcinogen Activity-B6C3F1 Mouse, an activity scheme based on cancer data obtained with the B6C3F1 mouse, and the Carcinogen Activity-Combined.

This is an abstract of a proposed presentation and does not reflect EPA policy.

ABSTRACTS
POSTERS

P1

USE OF THE POLYMERASE CHAIN REACTION TO AMPLIFY A SEGMENT OF THE hisD3052 GENE OF Salmonella typhimurium FOR DNA SEQUENCE ANALYSIS Douglas A. Bell[1], James E. Lee[2] and David M. DeMarini[3] [1]National Research Council, U.S. EPA, RTP, NC 27711; [2]Duke University Medical Center, Durham, NC 27710; [3]U.S. EPA, RTP, NC 27711 (U.S.A.)

Previous DNA sequence analyses of revertants of the hisD3052 gene of S. typhimurium TA98 or TA1538 have employed either a colony hybridization technique (Cebula et al., Environ. Mol. Mutagen. 11, Suppl. 11:21; 1988) or traditional cloning procedures (Fuscoe et al., Mutat. Res. 201:241; 1988). We have used the polymerase chain reaction (PCR) to rapidly process revertants for DNA sequence analysis. Briefly, a revertant is streaked onto minimal medium supplemented with biotin. After growth, a colony is boiled for 5 min in 200 µl of TE buffer and then centrifuged for 5 min. 5 µl of the supernatant is used for each 50-µl PCR reaction. The two amplification primers (AP) flank a 330-bp segment that contains the hisD3052 mutation. The sequence of AP1 is 5' CGT CTG AAG TAC TGG TGA TCG CA 3'; AP2 is TGC GGT GGT AAT CGC ATC CAC CA. Single-stranded DNA is obtained by asymetric PCR using a 1:100 primer ratio (0.5 pmol:50 pmol) and 40 PCR cycles. Excess primers are removed from the PCR mixture by a Centricon 30 filtration unit, and the ssDNA is sequenced using the Sequenase sequencing kit. We have preliminary sequence data for a group of spontaneous revertants (TA98 +/-S9) and revertants induced by exposure to 1-nitropyrene or an extract from ambient air particles. The most frequent mutation among revertants is a -2 deletion of GC or CG within the sequence CCGCGCGCGG, which occurs upstream of the hisD3052 site. This deletion occurs in 56% (5/9) of the spontaneous (-S9) revertants, 30% (3/10) of the spontaneous (+S9) revertants, 100% (8/8) of the 1-nitropyrene revertants, and 88% (7/8) of ambient air revertants.

P2

CELL CYCLE-SPECIFIC CHROMOSOMAL ABERRATIONS IN MOUSE FOLLOWING EXPOSURE TO THE DNA TOPOISOMERASE INHIBITORS CAMPTOTHECIN AND m-AMSA. D.R. Howard[1], L.C. Backer[1], D.M. DeMarini[2], A.D. Kligerman[2], and J.W. Allen[2], [1]EHRT, Inc., RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

We have shown previously that the topoisomerase inhibitors camptothecin (CAMP) and amsacrine (m-AMSA) induce primarily chromatid aberrations in mouse bone marrow cells following administration of either chemical by i.p. injection. The aim of the present study was to determine if the types of chromosome aberrations (CAs) induced at particular stages of the cell cycle would reflect certain differences in clastogenic mechanisms of CAMP (single-strand breaker) and m-AMSA (double-strand breaker). We assessed CAMP and m-AMSA induction of CAs in bone marrow 2 hr following exposure at the putative G_2 stage. Six animals per dose were injected i.p. with 0 or 9 mg/kg of CAMP or m-AMSA dissolved in DMSO. Thirty min later, 3 animals/dose were injected with colchicine, and the bone marrow was harvested 1.5 hr later. Both chemicals induced an increase in CAs. The response was limited to only chromatid-type breakage and rearrangements: CAMP induced 9 CAs per 50 cells/animal, and m-AMSA induced 28 CAs compared to < 1 in the DMSO controls. We also analyzed mouse peripheral blood lymphocytes (PBLs) exposed to CAMP or m-AMSA during G_0. Two hr after i.p injection, PBLs were harvested from the remaining 3 mice/dose via cardiac puncture, washed to remove traces of chemical, and cultured for 44 hr. The cultures were harvested, and 50 metaphases per animal were analyzed for the induction of CAs. Treatment of G_0 lymphocytes did not induce a statistically significant increase in the frequency of CAs. All aberrations that were observed were of the chromatid-type. Because of the qualitatively different mechanisms of interaction with DNA, we expected that CAMP and m-AMSA would induce different types of CAs. Our finding that both chemicals induce chromatid-type aberrations at G_2 and no aberrations during G_0 indicates that additional mechanisms beyond mediation of strand breakage by DNA topoisomerases may determine the ultimate clastogenic actions of these chemicals.

This is an abstract of a proposed poster and does not necessarily reflect U.S. EPA policy.

P3

SELECTION OF AN EXPERIMENTAL PROTOCOL FOR SCREENING TEST AGENTS IN THE MOUSE BONE MARROW MICRONUCLEUS TEST. G.L. Erexson[1], J.L. Huston[1]*, R.M. Boehm[1]*, D. Gulati[2], and M.D. Shelby[3]. [1]Environmental Health Res. & Testing, Inc., P.O. Box 12199, RTP, NC 27709 and [2]2514 Regency Road, Lexington, KY 40503; [3]NIEHS, NTP, Box 12874, RTP, NC.

