GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

SIXTH ANNUAL MEETING

PROGRAM AND ABSTRACTS

OCTOBER 6, 1988

SHERATON UNIVERSITY CENTER

DURHAM, NORTH CAROLINA

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

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GEMS

Genotoxicity and Environmental Mutagen Society

Dear GEMS Member:

Thank you for participating in the Sixth 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. Don Coffey** from Johns Hopkins Hospital as the Keytnote Speaker. He will talk on "The Nuclear Matrix." We also have four other Invited Talks. **Dr. Tom Kunkel** (NIEHS) will discuss "Analysis of Fidelity Mechanisms with Eukaryotic DNA Polymerases." **Dr. David DeMarini** (EPA) will discuss "Mediation of Mutagenesis by DNA Topoisomerases." **Dr. Heinrick Malling** (NIEHS) will talk about "Use of ϕX174 as a Shuttle Vector for the Study of in vivo Mammalian Mutagenesis." **Dr. Carl Barrett** (NIEHS) will talk on the "Role of Tumor Suppressor Genes in a Multistep Model of Carcinogenesis." In addition, scientists will be competing for the GEMS Travel Award to attend the International Environmental Mutagen Society Meeting in Cleveland, OH in July 1989.

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 **Dr. David DeMarini** for organizing this Annual Meeting; I also thank **Virginia Houk, Dr. Peter Working,** and **Dr. Robert Smart** for the time and effort they put into the Society during the last year. I congratulate the new Members of the Board, **Edilberto Bermudez, Dr. David Doolittle,** and **Dr. Wendell McKenzie.** I look forward to the continued growth and vitality of the Society. Welcome to the Sixth Annual Meeting of GEMS.

Sincerely,

Martha M. Moore

Martha M. Moore
President, GEMS

ACKNOWLEDGMENTS

GEMS gratefully acknowledges the following companies for providing additional financial support for the Sixth Annual Meeting.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC. (Bruce Casto) is cosponsoring, with **GEMS**, the coffee and rolls at 9:30 am.

INNOVATIVE PROGRAMMING ASSOCIATES, INC. (Morton S. Cohen) is cosponsoring, with **GEMS**, the coffee at 3:00 pm.

INTEGRATED LABORATORY SYSTEMS (T.K. Rao) is cosponsoring, with **GEMS**, the social hour at 5:00 pm.

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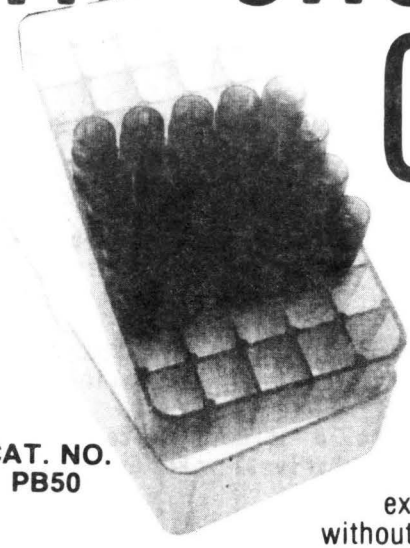

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PROGRAM
GEMS SIXTH ANNUAL MEETING
OCTOBER 6, 1988

Sheraton University Center
Durham, North Carolina

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

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

8:30 - 8:45 **T1** DRUG-RESISTANT T CELLS MAY CONTRIBUTE IN HUMAN GRAFT REJECTION. W.L. Stanford[a], G.H.S. Strauss[b], and O.J. Finn[c]. [a]EHRT, RTP, NC 27709; [b]U.S. EPA, RTP, NC 27711; [c]Duke University Medical Center, Durham, NC 27710.

8:45 - 9:00 **T2** ANTIMUTAGENICITY STUDIES IN SALMONELLA. J.R. Warner, T.J. Hughes, and M.E. Wall. RTI, RTP, NC 27709.

9:00 - 9:15 **T3** THE INDUCTION OF CHROMOSOME ABERRATIONS IN MOUSE BONE MARROW CELLS BY THE DNA TOPOISOMERASE INHIBITORS CAMPTOTHECIN AND AMSACRINE. D.R. Howard[a], L.C. Backer[a], D.M. DeMarini[b], and J.W. Allen[b]. [a]EHRT, RTP, NC 27709; [b]U.S. EPA, RTP, NC 27711.

9:15 - 9:30 **T4** MUTATION VS. MUTANT FREQUENCY IN THE MOUSE LYMPHOMA ASSAY. D. Daston[a], W. Caspary[a], C. Rudd[b], K. Pardo[b]. [a]NIEHS, RTP, NC 27709; [b]SRI, Menlo Park, CA 94025.

9:30 - 11:00 **Coffee, Posters, Exhibits**

11:00 - 11:15 **T5** ONCOGENE AMPLIFICATION, METHYLATION, EXPRESSION, AND MUTATION IN CHEMICALLY TRANSFORMED RESPIRATORY EPITHELIAL CELLS. J.A. Lasley[a], N.S. Schorschinsky[a], D.K. Beeman[b], and M.J. Mass[b]. [a]EHRT, RTP, NC 27709; [b]U.S. EPA, RTP, NC 27711.

11:15 - 11:30 **T6** CHEMICAL SYNTHESIS AND CHARACTERIZATION OF A MODEL CYCLOPENTA-FUSED PAH PRODUCT OF ADENOSINE. A.W. Bartczak[a], R. Sangaiah[a], S.D. Wyrick[a], G. Toney[b], and A. Gold[a]. [a]UNC, Chapel Hill, NC 27599; [b]NIEHS, RTP, NC 27709.

11:30 - 11:45 **T7** DETECTION AND COMPARISON OF DNA ADDUCTS IN ANIMAL TISSUES/CELLS FOLLOWING IN VIVO EXPOSURE TO THREE ENVIRONMENTALLY IMPORTANT COMPLEX MIXTURES. M. George[a], P. Rouse[a], J. Scott[a], J. Inmon[b], J. Gallagher[b], and J. Lewtas[b]. [a]EHRT, RTP, NC 27709; [b]U.S. EPA, RTP, NC 27711.

11:45 - Noon **T8** MOLECULAR AND CYTOGENETIC ANALYSIS OF SPONTANEOUS AND CYANOETHYLENE OXIDE (CNETO)-INDUCED TK$^{-/-}$ MUTANTS IN HUMAN CELLS. L. Recio, Y. Kodama, C.J. Boreiko, and T.R. Skopek. CIIT, RTP, NC 27709.

Noon - 1:30 **Lunch, Business Meeting, Recognition of Exhibitors and Sponsors, and Installation of New Members of the Board of Directors**

1:30 - 1:45 **T9** A METHOD FOR DETERMINING MUTATION SPECIFICITY IN HUMAN CELLS. D. Simpson[a], R. Crosby[a], L. Recio[a], R. Albertini[b], and T. Skopek[a]. [a]CIIT, RTP, NC 27709; [b]U. of Vermont, Burlington, VT 05405.

1:45 - 2:15 **Invited Talk:** ANALYSIS OF FIDELITY MECHANISMS WITH EUKARYOTIC DNA POLYMERASES. T.A. Kunkel. NIEHS, RTP, NC 27709.

2:15 - 2:30 **Invited Talk:** MEDIATION OF MUTAGENESIS BY DNA TOPOISOMERASES. D.M. DeMarini. U.S. EPA, RTP, NC 27711.

2:30 - 3:00 **Invited Talk:** USE OF ΦX174 AS A SHUTTLE VECTOR FOR THE STUDY OF IN VIVO MAMMALIAN MUTAGENESIS. H.V. Malling and J.G. Burkhart. NIEHS, RTP, NC 27709.

3:00 - 3:30 **Coffee and Exhibits**

3:30 - 4:00	**Invited Talk:** ROLE OF TUMOR SUPPRESSOR GENES IN A MULTISTEP MODEL OF CARCINOGENESIS. J.C. Barrett. NIEHS, RTP, NC 27709.
4:00 - 5:00	**Keynote Address:** THE NUCLEAR MATRIX. D. Coffey. The Johns Hopkins Hospital, Baltimore, MD 21205.
5:00 - 5:05	**Awards**
5:05 - 6:00	**Social: Cheese, fruits, and beverages**
6:00	**Adjourn**

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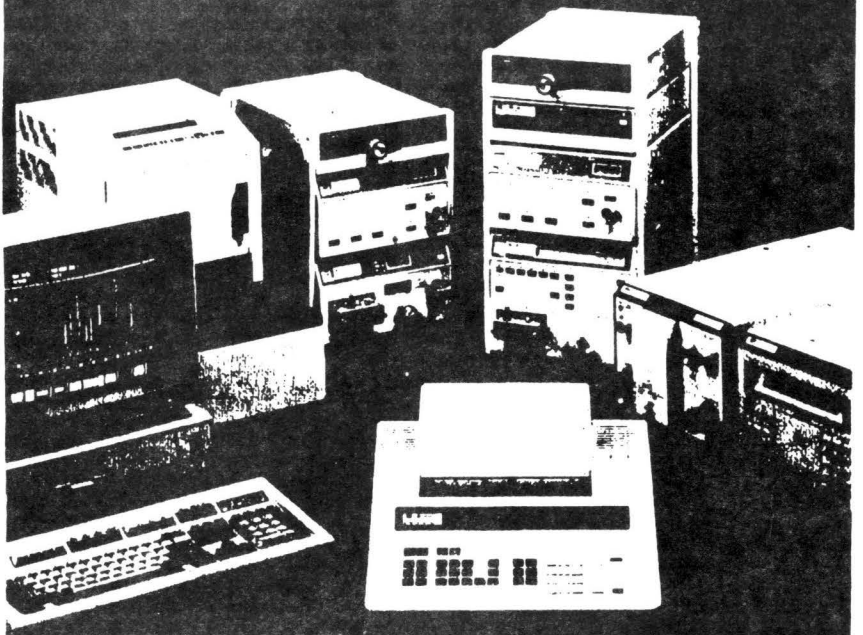

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POSTERS

P1 DEVELOPMENT AND VALIDATION OF THE SPIRAL SALMONELLA ASSAY: AN AUTOMATED APPROACH TO BACTERIAL MUTAGENICITY TESTING. V.S. Houk[a], S. Schalkowsky[b], and L.D. Claxton[a]. [a]U.S. EPA, RTP, NC 27711; [b]Spiral System Instruments, Inc., 4853 Cordell Ave., Suite A-10, Bethesda, MD 20814.

P2 THE USE OF THE AUTOMATED SPIRAL SALMONELLA ASSAY FOR THE DETECTION OF MUTAGENIC COMPLEX ENVIRONMENTAL SAMPLES. G. Early[a], V.S. Houk[b], and L.D. Claxton[b]. [a]St. Augustine's College, Raleigh, NC 27611; [b]U.S. EPA, RTP, NC 27711.

P3 THE MUTAGENIC IMPACT OF X-RAY TREATMENT ON ELECTROPHORETICALLY EXPRESSED LOCI IN THE MOUSE. S.E. Lewis[a], L.B. Barnett[a], R.A. Popp[b], and M.D. Shelby[c]. [a]RTI, RTP, NC 27709; [b]Biology Division, ORNL, Oak Ridge, TN 37831; [c]NIEHS, RTP, NC 27709.

P4 DEVELOPMENT OF AN ULTRAMICROSUSPENSION MUTAGENICITY ASSAY AND APPLICATION TO AIRBORNE PARTICULATES COLLECTED BY PERSONAL SAMPLERS. S. Goto[a], H. Matsushita[a], Y. Takagi[b], M. Murata[b], K. Williams[c], D.M. DeMarini [c], and J. Lewtas[c]. [a]Institute of Public Health, Tokyo, Japan; [b]Azabu University, Kanagawa, Japan; [c]U.S. EPA, RTP, NC 27711.

P5 THE CONSEQUENCES OF UNREPAIRED DOUBLE-STRAND BREAKS IN YEAST. C.B. Bennett[a], T. Nilsson-Tillgren[b], and M.A. Resnick[a]. [a]NIEHS, RTP, NC 27709; [b]U. of Copenhagen, Denmark.

P6 PATTERNS OF CHEMICAL-INDUCED DAMAGE TO THE SYNAPTONEMAL COMPLEX. J.W. Allen[a], L.C. Backer[b], P.A. Poorman[c], J.B. Gibson[d], B. Westbrook-Collins[a], and M.J. Moses[d]. [a]U.S. EPA, RTP, NC 27711; [b]EHRT, RTP, NC 27709; [c]Burroughs Wellcome Co, RTP, NC 27709; [d]Duke University Medical Center, Durham, NC 27710.

P7 COMPARISON OF SISTER CHROMATID EXCHANGE (SCE) INDUCTION IN SUBSETS OF MOUSE LYMPHOCYTES EXPOSED IN VITRO TO 4-HYDROXYCYCLOPHOSPHAMIDE (4-OHCP) OR PHOSPHORAMIDE MUSTARD (PAM). P. Kwanyuen[a], G.L. Erexson[a], M.F. Bryant[a], and A.D. Kligerman[b]. [a]EHRT, RTP, NC 27709; [b]U.S. EPA, RTP, NC 27711.