Due to methodological variations in the mouse bone marrow micronucleus (MN) test; one universal treatment protocol for in vivo chemical exposure is desireable. A common protocol among laboratories would simplify comparison of MN data. Mitomycin C (MMC) and 7,12-dimethylbenzathracene (DMBA) were tested i.p. at doses of 0, 0.5, 1, and 1.5 mg MMC/kg and 0, 25, 50, and 100 mg DMBA/kg using three different protocols. Male B6C3F1 mice (9 to 14 weeks of age, 5 mice/dose) were treated with either (1) one exposure with harvests for bone marrow polychromatic erythrocytes (PCEs) at 24, 48, or 72 h post-treatment; (2) two exposures separated by 24 h with harvests at 24 or 48 h after the second treatment; or (3) three exposures separated by 24 h with one harvest time at 24 h after the third treatment. For comparison, DMBA was administered also by gavage but only for the three-treatment protocol at the same doses used for the i.p. injections (0, 25, 50, and 100 mg/kg). Bone marrow smears were stained in acridine orange (pH = 7.4) and 2000 PCEs/mouse were scored for MN-containing PCEs. The percent PCEs was determined by counting 200 consecutive PCEs and normochromatics. Statistical analyses revealed that the three-treatment protocol was superior. Therefore, this protocol was selected by NTP for use in in vivo rodent bone marrow MN testing. Additional experiments were done using this protocol to select a positive control dose for both MMC and DMBA to be used in testing coded compounds. The positive control doses chosen are 0.2 mg/MMC and 12.5 mg DMBA/kg which induce about 8 MN-PCEs/1000 PCEs.
[This research was supported by a contract from NTP, #N01-ES-852081].

P4

EFFECT OF PENTACHLOROPHENOL ON 2,6-DINITROTOLUENE-INDUCED URINE MUTAGENICITY AND INFLUENCE ON INTESTINAL ENZYMES IN THE MALE CD-1 MOUSE, S.E. George*,[a], R.W. Chadwick[a], M.J. Kohan[a] and J.P. Dekker[b]. [a]EPA, MD68, and [b]EHRT, Inc., RTP, NC 27711

Pentachlorophenol (PCP) and 2,6-dinitrotoluene (DNT) are environmental contaminants with genotoxic properties. In this study, DNT urine mutagenicity was compared to intestinal enzyme activities to better understand the influence of PCP on DNT metabolism. Pentachlorophenol has been reported to inhibit sulfotransferase activity which is involved in DNT metabolism and detoxification. Mice were dosed p.o. daily for 4 weeks with PCP (42.8 mg/kg body weight). On 1, 2, and 4 weeks after the initial PCP dose, mice were dosed with DNT (75 mg/kg body weight) and urine collected for 24 hours. The urine was concentrated and aliquots tested for mutagenicity using Salmonella typhimurium strain TA98 without metabolic activation in a microsuspension bioassay. On weeks 2 and 4 after the initial dose, 1 set of mice were sacrificed, the intestines removed and nitroreductase, azo reductase, β-glucuronidase, dechlorinase, and dehydrochlorinase activities measured. A significant increase ($p<0.05$) in urine mutagenicity was observed in all mice treated with DNT with and without PCP. By week 4, there was a significant increase in urine mutagenicity in mice treated with PCP and DNT when compared to mice administered DNT alone. In the small and large intestines, there was a significant increase in β-glucuronidase activity by 4 weeks of PCP treatment. This same activity was increased in the cecum at week 2. PCP decreased nitroreductase and dechlorinase activities in the small intestine by week 4, accompanied by an increase in azo reductase activity in the cecum. Because PCP increases β-glucuronidase activity in all three sections of the intestinal tract, more genotoxic metabolites may be available for microbial reduction and subsequent reabsorption.

P5

DEVELOPMENT OF AN INDIRECT-ACTING (+S9) DATA BASE IN THE AMES/SALMONELLA ASSAY TO RANK THE MUTAGENICITY OF COMPLEX ENVIRONMENTAL MIXTURES. J.R. Warner[1], T.J. Hughes[1], L.E. Myers[1], V.S. Houk[2] and L.D. Claxton[2]. [1]Research Triangle Institute and [2]Environmental Protection Agency, Research Triangle Park, NC.

Data on 10 indirect-acting mutagens tested at RTI and EPA were compared under the following assay parameters: Salmonella typhimurium TA100, 10 indirect-acting chemicals tested with Aroclor-induced rat liver S9 standardized at 1.1 mg of protein per plate, 10 dose levels in the linear portion of the dose-response curves, and duplicate plates per dose. Four rounds were conducted in each laboratory. A different batch of S9 was utilized for every two rounds. The S9 lots were prepared at RTI and EPA. The rounds were conducted at least one week apart. The results suggested: (1) rankings of nine of the 10 chemicals were identical in both laboratories when the boundaries were set at an order of magnitude difference in slope values (revertants/ug); (2) 2AN had a mean slope greater than 1000; BAP, MC, and DBP had slope values between 1000 and 100; and ABP, FG, BAA, AAF and AO had slope values between 100 and 10; (3) DMBA had a slope value on either side of 100 and thereby was ranked differently in each laboratory. Variabilities were low (below 25% in most cases), which may be accounted for by two parameters of the study: the amount of S9 was calibrated to a set amount of protein per plate and not to a percentage of S9 in the S9 mix, and the test doses were in the initial, linear, nontoxic portion of the dose-response curves. (Supported by EPA 68-02-4186-52)

P6

PLASMA MEMBRANE CHARACTERISTICS AS INDICES OF IN VITRO TOXICITY. D. W. Bombick and D. J. Doolittle. R. J. Reynolds Tobacco Company, Winston-Salem, NC.