P8 THE MUTAGENIC ACTIVITY OF HYDROGEN PEROXIDE IN SALMONELLA TYPHIMURIUM. A. Abu-Shakra and E. Zeiger, NIEHS, RTP, NC 27709.

P9 INTRA- AND INTERLABORATORY VARIABILITY STUDIES IN THE AMES/SALMONELLA MUTAGENICITY ASSAY. T.J. Hughes[a], L.E. Myers[a], L.G. Monteith[a], V.S. Houk[b], and L.D. Claxton[b]. [a]RTI, RTP, NC 27709; [b]U.S. EPA, RTP, NC 27711.

P10 A MULTIPLE SAR DATA BASE APPROACH TO PREDICTING SALMONELLA MUTAGENICITY. D. Walsh[a], K.L. Dearfield[b], E. Perry[a], and L.D. Claxton[c]. [a]EHRT, RTP, NC 27709; [b]U.S. EPA, Washington, DC 20460; [c]U.S. EPA, RTP, NC 27711.

P11 SPONTANEOUS ad-3 MUTANTS RECOVERED FROM A TWO-COMPONENT HETEROKARYON (H-12) OF NEUROSPORA CRASSA CONSIST OF GENE/POINT MUTATIONS AND MULTILOCUS DELETIONS. F.J. de Serres. RTI, RTP, NC 27709.

P12 MOST X RAY-INDUCED ad-3 IRREPARABLE MUTANTS IN NEUROSPORA CRASSA MAP AS A SERIES OF OVERLAPPING MULTILOCUS DELETIONS BUT AN UNEXPECTEDLY HIGH NUMBER RESULT FROM CLOSELY LINKED INDEPENDENT SITES OF GENETIC DAMAGE. F.J. de Serres. RTI, RTP, NC 27709.

P13 MOLECULAR AND GENETIC CHARACTERIZATION OF X RAY-INDUCED SPECIFIC-LOCUS MUTATIONS IN THE ad-3 REGION OF NEUROSPORA CRASSA. F.J. de Serres, L.K. Overton, and J.S. Dubins. RTI, RTP, NC 27709.

P14 ANALYSIS OF THE ABILITY OF THE L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA ASSAY TO DETECT AND QUANTITATE INDUCED GENETIC EVENTS. M.M. Moore[a], K. Brock[b], C. Doerr[b], M. Applegate[c], and J.C. Hozier[c]. [a]U.S. EPA, RTP, NC 27711; [b]EHRT, RTP, NC 27709; [c]Florida State University, Tallahassee, FL 32306.

P15 SYNAPTONEMAL COMPLEX DAMAGE AND ITS RELATIONSHIP TO GERM CELL CHROMOSOME ABERRATIONS. L.C. Backer[a], J.B. Gibson[b], D.R. Howard[a], G. Honore[b], M.J. Moses[b], and J.W. Allen[c]. [a]EHRT, RTP, NC 27709; [b]Duke University Medical Center Durham, NC 27710; [c]U.S. EPA, RTP, NC 27711.

P16 SISTER CHROMATID EXCHANGE (SCE) INDUCTION AND DNA ADDUCT FORMATION IN RODENT PERIPHERAL BLOOD LYMPHOCYTES (PBLs). A.D. Kligerman[a], S. Nesnow[a], G.L. Erexson[b], and R. Gupta[c]. [a]U.S. EPA, RTP, NC 27711; [b]EHRT, RTP, NC 27709; [c]Baylor College of Medicine, Houston, TX 77030.

P17 THE CHINESE HAMSTER V79 METABOLIC COOPERATION ASSAY: APPLICATIONS IN TOXICOLOGY. G.P. Wyatt and E. Elmore. NSI Technology Services Corp., RTP, NC 27709.

P18 ACTIVITIES OF SELECTED CYCLIC HYDROCARBONS OF KNOWN CARCINOGENICITY IN THE V79 METABOLIC COOPERATION ASSAY. E. Elmore[a], H.A. Milman[b], B.P. Wilkinson[a], and G.P. Wyatt[a]. [a]NSI Technology Services Corp., RTP, NC 27709; [b]U.S. EPA, Washington, DC 20460.

P19 INHIBITION OF BENZO(a)PYRENE-INDUCED MALIGNANT TRANSFORMATION IN CULTURED TRACHEAL EPITHELIAL CELLS BY RETINOIC ACID. B. Wilkinson, J. Arnold, and V. Steele. NSI Technology Services Corp., RTP, NC 27709.

P20 APPLICATION OF WHEAT SEEDLING ASSAY FOR DETECTING CHEMICALLY INDUCED ANEUPLOIDY. S.S. Sandhu, J.S. Dhesi[b], and G.P. Redei[c]. [a]U.S. EPA, RTP, NC 27711; [b]EHRT, RTP, NC 27709; [c]U. of Missouri, Columbia, MO 65211.

P21 DIRECT DNA SCREENING FOR SPONTANEOUS AND INDUCED MUTATIONS. E. Korytynski, J. Dubins, S.E. Lewis, L.B. Barnett, and R.R. Cobb. RTI, RTP, NC 27709.

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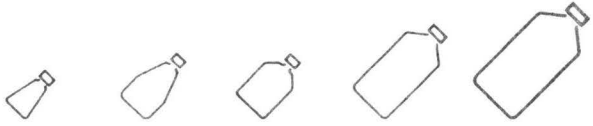

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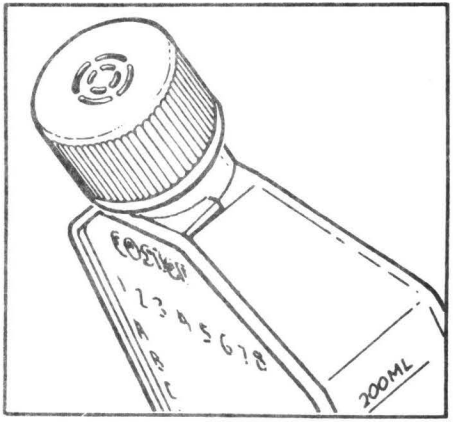

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ABSTRACTS
TALKS

T1

DRUG RESISTANT T CELLS MAY CONTRIBUTE IN HUMAN GRAFT REJECTION, W.L. Stanford[a], G.H.S. Strauss[b], and O.J. Finn[c], [a]EHRT, RTP, NC, 27709; [b]U.S. EPA, GTD, RTP, NC 27711; and [c]DUMC, Durham, NC 27710.

The objective of our study was to determine whether drug resistance by transplant recipient T cells contribute to rejection of human organ transplants and to further characterize the mutant phenotype in the Strauss-Albertini Test. Following transplantation, the organ recipients (kidney or liver) received chemical immunosuppressive therapy, including the purine analog azathioprine (AZ), to prevent graft rejection. The T cell lines used in this series of experiments were originally established by obtaining (from biopsies) recipient T cells which have infiltrated transplanted grafts and initiated rejections. These T cells, which are allo-specific for donor HLA antigens, were subsequently expanded into T cell lines using EBV-transformed donor B cell lines as antigen. The Strauss-Albertini Test uses 6-thioguanine, which is closely related to AZ, as the selective agent to measure the genotoxic effects of *in vivo* mutagen exposure by enumerating presumed HGPRT mutants *in vitro*. The Strauss-Albertini Test was modified to enumerate thioguanine-resitant (TGR) T cells from human graft recipients which arise *in vivo* in response to antigenic stimulation by the graft and selection, we believe, by AZ. Although the frequency of TGR variants in the majority of T cell lines has closely resembled the frequency of 5×10^{-6} found in peripheral blood lymphocytes of controls, we have encountered four TGR T cell lines, including two lines with variant frequencies of 0.663 and 0.690, respectively. We shall discuss the relationship between variant frequencies and graft rejection in the face of high doses of purine analog immunosuppressants. (This abstract does not necessarily reflect U.S. EPA policy.)

T2

ANTIMUTAGENICITY STUDIES IN SALMONELLA. J.R. Warner, T.J. Hughes and M.E. Wall. Research Triangle Institute (RTI), RTP, NC 27709.

The purpose of this research was to conduct preliminary studies into the mechanisms of antimutagenesis in Salmonella typhimurium. Mutagens were aflatoxin B_1 (AFB_1) and 2-anthramine (2AN); antimutagens were coumarin (C), psoralen (P), 8-methoxypsoralen (8MP) and 5-methoxypsoralen (5MP). Assay parameters were: TA100, Aroclor-induced rat liver S9 (5% S9 mix), plate incorporation technique (AFB_1) or preincubation technique (2AN). The antimutagens were neither toxic nor mutagenic under the test conditions. The endpoint of the antimutagenicity assay was a reduction in the number of revertants on mutagen treated plates upon addition of the antimutagens. AFB_1 (0.05 ug/plate) produced 1200 revertants, and 2AN (2.5 ug/plate) produced 2400 revertants. Initial results suggest: 1) antimutagenicity against AFB_1 was in the order of 8MP>P>5MP>C; 2) antimutagenicity against 2AN was in the order of P>8MP>5MP>C; and 3) equivalent doses of antimutagens inbibited AFB_1 more significantly than did 2AN (e.g., 150 ug of 8MP inhibited AFB_1 55%, but inhibited 2AN only 10%). This differential inhibition may be due to the structural similarities between the antimutagens and AFB_1. In the second part of this research, the order in which the mutagens and antimutagens are added and the combinations that are used will be adjusted to determine if these antimutagens are acting as protective agents, interacting with the mutagens or S9, or interacting with the metabolites of the mutagens. Results of these studies will be discussed.

T3

THE INDUCTION OF CHROMOSOME ABERRATIONS IN MOUSE BONE MARROW CELLS BY THE DNA TOPOISOMERASE INHIBITORS CAMPTOTHECIN AND AMSACRINE. D.R.Howard[1], L.C. Backer[1], D.M. DeMarini[2], J.W. Allen[2], [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

Topoisomerases are enzymes that control supercoiling, breakage, and reunion of DNA strands. There is evidence to suggest that two antitumor drugs, camptothecin and amsacrine (m-AMSA), inhibit topoisomerase activity by binding to the DNA-topoisomerase complex and preventing reunion of the broken DNA strands. m-AMSA is a known clastogen that binds to topoisomerase II to induce double-strand breaks in DNA. Camptothecin inhibits the activity of topoisomerase I, inducing single-strand DNA breaks. Although m-AMSA induces chromosome aberrations, camptothecin has not been characterized for this effect. In this experiment, camptothecin and m-AMSA were compared for their capacity to induce chromosome- and chromatid-type aberrations in mouse bone marrow cells. Male mice were exposed by i.p. injection to 0, 0.5, 1.5, or 3.0 mg/kg camptothecin or m-AMSA in DMSO. Animals were injected with colchicine at 15 h, and bone marrow was harvested at 18 h. BrdUrd tablets were implanted to assure identification of first division cells. Four animals/dose and 100 cells/animal were scored for chromosome aberrations. Both chemicals induced approximately 50 chromatid and 10 chromosome aberrations per animal at the highest dose. Despite the mechanistic differences between camptothecin (single-strand DNA breaker) and m-AMSA (double-strand DNA breaker), there were no differences observed in either the capabilities of these two chemicals to induce chromosome aberrations, or in the types of aberrations they induced.

This abstract does not necessarily reflect U.S. EPA policy.

T4

MUTATION VS MUTANT FREQUENCY IN THE MOUSE LYMPHOMA ASSAY. D. Daston[a], W. Caspary[a], C. Rudd[b], K. Pardo[b], [a]NIEHS/NIH, RTP, NC, [b]SRI Int, Menlo Park, CA

Mutations in mammalian cells can be detected by measuring phenotypic changes which occur after products of the altered gene disappear or are synthesized. In the mouse lymphoma L5178Y cell, mutation at the thymidine kinase (tk) locus results in tk deficiency (TK +/- → TK -/-) i.e., loss of thymidine kinase enzyme activity. The time necessary to acquire the new phenotype is called the expression period. Ideally, the frequency of mutants present before expression is equal to that detected at the end of the expression period. However, if the mutagenic event is associated with a change in cell growth rate, changes in the ratio of mutant to wild-type cells can occur. We developed a method which segregates and immobilizes cells in agar during expression. Because slow growing mutants are not diluted out and lost, this procedure more accurately determines the frequency of mutations at the tk locus in mouse lymphoma cells than procedures in which cells are mixed during expression. Thus, the calculation of mutation frequency (proportion of new mutations formed in a population of cells) is possible, rather than the mutant frequency (proportion of mutants in a population of cells) which is usually determined in mammalian cell assays. The expression time of newly formed spontaneous mutations was calculated to be 30 hours, and the mutation rate was approximately 50 per million cell divisions. Fast and slow growing mutants were detected as large and small TFT-resistant colonies, respectively. The rate of formation of the small colonies was 3-4 times that of the large colonies. The predominance of slower growing mutants in this procedure may partially account for the much higher spontaneous mutation rates observed as compared with those reported in earlier studies.