The development of mechanistically oriented in vitro tests to evaluate relative chemical cytotoxicity will help determine probable mechanisms of chemical cytotoxicity. The cellular plasma membrane is one potential site of action for chemically induced cytotoxicity. Adverse changes in the plasma membrane can reduce the function of this membrane as a protective barrier and a communication interface with the extracellular environment. The objective of this study was to evaluate the relative sensitivity of several indicators of plasma membrane function as indicators of in vitro cytotoxicity. Two cell types (human fibroblasts and rat liver cells) treated with several chemicals (TPA, dieldrin, MNNG, MMS, ethanol and DMSO) at short (1 hour) and long (24 hours) exposures were used. Plasma membrane parameters examined include assessments of cell shape, membrane fluidity, LDH release and exclusion of a fluorescent dye. Clonogenicity assays to determine cell killing also were conducted. The relative sensitivity of these parameters to chemically induced toxicity varied and was dependent upon both cell type and chemical used. For example, the solvents ethanol and DMSO principally increased LDH release while TPA had greater effects on fluorescent dye uptake and cellular morphology. In addition, two of the chemicals (MNNG and MMS) caused cell death but had little effect on these plasma membrane parameters, indicating another cytotoxic mechanism, not related specifically to the plasma membrane is involved. The development of these types of mechanistically oriented cytotoxicity indices should be valuable for establishing strategies for toxicological evaluation.

P7

HETEROZYGOUS EFFECTS OF X-RAY-INDUCED SPECIFIC LOCUS MUTATIONS IN THE ad-3 REGION OF Neurospora crassa.

F.J. de Serres[a], L.K. Overton[a] and B.M. Sadler[b], [a]Center for Life Sciences and Toxicology, [b]Center for Bioorganic Chemistry, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, NC 27709 (USA)

The morphological specific-locus assay in a two-component heterokaryon (H-12) of Neurospora crassa has been used to recover both gene/point mutations and multilocus deletions in the adenine-3 (ad-3) region. H-12 is heterozygous for mutants at 3 closely linked loci, ad-3A, ad-3B and nic-2 (nicotinamide-requiring); thus, X-ray-induced mutants in this region can be of 5 different genotypes: ad-3A, ad-3B, ad-3A ad-3B, ad-3A ad-3B nic-2, and ad-3B nic-2. All 5 classes can result from multilocus deletion (ad-3^{IR}); gene/point mutations (ad-3^{R}) are usually restricted to the first 2 classes. Multilocus deletions usually result from deletion of one or both of these loci as well as other loci with known or unknown requirements in the immediately adjacent regions (de Serres, Mutation Res. 211, 89, 1989). Complementation studies on a series of X-ray-induced ad-3A or ad-3B mutants (de Serres, Natl. Cancer Inst. Monogr., 18, 33, 1965) showed that they were not recessive and had heterozygous effects in terms of markedly reduced linear growth rates. Present studies were performed on a larger sample of X-ray-induced ad-3A or ad-3B mutants (de Serres, Mutation Res. 210, 281, 1989) to determine whether mutations of either type (ad-3^{R} or ad-3^{IR}) show heterozygous effects. For example, ad-3A^{IR} mutants were combined with an ad-3B^{R} mutant or an ad-3B^{IR} mutant, and the linear growth rates of the resulting forced dikaryons (ad-3A^{IR} + ad-3B^{R}, or ad-3A^{IR} + ad-3B^{IR}) were determined. ad-3A^{IR} mutants showed more pronounced heterozygous effects in combination with an ad-3B^{IR} mutant than an ad-3B^{R} mutant; similar results were found with ad-3B^{IR} mutants. Additional comparisons were made with gene/point mutations at each locus.

Such studies have shown that at loci that are expected to show recessive inheritance that dominance is allele-specific. Some X-ray-induced alleles at the ad-3A locus or at the ad-3B locus show partial dominance in terms of altered linear growth rates.

P8

CASE-SAR ANALYSIS OF PAH CARCINOGENICITY, A. Richard[a], Y. Woo[b] and G. Klopman[c]; [a]EPA, MD-68, RTP, NC 27711, [b]EPA, TS-796, Washington DC 20460, and [c]Dept. of Chemistry, Case Western Reserve University, Cleveland, OH 44106.

A CASE (Computer Automated Structure Evaluation) structure-activity analysis was performed on an extensive data base of polyaromatic hydrocarbons (PAH) for possible application to preliminary assessment of PAH's located at hazardous waste sites. A TRAINING set, consisting of 78 PAH's and their experimental carcinogenicities, was used to derive the CASE fragments; a TEST set of 108 PAH's with activity assignments based on "expert judgement" were compared to the CASE predictions; and (3) a VALIDATE set of 30 PAH's with experimental carcinogenicities was used to independently validate the CASE results. CASE determined 8 fragments to be significantly associated with activity and 4 with inactivity. Three active fragments include an unsubstituted bay region with an exposed terminal ring, and other fragments are consistent with current theories of PAH activity. Using as few as 6 of these fragments, CASE correctly predicted the activities of 96% of the TRAINING set of PAH's (excluding PAH's with marginal activity). In contrast, CASE predictions agreed with "expert judgement" for only 63% of the TEST PAH's. Upon closer examination, this was attributed to inadequate representation of PAH subclasses with fewer than 4 rings in the TRAINING set. When these subclasses were excluded from TEST, the CASE prediction agreement with "expert judgement" improved dramatically. The results highlight an important data need and indicate that an SAR analysis should be performed on the < 4 ring subclasses only when more data on these subclasses becomes available. Using the CASE fragments to predict carcinogenicities for the VALIDATE set yielded 77% agreement with experimental results which provided independent evidence of the general utility of the CASE results to > 3 ring PAH structures. The CASE fragment descriptors should prove useful for aiding in the preliminary assessment of the carcinogenicities of > 3 ring PAH's for which activity data is unavailable.

THIS ABSTRACT DOES NOT REFLECT EPA POLICY.

P9

EXPRESSION OF MUTATIONS AT THE HGPRT LOCUS IN CHINESE HAMSTER OVARY (CHO) CELLS USING SEMISOLID MEDIUM TO SEGREGATE CELLS AFTER MUTATION INDUCTION. P.S. Lee and C.J. Rudd. SRI International, Menlo Park, CA. 94025

We are developing a modified procedure for quantitation of mutations at the HGPRT locus to reduce the cost and potential errors associated with the subculturing of cells during the expression period. This procedure eliminates the need for subculturing during the expression period by maintaining cells in semisolid medium after the induction of mutations. Thus, clonal populations are maintained for the duration of the experiment. Using this method, the number of TG-resistant colonies identified should more closely represent the number of cells with a mutation at the HGPRT locus at the beginning of the expression period. Standard techniques with attached cells were used for induction of mutations in CHO cells. Cells were treated with 500 µg/ml ethylmethanesulfonate (EMS) for 4 hr. The cells were incubated overnight in fresh medium, then trypsinized and suspended in semisolid medium containing 0.22% BBL agar and 2% fetal bovine serum. The initial cell density was 2 x 10^4 cells/25 ml medium/dish. Mutant colonies were selected after 1 to 8 days by adding TG (final concentration 30 µM) as an overlay with additional medium. Serum concentration was increased to 5% during the selection of TG^r colonies to increase their growth rate. MTT-stained colonies were counted after a total of 25 days incubation. The recovery of HGPRT- cells in reconstruction experiments was equivalent in the presence or absence of wild-type (HGPRT+) cells at all expression days under these conditions. The results indicated that an expression period of 7 days was optimal for this assay. The mutant frequency of cultures treated with 500 µg/ml EMS and selected at expression Day 7 was 236 TG^r colonies per 10^5 colony-forming cells (CFC), compared to 8 TG^r colonies per 10^5 CFC for the solvent control cultures.

P10

AN EVALUATION OF THE METABOLISM OF 1-NITRO[^{14}C]PYRENE BY RABBIT TRACHEAL EPITHELIAL CELLS: KINETIC ANALYSIS. Leon C. King[1,2], Ernest Hodgson[2] and Joellen Lewtas[1] [1]U. S. Environmental Protection Agency, Research Triangle Park, NC; [2]Department of Toxicology, North Carolina State University, Raleigh, NC

The metabolism of 1-nitro[^{14}C]pyrene (^{14}C-1-NP) by freshly isolated rabbit tracheal cells has been investigated in order to determine the kinetic parameters K_M amd V_{Max}. Experiments to optimize the cell number and the incubation time yielding maximum metabolite formation following incubations with ^{14}C-1-NP were also performed. Metabolites from the incubation medium and cell lysates at each stage of this investigation were extracted, analyzed and quantitated by HPLC and liquid scintillation spectrophotometry. Maximum rate of metabolite production was attained at 2 x 10^6 cells and 4 hours incubation, with no significant increase at 20 hours. Using these optimized conditions, experiments were performed to assess the kinetics of the metabolism of ^{14}C-1-NP over a concentration range of (0.1 to 50µM). The apparent K_M ranged from 2.43 ± 1.81 x 10^{-1}µM (S.E.) to 3.89 ± 7.31 x 10^{-1}µM (S.E.). V_{Max} was determined to range from 5.62 x 10^{-3} ± 3.9 x 10^{-4}µmoles/mg/hr (S.E.) to 7.65 x 10^{-3} ± 8.7 x 10^{-4} µmoles/mg/hr (S.E.). The apparent K_M and V_{Max} were determined using three linear transformation models (Lineweaver-Burk, Hofstee, Woolf and a nonlinear model). Scatchard analysis of the kinetic data resulted in a curvilinear plot indicating the presence of two enzymes, one with a low affinity (K_M = 0.584µM) and high capacity (Vmax = 2.38 x 10^{-3}µmoles/mg/hr) and another with a high affinity (K_M = 0.062µM) and low capacity (V_{Max}= 8.1 x 10^{-4}µmoles/mg/hr). The results of this study provide information needed to optimize experimental conditions in evaluating the comparative metabolism and genotoxicity of ^{14}C-1-NP by respiratory tract cells from various species.

P11

APPLICATION OF BIOASSAY-DIRECTED CHEMICAL ANALYSIS TO INCINERATOR EFFLUENTS AND OTHER COMBUSTION EMISSIONS. D.M. DeMarini[1], P.M. Lemieux[1], J.A. McSorley[1], W.P. Linak[1], E. Perry[2], and R.W. Williams[2]. [1]U.S. EPA, RTP, NC 27711, [2]Environmental Health Research and Testing, Durham, NC 27713 (U.S.A.).

We have used high-pressure liquid chromatography (HPLC) coupled to a microsuspension mutagenicity assay in order to fractionate and determine the mutagenicity of the fractions from various combustion emissions. Some of the incinerator effluents were from full-scale municipal waste incinerators, and others were from simulated hazardous waste incinerators operating at sub-optimum conditions. For these later experiments, pure compounds such as polyethylene, polyvinylchloride, toluene, and carbon tetrachloride were combusted in a pilot-scale rotary-kiln incinerator. Particles collected on filters and semi-volatiles collected on XAD-2 resin were extracted with dichloromethane, and these extracts were fractionated by HPLC using a cyanopropyl column with a dichloromethane/pentane/methanol solvent gradient. The fractions were solvent exchanged into dimethyl sulfoxide prior to bioassay in strain TA98 of Salmonella in a total reaction volume of 100 µl using a 90-min incubation. Reproducible bioassay chromatograms (mutagrams) were generated for the combustion effluents, each of which produced a unique mutagram. Combustion of pure compounds under sub-optimal conditions produced complex gas chromatograms (GC) and HPLC chromatograms, indicating that many products of incomplete combustion were produced. The mutagrams identified fractions that accounted for much of the mutagenic activity of the sample but that represented a small amount of mass based on the GC and HPLC chromatograms.

[This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.]