T5

ONCOGENE AMPLIFICATION, METHYLATION, EXPRESSION, AND MUTATION IN CHEMICALLY-TRANSFORMED RESPIRATORY EPITHELIAL CELLS. Jessica A. Lasley[1], Nancy S. Schorschinsky[1], Diane K. Beeman[2], and Marc J. Mass[2], [1]Environmental Health Research and Testing, Inc., Research Triangle Park, NC 27709 and [2]Carcinogenesis and Metabolism Branch, MD-68, U.S.E.P.A., Research Triangle Park, NC 27711.

Considerable evidence has accumulated linking alterations in oncogenes and the development of cancer. Most of these studies have utilized tumors, which are late stages in the neoplastic process. We utilized the rat tracheal epithelial (RTE) cell transformation system to observe the participation of oncogenes in comparatively early stages of cell transformation in vitro. Eight cell lines were studied for alterations in K-ras, H-ras, and c-myc. These lines were transformed in culture with the carcinogens benzo(a)pyrene (BP), 7,12-dimethylbenz(a)anthracene (DMBA), and/or the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Southern blot hybridizations indicated no amplification of H-ras, K-ras, or c-myc in the lines studied. However, restriction length polymorphisms (RFLPs) were observed in 2 cell lines digested with Eco RI and probed with K-ras . We did not find alterations in H-ras codon 61 that have been found in DMBA-induced tumors in vivo. All cell lines had fewer restriction sites when digested with Hpa II and probed with H-ras than did normal cells. One cell line had 2 types of c-myc RFLPs when digested with Bam Hl or Hind III, and also exhibited methylation changes in CCGG sequences compared with normal cells. In contrast to changes in DNA restriction patterns, we did not observe elevations in expression levels in any oncogenes using RNA dot blots. We conclude that transformed RTE cells can demonstrate a number of oncogene alterations, however, the relationship of each to the transformed state requires further study. *This is an abstract of a proposed presentation and does not constitute EPA policy.*

T6

CHEMICAL SYNTHESIS AND CHARACTERIZATION OF A MODEL CYCLOPENTA-FUSED PAH PRODUCT OF ADENOSINE, A.W. Bartczak[a], R. Sangaiah[a], S.D. Wyrick[a], G. Toney[b], and Avram Gold[a]. [a]UNC-CH, Chapel Hill, NC 27599-7400, [b]NIEHS, Research Triangle Park, NC 27709

The covalent binding of carcinogenic polycyclic aromatic hydrocarbons (PAH) to DNA in intact organisms has been shown to correlate with carcinogenic potency. Most of the carcinogenic PAH investigated contain a bay region diolepoxide. Several carcinogenic members of a unique family of PAH, the cyclopenta-fused PAH (cpPAH), have been found in the environment and have been shown to be metabolically activated via a cyclopenta ring epoxide. Despite the biological activity of several cpPAH, no adducts have yet been characterized. Sufficient quantities of adducts could not be obtained by modification of DNA or deoxynucleosides with a cyclopenta PAH epoxide. Therefore we have undertaken the chemical synthesis of cpPAH-modified nucleosides by alternative routes. We report synthesis of the nucleoside adducts predicted from the interaction of adenosine and the cyclopenta oxide of acenaphthene. Milligram quantities of product have allowed structural characterization of the four diastereoisomeric adducts by NMR and computer-assisted molecular modeling studies. Comparison of the NMR spectra indicate two distinct groups, based on the chemical shifts of protons. Computer-assisted molecular modeling rationalizes the differences in the chemical shifts of the protons by providing estimates of critical distances and angles from the minimum energy configurations of the four adducts.

T7

DETECTION AND COMPARISION OF DNA ADDUCTS IN ANIMAL TISSUES/CELLS FOLLOWING IN VIVO EXPOSURE TO THREE ENVIRONMENTALLY IMPORTANT COMPLEX MIXTURES, M.George,[1] P.Rouse,[1] J.Scott,[1] J.Inmon,[2] J.Gallagher[2] and J.Lewtas.[2] [1]Environmental Health Research and Testing Inc. RTP,NC 27709 and [2]U.S. Environmental Protection Agency RTP,NC 27711.

Animal models need to be utilized to better study the relationship between genotoxic complex mixtures and adverse consequence as measured by DNA adduct formation. In these studies we have used ^{32}P-postlabeling to detect and compare DNA adduct levels and profiles in various mouse/rat tissues and isolated cells following three separate routes of exposure with three environmentally important complex mixtures. Mice were skin painted and rats interperitoneally (IP) injected or intratracheally instilled with smoky coal, coke oven main or diesel extract. Persistence of the resulting DNA adducts was monitored over time in the IP treated animals. A zone of DNA adducts was observed for all three complex mixtures for the skin painted and IP injected animals, however, these adduct zones were not seen with the intratracheally instilled animals. Each complex mixture resulted in a characteristic DNA adduct profile. Adduct levels, when compared on a mg/kg basis, were higher in IP injected animals compared to skin painted animals. Moreover, lung DNA was shown to have higher adduct levels compared to liver DNA for all three complex mixtures examined. DNA adducts were also shown to persist 21 days following the initial exposure for those animals interperitoneally injected with diesel or smoky coal extract. Studies of this type can provide important information regarding the genotoxic potential of environmentally important complex mixtures. [This abstract does not necessarily reflect EPA policy.]

T8

MOLECULAR AND CYTOGENETIC ANALYSIS OF SPONTANEOUS AND CYANOETHYLENE OXIDE (CNETO)-INDUCED TK -/- MUTANTS IN HUMAN CELLS

L. Recio, Y. Kodama, C.J. Boreiko, and T.R. Skopek Chemical Industry Institute of Toxicology, RTP, NC

Two phenotypic classes of tk-/- mutants are observed in human TK6 lymphoblasts; one class of mutants (tkn) has a normal growth rate relative to wild type while the second class (tks) grows at a slower rate. The molecular nature of spontaneous and CNETO-induced (the proposed ultimate mutagenic metabbolite of the rat carcinogen acrylonitrile) tk-/- mutants from these two phenotypic classes was investigated by Southern blot analysis. 7/9 tkn spontaneous mutants and 11/12 CNETO-induced tkn had no detectable alterations in their tk restriction fragment pattern. In contrast, 25/26 tks mutants analyzed (spontaneous and CNETO-induced) had lost a 14.8kb polymorphic fragment of the +tk allele. The nature of the tks mutant phenotype was further investigated using karyotype analysis. G-banding and/or Q-banding analysis of metaphase chromosomes was performed on the parental TK6 cell line, 16 tks mutants (8 spontaneous and 8 CNETO-induced) and 2 tkn mutants that had also lost the 14.8kb polymorphic band. Chromosome 17, the chromosome to which tk has been localized, was normal with respect to the parental cell line in 15/16 tks mutants. An apparent duplication was present in one tks mutant, with a breakpoint at or near the chromosome band to which tk has been localized. Although the tks mutant phenotype was strongly associated with loss of a 14.8kb polymorphic fragment (96% of all tks mutant analyzed), there does not appear to be chromosome 17 aberrations visible at the cytogenetic level in tks mutants. These observations are different from those made in mouse lymphoma cells, where chromosome 11 in tks mutants has a high frequency of aberrations, and sugggests differences in the stability of the human and rodent chromosome complements.