P12

COMPARISON OF DNA ADDUCTS IN MOUSE TISSUES FOLLOWING IN VIVO EXPOSURE TO COMPLEX AIR POLLUTION SOURCES M. George[1], M. Jackson[1], J. Gallagher[2], J. Inmon[2], J. Scott,[1] T. Shank,[1] and J. Lewtas[2] [1]Environmental Health Research and Testing, Research Triangle Park, NC; [2]U.S. EPA, Research Triangle Park, NC.

Dose-related differences in the binding of DNA reactive intermediates for five environmentally important complex particulate extracts and a well studied carcinogen Benzo(a)Pyrene (B(a)P) were examined in female C-57 or Sencar mice following the topical application ranging from 1-120 mg per mouse. Particulate extracts from coke oven, coal soot diesel, urban and incinerator exhaust were selected as model complex mixtures based on their environmental importance. Positive and negative control animals were treated with either 1.2 mg B(a)P or acetone alone. DNA was isolated from skin, lung and liver DNA 24 hours following the last application and analyzed for DNA adducts using the ^{32}P-postlabeling assay. Each of the particle extracts produced distinct patterns of DNA adducts. A diagonal zone of radioactivity, presumably representing multiple DNA adducts, was observed for all complex mixture extracts. One adduct common to all five complex mixture modified DNA samples, comigrated with the major B(a)P adduct observed following treatment with B(a)P alone. Based on the B(a)P concentration of some of these extracts it seems unlikely that this adduct is derived from B(a)P alone. DNA adduct levels were generally higher in skin DNA as opposed to lung and liver DNA. At the 20 mg dose the highest total number of skin DNA adducts resulted from the metabolism of incinerator extract followed by coke oven, smoky coal, urban air and diesel treatments. Important dose-related differences in DNA adduct formation are presented.

P13

AMBIENT AIR SAMPLING AND SAMPLE PREPARATION FOR MUTAGENICITY BIOASSAY.
Randall R. Watts[1] and Ron W. Williams[2]. [1]US Environmental Protection Agency, MD-68, Research Triangle Park, NC 27711, [2]Environmental Health Research and Testing, P.O. Box 12199, Research Triangle Park, NC 27709

The regulation of air pollutants is a vital part of the mission of the US Environmental Protection Agency, and Agency research programs are directed toward improving sampling and assay procedures. This presentation describes portions of that research dealing with the planning of biomonitoring studies designed to collect particle-bound organics and semi-volatile organics for subsequent determination of air mutagenicity using short-term bioassay techniques. Practical aspects of sampling, sample handling, and sample preparation for mutagenicity bioassay are discussed. Examples are given from recent field studies.

This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.

P14

A SOLID-PHASE EXTRACTION (SPE) APPROACH FOR CHEMICAL SEPARATION OF ACIDIC COMPONENTS OF COMPLEX MIXTURES FOR BIOASSAY-DIRECTED CHEMICAL ANALYSIS. D. Bell[1], R. Williams[2], D. Thompson[3], L. Brooks[2], R. Zwiedinger[4], and J. Lewtas[4]. [1]National Research Council, U.S. EPA, RTP, NC 27711, [2]EHRT, 631 United Dr., Durham, NC 27713, [3]NSI, RTP, NC 27709, [4]U.S. EPA, RTP, NC 27711 (U.S.A.).

Chemical class separation of urban air particle extracts impacted by residential wood combustion is technically difficult due to the large quantity of polar and acidic components in these mixtures. In this work, solid-phase extraction with anion exchange resin (AG-MP1) was combined with organic elution solvents allowing separation of samples into neutral/base, polar neutral, weak acid, and strong acid fractions. The respective solvents used were dichloromethane (DCM), methanol (MEOH), methanol modified with CO_2 (CO_2), and 2% trifluoroacetic acid (TFA) in methanol. Good qualitative separation of a mixture of authentic standards was achieved. Neutral and basic components appeared only in the DCM fraction. Strong acids, benzoic acid, and 2-nitro-benzoic acid were eluted in the TFA fraction. Weaker acids were split among the MeOH, CO_2, and TFA fractions. Total mass recovery through the composite (MSC) and wood smoke-impacted ambient composite (WSC) samples was 89-103%. Mutagenicity was measured in the standard plate-incorporation assay with Salmonella TA98 (+/- S9). The DCM fraction was generally the most potent fraction with or without S9. It contained 42-50% of the direct-acting mutagenicity and 57-71% of the S9-mediated mutagenicity of the WSC and MSC samples. The TFA fraction also contributed significant mutagenicity. Recovery of mutagenicity from reconstituted samples varied from 82-137%.
[This is an abstract of a proposed presentation and does not represent EPA policy.]

P15

RELATIONSHIP BETWEEN INDOOR AND OUTDOOR MUTAGENICITY IN A WOODSMOKE IMPACTED AIRSHED. S. Warren, R. Zweidinger, L. Claxton, J. Dorsey, R. Highsmith, L. Cupitt and J. Lewtas. USEPA, RTP, NC and EHRT, RTP, NC.

The Integrated Air Cancer Project conducted a major field study in Boise, ID examining the effects of outdoor air and woodstoves on the mutagenicity of indoor air. Simultaneous air samples were taken inside and outside 10 paired homes with and without woodstoves and at other locations across the city. Particulate matter was collected on teflon coated glass fiber filters and semi-volatile organic matter using XAD-2. Extracted samples were bioassayed in the *Salmonella typhimurium* microsuspension histidine reversion assay using strain TA98 with metabolic activation. The total (particles and XAD-2) average concentration of mutagenicity outdoors was 2-fold higher than indoors. Semi-volatile organics consistently made a higher contribution to the total mutagenicity measured indoors, ranging from 26 to 56%. Indoor particulate mutagenicity increased in proportion to outdoor particulate mutagenicity. The presence or absence of woodstoves in the homes did not appear to have any consistent influence on the concentration of indoor mutagens.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

P16

THE COMPARISON OF MUTAGEN-INDUCED THYMIDINE KINASE (TK) MUTANT FREQUENCIES IN HUMAN AND MOUSE LYMPHOMA TESTING CELLS. M.M. Moore[1] K. Harrington-Brock[2], L. Parker[2]. [1]U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 USA; [2]Environmental Health Research and Testing, Inc., Research Triangle Park, NC 27709 USA.