T9

A METHOD FOR DETERMINING MUTATION SPECIFICITY IN HUMAN CELLS, D. Simpson, R. Crosby, L. Recio, R. Albertini[a] and T. Skopek, Chemical Industry Institute of Toxicology Research Triangle Park, NC 27709 and [a]University of Vermont, Burlington, VT.

A method has been developed for the specific amplification and cloning of human hprt cDNA. This general cloning/sequencing scheme can be used to analyze hprt mutation in human cells obtained both *in vitro* and *in vivo*. In this procedure messenger RNA is isolated from mutant lymphoblasts or lymphocytes and is used to produce a cDNA copy of hprt mRNA using reverse transcriptase primed with a synthetic oligonucleotide which is specific for the 3' end of the gene. Second strand synthesis and subsequent specific amplification of hprt sequences is accomplished using the *Thermus aquaticus* DNA polymerase in the polymerase chain reaction (PCR). The resulting hprt fragments are cloned into M13mp19 using restriction sites built into the 5' end of each primer. Dideoxy sequencing is carried out on the cloned products to reveal the nature of the mutation. Ongoing *in vitro* studies include the analysis of formaldehyde and cyanoethylene oxide induced mutants. Six of seven formaldehyde-induced mutants displayed a base pair substitution at AT base pairs with 4 occuring at a single site in the gene. The majority of cyanoethylene oxide mutants sequenced thus far revealed exon loss in the hprt sequence. To demonstrate the feasibility of *in vivo* analysis, we have determined the hprt sequences in T-lymphocytes from a Lesch-Nyhan ($hprt^-$) patient. We have detected a single AT to GC transition in the hprt coding region resulting in a methionine to threonine change in the hprt protein.

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ABSTRACTS
POSTERS

P1

DEVELOPMENT AND VALIDATION OF THE SPIRAL SALMONELLA ASSAY: AN AUTOMATED APPROACH TO BACTERIAL MUTAGENICITY TESTING. V.S. Houk[a], S. Schalkowsky[b], and L.D. Claxton[a]. [a]US EPA, RTP, NC 27711, and [b]Spiral System Instruments, Inc., 4853 Cordell Ave., Suite A-10, Bethesda, MD 20814.

Since its development by Dr. Bruce Ames and his colleagues more than a decade ago, the Salmonella/ mammalian microsome mutagenicity assay has become a widely accepted tool for the identification of chemicals with mutagenic and carcinogenic potential. Several automated approaches to Salmonella testing have been proposed in recent years but have failed to gain acceptance in the scientific community due to poor performance or lack of demonstrated usefulness. In this paper we report on an automated system that successfully generates dose-response data and, moreover, reduces the labor, materials, and sample mass required to obtain such information. In the standard plate-incorporation assay, dose-response relationships are defined by testing discrete doses of the test agent on a series of agar plates. In contrast, the spiral Salmonella assay generates dose-response data from a continuous concentration gradient on a single agar plate. Upon analysis, each spiral plate yields a dose-response curve consisting of 13 data points that span a concentration range equivalent to about 5 two-fold serial dilutions. The performance of the spiral Salmonella assay was compared to that of the conventional plate-incorporation assay using 13 mutagens and 7 nonmutagens selected from a variety of chemical classes. Concordant qualitative responses were obtained for all compounds tested, and comparable dose-response relationships were generated by all mutagens with the exception of sodium azide and cyclophosphamide, which are highly water soluble and, thus, are unable to maintain a well-defined concentration gradient on a spiral plate due to rapid diffusion. In general, toxicity was expressed at a lower dose in the spiral assay, and the mutagenic potencies (slopes of the dose-response curves) were greater in the spiral assay relative to the plate-incorporation assay. These differences will be discussed, as will the applicability of the spiral plating technique to routine screening and its relevancy to future mutagenesis testing.

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

P2

THE USE OF THE AUTOMATED SPIRAL SALMONELLA ASSAY FOR THE DETECTION OF MUTAGENIC COMPLEX ENVIRONMENTAL SAMPLES. G. Early[1], V.S. Houk[2], and L.D. Claxton[2]. [1]St. Augustine's College, Raleigh, N.C. 27611 and [2]US EPA, RTP, NC 27711.

An automated approach to Salmonella mutagenicity testing, which eliminates the serial dilutions and multiple plates required by the conventional plate-incorporation assay to generate dose-response data, was recently developed (see DEVELOPMENT AND VALIDATION OF THE SPIRAL SALMONELLA ASSAY: AN AUTOMATED APPROACH TO BACTERIAL MUTAGENICITY TESTING by V.S. Houk et al.). In the spiral Salmonella assay, the bacteria are exposed in the presence or absence of S9 to a continuous concentration gradient of the compound deposited on a single agar plate. From each plate a dose-response curve comprised of 13 data points is generated. In this study, we have examined the effectiveness of the spiral assay for use on complex environmental samples. Four sets of combustion particle emissions were selected for testing: an automotive diesel exhaust sample, 2 wood combustion emissions, and a sample obtained from the controlled combustion of a bituminous-like coal from China. Preliminary comparisons of mutagenesis data generated by these complex mixtures in the spiral and standard Salmonella assays have demonstrated that a 40- to 80-fold reduction in the sample mass required for dose responsiveness can be achieved by the spiral assay. Furthermore, due to differences in sample deposition practices between the 2 techniques, it was possible in the spiral assay to omit the solvent exchange step (to DMSO) and test the dichloromethane extract directly.

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

P3

THE MUTAGENIC IMPACT OF X-RAY TREATMENT ON ELECTROPHORETICALLY EXPRESSED LOCI IN THE MOUSE. Susan E. Lewis[1], Lois B. Barnett[1], Raymond A. Popp[2], and Michael D. Shelby[3]; [1]RTI, RTP, NC 27709; [2]Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; [3]NIEHS, RTP, NC 27709.