The TK6 line of human lymphoblastoid cells can be used to detect mutants at the heterozygous thymidine kinase (tk) locus. Little et al. (1987, Banbury Report 28: Mammalian Cell Mutagenesis, p. 225) have reported that a class of slow-growing TK mutants can be recovered and that at least some of these mutants may result from mitotic recombination. These results are similar to our findings for small-colony TK-deficient mutants of mouse lymphoma cells. We wished to make quantitative comparisons between the induced mutant frequency in the human and mouse lymphoma cells. Slow-growing mutants are difficult to recover and count using the procedures standardly used with the TK6 cell line. We are investigating modifications which might optimize growth and quantitation of slow-growing mutants. Modifications include using 24 well plates rather than 96 well plates and plating cells at $1x10^3$ to $5x10^3$ rather than $4x10^4$ cells per well. Using these procedures, we are comparing the ICR-170-, EMS-, and MMS-induced TK mutant frequencies in human and mouse lymphoma cells. (This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.)

P17

CORRELATION BETWEEN CHROMOSOME ABERRATION FREQUENCY AND SMALL-COLONY TK-DEFICIENT MUTANT FREQUENCY IN L5178Y/TK$^{+/-}$ -3.7.2C CELLS. C.L. Doerr[1] and M.M. Moore[2]. [1]Environmental Health Research and Testing, Inc., Research Triangle Park, NC 27709 USA; [2]U.S. Environmental Potection Agency, Research Triangle Park, NC 27711 USA.

The L5178Y mouse lymphoma assay quantitates gene mutation at the thymidine kinase locus. Karyotypic and molecular analyses of mutants have shown an association between small-colony phenotype and chromosomal events. We have been evaluating the association of small-colony induction with gross aberration analysis to determine if the mouse lymphoma assay can be used directly to determine potential clastogenicity of test compounds. Based on data from 36 compounds, we find a clear association between clastogenicity and small-colony induction. There is, as expected, no simple mathematical correlation between the two endpoints. Many gross aberrations are incompatible with cell survival and colony formation, and some small-colony TK mutants are products of events which cannot be scored visually as chromosome aberrations when small changes in some essential DNA sequence(s) results in slow growth. Based on these analyses and previous studies, we feel that the small-colony TK mutant frequency is the most useful measure of the clastogenicity of test compounds. It reflects cytogenetic events compatible with long-term cell viability, and these are the types of events important in human disease. (This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.)

P18

Molecular Analysis of hprt Mutations Induced by 2-Cyanoethylene Oxide in Human Cells: Mutations Resulting in Aberrant Splicing of mRNA Occur Frequently in Human Cells. Leslie Recio[1], Deborah Simpson, Judi Cochrane, Howard Liber[2], and Thomas R. Skopek. [1] CIIT, RTP, NC and [2]Harvard School of Public Health, Boston, MA.

The proposed mutagenic metabolite of the rat carcinogen acrylonitrile, 2-cyanoethylene oxide (ANO), is mutagenic at both the human tk and hprt loci. To develop a better understanding of its mechanism of action in human cells, our group has determined the specific DNA sequence alterations induced by ANO treatment. A collection of hprt-human TK6 lymphoblasts was isolated following treatment with 150μM x 2h ANO and characterized by dideoxy sequencing of cloned hprt cDNA. Base pair substitution mutations in the hprt coding region were observed in 19/39 of hprt mutants; 11 occurred at AT base pairs and 8 at GC base pairs. Two -1 frameshift mutations involving GC bases were also observed. Approximately half (17/39) of the hprt mutants displayed the complete loss of single and multiple exons from hprt cDNA, as well as small deletions, some extending from exon/exon junctions. Southern blot analysis of five mutants with single exon losses revealed no visible alterations. Analysis of one mutant missing exons 3-6 in its hprt mRNA revealed a visible deletion in the corresponding region in its genomic DNA. The missing exon regions of four mutants (one each with exon 6,7 and 8 loss and one mutant with a 17 base deletion of the 5' region of exon 9) were PCR amplified from genomic DNA and analyzed by Southern blot using exon-specific probes. The exons missing from the hprt mRNA were present in the genomic hprt sequence. The appropriate intron/exon regions of hprt genomic DNA from a mutant with exon 8 loss and a mutant exhibiting cryptic splicing in exon 9 were cloned into M13mp19 and sequenced. Point mutations in the splice acceptor site of exon 8 (AT → TA) and exon 9 (AT → GC) were observed. These observations indicate that ANO induces primarily point mutations in human cells at both AT and GC base pairs, and that splice acceptor sites are prone to ANO mutagenesis. This work also suggests that there are at least two promutagenic lesions induced by ANO.

P19

RAT PLEURAL MESOTHELIAL CELLS: CHARACTERIZATION OF A MODEL FOR FIBER- INDUCED EFFECTS.

E Bermudez, J Everitt, and C Walker. CIIT, RTP, NC.

Human exposure to a variety of natural mineral fibers has been linked to neoplastic transformation of mesothelial tissue. Tumors histologically similar to those observed in humans are induced in the pleural and peritoneal cavities of rats exposed to a variety of natural and man-made mineral fibers. Rat mesothelial cell strains were established and surveyed for gene expression of relevant growth factors and receptors. Rat parietal pleural mesothelial cells were isolated by digestion with collagenase and grown in medium consisting of Ham's F-12 supplemented with 10% heat inactivated fetal bovine serum. The cell strains produced by this procedure were judged to be of mesothelial origin by virtue of their epithelial morphology, the presence of gap junctions and microvilli, and the coexpression of keratins and vimentin. In addition, the cells had a normal diploid chromosomal complement of 42 and exhibited contact inhibition of growth. The expression of transforming growth factor β, fibroblast growth factor, and receptors for epidermal growth factor, insulin and platelet derived growth factor were detected by Northern blot analysis. No expression of transforming growth factor α and platelet derived growth factor-A chain transcripts could be detected. This information is important for understanding mesothelial cell biology and will serve as the baseline for detecting alterations in these parameters in studies designed to examine fiber toxicity in mesothelial cells.

P20

EVALUATION OF GENOTOXICITY AND INDUCED CELL PROLIFERATION IN HEPATOCYTES FROM RATS AND MICE TREATED WITH FURAN. D. M. Wilson, and B. E. Butterworth, CIIT, Research Triangle Park, NC (USA).

Initial observations from bioassays by the National Toxicology Program indicate that furan produces hepatocellular carcinomas in male F344 rats and in male and female B6C3F1 mice. Although furan is negative in the Ames test, the profile of mutations produced in oncogenes from furan-induced tumors are different from those of spontaneous tumors (Reynolds et al, Science 237, 1309 (1987)). To investigate the possible mechanisms by which furan causes tumors, genotoxicity, as indicated by DNA repair, and chemically-induced cell proliferation were examined. Primary hepatocyte cultures from male F344 rats were incubated with furan and 10 μCi/ml ^{3}H-thymidine. DNA repair as unscheduled DNA synthesis (UDS) was quantitated autoradiographically as net grains per nucleus (NG). Concentrations from 1 μM to 10 mM furan yielded no UDS in vitro. To examine UDS in vivo, furan was administered by gavage to male rats (5, 30 and 100 mg/kg) and to male B6C3F1 mice (10, 50, 100 and 200 mg/kg) twelve hours prior to isolation of hepatocytes. No increase in NG was observed in hepatocytes from furan-treated animals compared to controls. Cell proliferation in vivo was evaluated by autoradiographic analysis of ^{3}H-thymidine incorporation in cultures of primary hepatocytes isolated 36 hours after a single gavage administration of furan (30 mg/kg) to male rats. Hepatocytes from vehicle treated rats had a labeling index (LI) of 0.2±0.1% while that of furan-treated rats was 17±5%. Taken together, available data suggest that the distinct profile of oncogenes from furan-induced tumors may have resulted from enhanced susceptibility of those genes to mutations related to forced cell proliferation rather than from direct mutagenesis by furan. Furan-induced cell proliferation may also have been a driving force in tumor promotion and progression.

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CYTOTOXICITY OF HAZARDOUS INDUSTRIAL WASTES IN A RAT TRACHEAL EPITHELIAL CELL LINE. D.K. Beeman, D.M. DeMarini, and M.J. Mass. U.S. EPA, RTP, NC 27711.

The cytotoxicities of ten hazardous industrial waste samples were evaluated using an established rat tracheal epithelial cell line. One waste sample was from a petrochemical plant, a second was from a pharmaceutical plant, and the remaining eight were mixtures of wastes from a variety of industrial sources that had been collected by four commercial hazardous waste incineration facilities. All 10 waste samples were chemically characterized for the presence of organic chemicals and various metals found on the EPA Appendix XIII list. For each waste sample, 5, 35-mm dishes were seeded with 200 cells each, and the cells were allowed to attach overnight. The next day, medium was removed, and 2 ml of medium containing various concentrations of the wastes were added. The cells were incubated for 5-7 days, after which colonies were fixed, stained, and counted. Preliminary data indicate that the wastes display a range of cytotoxic potencies covering four orders of magnitude. Previous work has determined the cytotoxicities of these waste samples to Escherichia coli and F-344 rats. The cytotoxic potencies of these wastes in the rat tracheal epithelial cell line will be compared to results in the other biological systems.

P22

EFFECT OF THE ANTI-CARCINOGENIC BOWMAN-BIRK PROTEASE INHIBITOR ON ONCOGENE EXPRESSION AND EPITHELIAL CELL PROLIFERATION IN IRRADIATED RAT COLON, WILLIAM H. ST. CLAIR, DEPARTMENT OF RADIOLOGY, BOWMAN GRAY SCHOOL OF MEDICINE, WINSTON-SALEM, NC 27103.

Administration of 11 Gy of abdominal irradiation to rats causes a depletion of the cells in the proliferative compartment of the crypt after 3 days. The cellular depletion is followed by an increase in cell proliferation and regeneration of the epithelium. It has been shown that protease inhibitors can prevent the overexpression of the c-myc in irradiated or serum stimulated fibroblasts. Protease inhibitors have also been shown to suppress chemically induced carcinogenesis in rodents. In the present study, Fisher 344 rats received either 0 or 11 Gy of γ-irradiation to their entire abdomens with or without intraperitoneal administration of the Bowman-Birk protease inhibitor (BBI). Rat colons were collected 3,7,10,14,21 and 35 days after irradiation. Uptake of tritiated thymidine was monitored in crypts of the transverse colon to determine their proliferative status. Total cellular RNA was collected from the remainder of the colonic mucosa. The levels of c-fos, c-myc, c-Ha-ras, c-erbB and c-actin mRNA were examined by a standard dot and Northern blot analysis. The data demonstrates that BBI is capable of inhibiting the overexpression of two nuclear proto-oncogenes without interfering with the compensatory increase in crypt cell proliferation after a high dose of abdominal irradiation. (Supported by NIH grant RR-05404).