The induction of mutations by various doses of x-rays has been studied in the Electrophoretic Specific Locus Test in the mouse. Both female and male germ cells were studied. Following x-ray treatment, matings to produce (C57BL/6J X 2J) F_1 hybrid mice for electrophoretic analysis were made at appropriate times in order to study effects on specific germ cell stages. The effects on electrophoretically expressed loci were considerably less than on loci in the visible specific-locus test. Spermatogonia treated with a single dose of 300R gave higher frequencies of mutations on a per locus basis than did the 600R dose. The internal comparison of visible and electrophoretic loci in treated C57Bl/6J females shows that the visible loci are more susceptible to mutation at this dose than are the electrophoretic loci. Mutation frequencies are currently being studied in progeny whose male parent was treated with a fractionated dose of x-rays. [Supported in part by Contract #NO1-ES-2-5012 from NIEHS.]

P4

DEVELOPMENT OF AN ULTRAMICROSUSPENSION MUTAGENICITY ASSAY AND APPLICATION TO AIRBORNE PARTICULATES COLLECTED BY PERSONAL SAMPLERS. S. Goto[1], H. Matsushita[1], Y. Takagi[2], M. Murata[2], K. Williams[3], D.M. DeMarini[3], and J. Lewtas[3]. [1]Institute of Public Health, Tokyo, Japan; [2]Azabu University, Kanagawa, Japan; [3]U.S.Environmental Protection Agency, Research Triangle Park, NC 27711.

A new reduced-volume microsuspension mutagenicity assay employing 100-μl vials has been developed using a modification of two current microsuspension assays of *Salmonella typhimurium*. *S. typhimurium* strain TM677 used in the 8-AG-resistance forward-mutation assay and strain TA98 used in the histidine-reversion assay are currently being evaluated. The assays are conducted in 100-μl vials in a total volume of only 10 μl. The suspension is incubated for 90 min by rotating the vials. Evaporation is eliminated by means of tight-fitting Teflon plugs. The ultramicrosuspension assay showed good reproducibility (coefficient of variation: 8.3% - 14.3%) when 4NQO was tested. The forward-mutation version of this assay required approximately one-tenth the sample size of the microsuspension forward-mutation assay that had been used previously for indoor air samples (Lewtas *et al.* Atmos. Environ. 21:443, 1987). Furthermore, samples of airborne particulate extracts collected by personal samplers (2 liters/min X 24 hr) were found to be adequate for the measurement of mutagenicity. Samples collected from smokers showed higher mutagenic responses than those from nonsmokers. Current studies are in progress to evaluate the same ultramicrosuspension assay in *S. typhimurium* TA98 using further modifications of a microsuspension assay developed by Kado *et al.* (Mutat. Res. 121:25, 1983) and modified by DeMarini *et al.* (Environ. Mol. Mutagen. 11, Suppl. 11:27, 1988).

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

P5

THE CONSEQUENCES OF UNREPAIRED DOUBLE-STRAND BREAKS IN YEAST. C.B. Bennett[1], T. Nilsson-Tillgren[2], and M.A. Resnick[1]; [1]NIEHS, RTP, NC 27709 and [2]U. of Copenhagen, Denmark.

Recombinational repair mechanisms for eukaryotes have been proposed that require direct participation of both double-strand breaks (DSB), which produce invasive free duplex end(s), and a homologous chromosome or sister chromatid that pairs with the damaged chromosome, supplying a template for the repair event. We are examining the biological effects of DSBs in yeast systems where the possibility for recombinational repair is greatly reduced. For instance, gamma-ray-induced damage in homologous chromosomes is efficiently repaired. However, damage (presumably DSBs) induced in a pair of divergent chromosomes (S. carlsbergensis chromosome III/S. cerevisiae chromosome III) present in an otherwise homologous chromosome background (i.e., S. cerevisiae) leads to a high level of aneuploidy for the divergent chromosomes (Resnick, Skaanild, and Nilson-Tillgren, submitted). The presence of a DSB in a pair of chromosomes under conditions where recombinational repair is prevented because of lack of homology does not affect cell survival. In order to determine if DSBs function as transducing signals in the cell, we have developed a shuttle vector in yeast that contains a synthetic YZ junction (from MAT) flanked by nonyeast sequences (SV40 and E. coli). In the presence of galactose, HO endonuclease (fused to the GAL) promoter) is induced from a second selectable plasmid (pSE271) and cuts the target YZ junction resulting in a DSB in a nonintegrating and nongenomic region (the genomic YZ junctions are mutated). The YZ target plasmid contains CEN3 and is stably maintained at low copy number; however, higher copy numbers can be obtained and stably maintained due to the presence of the CUP1 gene on the plasmid. Thus, the indirect effects of a known DSB on recombination, repair, and various biological endpoints can be examined in a dose-dependent manner without the lethal effects associated with genomic damage.

P6

PATTERNS OF CHEMICAL-INDUCED DAMAGE TO THE SYNAPTONEMAL COMPLEX. J.W. Allen[1], L.C. Backer[2], P.A. Poorman[3], J.B. Gibson[4], B. Westbrook-Collins[1], M.J. Moses[4]. [1]U.S. EPA, RTP, NC 27711; [2]EHRT, Inc., P.O. Box 12199, RTP, NC 27709; [3]Wellcome Research Lab., RTP, NC 27709; [4]Duke Univ. Medical Center, Durham, NC 27710.

Clastogenic and anti-mitotic chemicals were evaluated for induction of synaptonemal complex (SC) aberrations in mouse spermatocytes. Following an intraperitoneal (ip.) or intratesticular (it.) injection of mitomycin-C, cyclophosphamide, colchicine, vinblastine sulfate, amsacrine, or bleomycin, a spectrum of damage was produced: all agents caused SC breakage, asynapsis, multi-axis configurations, and various forms of mispairing. However, chemical-specific propensities for inducing certain types of damage at S-phase or prophase were also evident. As examples, the alkylating agent, cyclophosphamide (10-200 mg/kg, ip.), was premeiotic S-phase dependent for maximal activities to induce SC breakage, rearrangements, and synaptic irregularities. The anti-tubulin alkaloid, colchicine (0.02-50 ug, it.), was especially efficient at early prophase for inducing synaptic aberrations. The radiomimetic, bleomycin (25-100 mg/kg, ip.), was exceptional in producing extensive SC breakage, rearrangement, and mispairing events in mid-late prophase. These observations suggest that patterns of SC aberrations induction can reflect different chemical mechanisms of interaction with germ cells.