P23

MAMMALIAN CELL MUTAGENESIS: A MAJOR ROLE FOR NON-DNA TARGETS. D. Clive, Wellcome Research Laboratories, Research Triangle Park, NC 27709 USA

Evidence is accumulating for major non-DNA targets for specific locus mutagenicity in mammalian cells involving heritable DNA alterations. Such a possibility (a) is consistent with radial-loop models of chromosome structure in mammalian cells (Marsden and Laemmli, *Cell* **17**: 849, 1979); (b) is based upon non-DNA structural components of the eukaryotic chromosome (*e.g.*, topoisomerase II; TP) (Gaulden, Mutagenesis **2** (1987) 357; Evans *et al.*, Mutation Res. **217**: 53, 1989); (c) invokes genetic alterations quantitatively larger than, and mechanistically different from, classical point mutations as a major class of gene mutations (Yandell *et al.*, *Som. Cell Mol. Gen.* **12**: 255, 1987; Applegate and Hozier, *Banbury Report* **#28**: 213, 1987) which (d) are of the types seen in rodent and human tumors (Ali *et al.*, *Science* **236**: 933, 1987); (e) attributes the relative insensitivity of some genotoxicity tests to the wrong chromosomal architecture (prokaryotes, including the Ames assay), the wrong chemistry (DNA repair synthesis incapable of detecting TP redimerization) or the wrong endpoint (dominant or X-linked loci fail to detect large scale recombination events); (f) explains the higher sensitivities of other assays (SCE assays, chromosome aberration tests, L5178Y/*tk*$^{+/-}$ mouse lymphoma assay [MLA]); and (g) implies the existence of genotoxic/carcinogenic chemical structures different from those implicated in DNA reactivity. The cytogenetic and molecular mutational spectra of a number of chemically diverse mutagens in the MLA indicates that only a few mutagens (*e.g.*, EMS, 2-amino-6N-hydroxyadenine) induce a significant proportion of subgenic DNA alterations at the heterozygous *tk* locus, whereas most induce predominantly DNA alterations which are at least many kilobases (and probably multigenic) in extent (Glover *et al.*, these abstracts). These results will be discussed in terms of genotoxicity models and mechanisms which include non-DNA targets.

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MOLECULAR SPECTRA OF L5178Y/*tk*$^{-/-}$ MUTANTS INDUCED BY DIVERSE MUTAGENS. P. Glover, R. Krehl and D. Clive, Wellcome Research Laboratories, Research Triangle Park, NC 27709 USA

Southern blot analyses were performed on DNA from at least 10 large and 10 small colony *tk*$^{-/-}$ mutants induced by each of 10 mutagens [2-amino-N6-hydroxyadenine (AHA), EMS, MMS, 2-AAF, methotrexate (Mtx), caffeine, methapyrilene (MP), *m*-AMSA, hycanthone methanesulfonate, procarbazine (Proc)]. Two molecular mutant genotypes were recognized upon digestion with *Nco-1* and subsequent probing with 1.1 kb cDNA insert from plasmid pMtk 4 (ATCC #37556): (1) no detectable alteration and (2) absence of the newly mutated *tk* allele as indicated by the absence of the 6.2 kb fragment (Applegate and Hozier, Banbury **#28**: page 213, 1987). In combination with the previously established chromosomal nature of most small colony *tk*$^{-/-}$ mutants (Moore *et al.*, 1985), this permitted the classification of these 10 mutagens according to the relative proportions of each of 4 classes of genetic damage they induced. AHA and EMS gave mutational spectra consistent with their point mutational effects in other systems. The other 8 mutagens induced mostly small colony mutants, most of which had lost the entire *tk* allele. Mtx induced high frequencies of large colony mutants at the *tk* locus, mostly lacking the *tk* allele, and was weakly or nonmutagenic at the hemizygous *hprt* locus. Four mutagens--Mtx, caffeine, MP and Proc--lack structural alerts for DNA reactivity (Ashby and Tennant, Mutation Res., **204**: 17-115, 1988) implying a major class of non-DNA targets for mutagenicity in mammalian cells (Clive, these abstracts). The mutagenicity spectra for 2-AAF, Hyc and caffeine are quite similar, raising the possibility that the DNA adducts of 2-AAF and the intercalating activity of Hyc may not be their principle mechanisms of mutagenicity in mammalian cells.

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SMOKE EXPOSURE, AGE, SEX, RACE, AND POTENTIATION AS VARIABLES AFFECTING SISTER CHROMATID EXCHANGE INDUCTION IN HUMANS, D. A. Tulis, J. K. Smollinger, and W. H. McKenzie, North Carolina State University, Genetics Dept., Raleigh, NC 27695 (USA).

In vitro cytogenetic analysis was performed on peripheral lymphocytes of 49 passively smoking children, ages 6 mo to 5 yrs. Mean SCE for non-smokers (7.60 ± 0.46) was not significantly different ($p > 0.746$) from mean SCE for passive smokers (7.85 ± 0.39). However, passively smoking children demonstrated a highly significant ($p < 0.001$) SCE increase to in vitro α-naphthoflavone (ANF) exposure, while the non-smoking children showed a much lower but still significant SCE increase to in vitro ANF exposure ($p < 0.03$). These results suggest that ANF has the potential to magnify and therefore detect an SCE insult experienced by passively smoking children. Age, sex, and race influences were also investigated for their effects on SCE. A significant ($p < 0.001$) SCE age effect was observed, with SCE frequency increasing with age. A significant ($p < 0.008$) SCE sex effect was also observed, with average female SCE (8.76 ± 0.26) higher than average male SCE (7.85 ± 0.23). However, no significant ($p > 0.94$) SCE race effect was detected, although the SCE race*smoking interaction was significant ($p < 0.01$). Urinary cotinine was determined to be highly correlated ($r = 0.70$) with the number of cigarettes smoked by the children's parents, and was used as an estimator of the actual amount of smoke inhaled by the children. This continuing study is presently investigating the role of ANF as a potentiator of SCE induction in non-smoking, passively smoking, and actively smoking young adults, and is also using cotinine as an indicator of cumulative smoke exposure for each subject.

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(P = Poster; T = Talk)

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