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

P7

COMPARISON OF SISTER CHROMATID EXCHANGE (SCE) INDUCTION IN SUBSETS OF MOUSE LYMPHOCYTES EXPOSED IN VITRO TO 4-HYDROXYCYCLOPHOSPHAMIDE (4-OHCP) OR PHOSPHORAMIDE MUSTARD (PAM). P. Kwanyuen[1], G.L. Erexson[1], M.F. Bryant[1], and A.D. Kligerman[2]. [1]EHRT,Inc.,RTP,NC 27709, [2]US EPA,RTP,NC 27711.

Experiments were designed to investigate the induction of SCEs in different subsets of mouse lymphocytes after exposure in vitro to 4-OHCP or PAM. Whole blood was obtained from several C57BL/6 male mice and pooled. Mononuclear cells (MNLs) were isolated using a Ficoll-Hypaque density gradient. Phytohemagglutinin (PHA; T-helper cells), concanavalin A (CON-A; T-suppressor cells), or lipopolysaccharide (LPS; B-cells) were used to stimulate the MNLs to divide. Bromodeoxyuridine (5 uM) and either PAM or 4-OHCP were added at 19 h and 21 h, respectively. Either 4-OHCP or PAM was added at equal concentrations (0.1, 0.3, and 1 ug/ml) to replicate 1 ml cultures containing 5 x 10^5 MNLs. Colcemid (0.5 ug/ml) was added at 46 h and the cultures were harvested at 50 h. Slides were prepared and stained using the fluorescence-plus-Giemsa technique. A total of fifty metaphases/concentration were scored for SCEs (25 cells/replicate culture). A significant concentration-dependent increase in SCE was observed with each chemical and mitogen. In general, for all 3 mitogens, 4-OHCP induced more SCEs than PAM. There was no significant difference in the number of SCEs induced by PAM in the B-lymphocytes compared to the T-lymphocytes. However, B-lymphocytes were more sensitive to 4-OHCP than T-lymphocytes. A possible explanation for this is that a suppressor population of B-lymphocytes is selectively sensitive to 4-OHCP. [This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy].

P8

THE MUTAGENIC ACTIVITY OF HYDROGEN PEROXIDE IN SALMONELLA TYPHIMURIUM. A. Abu-Shakra and E. Zeiger, Cellular and Genetic Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Hydrogen peroxide causes oxidative damage to DNA, and this damage is manifested as a mutagenic response in the Salmonella test. In the present study, H_2O_2 was tested for mutagenicity in a spectrum of Salmonella typhimurium tester strains. The aim was to select the strain and exposure conditions that produce the highest mutagenic response to H_2O_2 for use in further investigations into DNA damage and mutagenicity by H_2O_2 or H_2O_2-generating systems. The strongest mutagenic response was detected in TA104 (hisG428, rfa, uvrB, pKM101). Strain TA102, which carries the hisG428 gene on the multicopy plasmid pAQ1 and is excision repair proficient, was less sensitive. Of the strains carrying the frameshift mutations hisD3052 or hisD6610, only TA97 (hisD6610, hisO1242, rfa, uvrB, pKM101) was significantly reverted by H_2O_2. The presence of the hisO1242 mutation also had an enhancing effect on the mutagenic response to H_2O_2 in the hisG3108 strains SB1106 (hisO$^-$) and SB1111 (hisO$^+$). In the hisG46 strains, the presence of the plasmid pKM101 (TA100 and TA92) helped to produce a modest but detectable response, in contrast to the insensitive plasmid-free strains TA1535 and G46. The preincubation procedure was more effective than the standard plate procedure, yielding equivalent responses, but at approximately one-third of the dose. Based on these studies, TA104 was selected as the strain to use for studying H_2O_2-induced DNA damage and mutagenicity.

P9

INTRA AND INTERLABORATORY VARIABILITY STUDIES IN THE AMES/SALMONELLA MUTAGENICITY ASSAY T.J. Hughes[1], L.E. Myers[1], L.G. Monteith[1], V.S. Houk[2], and L.D. Claxton[2]. [1]RTI, RTP, NC 27709 and [2]EPA, RTP, NC 27711.

The purpose of this research was to determine if methods could be developed to evaluate and reduce the variability, and thereby accurately rank mutagenic activity, in the Ames/Salmonella mutagenicity assay. We compared data on ten (10) direct-acting mutagens tested at RTI and EPA using the following assay conditions: Salmonella typhimurium TA100, ten pure direct-acting mutagens (tested without S9), ten dose levels in the linear portion of the dose response curve, duplicate plates per dose, and three rounds of testing spaced at least one month apart. The ten test chemicals were: DMCC, EMS, EP, MMS, MNNG, NBP, NF, SA, SO and 4NQO. Results suggested: 1) intralaboratory variability did not exceed 40% in either laboratory; 2) interlaboratory variability was 60% or less for seven of the chemicals; 3) variability was significantly lower than in previous studies reported in the literature; 4) variability changed with chemical; 5) rankings of the ten chemicals based on slope values (revertants/ug) in the RTI and EPA laboratories were similiar; and (6) these data provide a basis for intralaboratory and interlaboratory evaluation of Ames mutagenicity data. Slope values (revertants/ug) ranged from a low of 0.06 (EMS) to a high of 4464.0 (4NQO), which provides a data base of five orders of magnitude for evaluation of mutagenic activity. Concurrent testing (same day, same researcher, same bacteria and media) is the most acceptable method to control variability. However, this is not always possible. The ability to quantify and rank Salmonella mutagenicity data generated in different laboratories or data generated within the same laboratory on different days will greatly assist in comparative risk assessment evaluations. Research sponsored by EPA Contract No. 68-02-4186-052.

P10

A MULTIPLE SAR DATA BASE APPROACH TO PREDICTING SALMONELLA MUTAGENICITY. D. Walsh[1], K.L. Dearfield[2], E. Perry[1], and L.D. Claxton[3]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, Washington, DC 20460; and [3]U.S. EPA, RTP, NC 27711.

The relationship between Salmonella mutagenicity and molecular structure of halogenated pyridines was studied by evaluating separate data bases focusing on different structural criteria. The computerized SAR system (ADAPT) was used to classify the data bases involved in this study. A compound was considered a nonmutagen only if there was a negative response in Salmonella tester strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation. A positive reponse in any of the Salmonella strains either with or without activation classified the compound a mutagen. A halogenated aromatic data base consisting of 70 compounds (25 mutagens, 45 nonmutagens) was correctly classified using 9 molecular descriptors. The correct internal predictability of these descriptors was determined to be 92%. A second data base with the focus on the nitrogenous function was constructed of 114 nitrogenous cyclic compounds (64 mutagens, 50 nonmutagens). This data base was successfully classified using 19 molecular descriptors and had a correct internal predictability of 89%. A third approach blended the criteria used in these two data bases. This data base contained aromatic nitrogenous compounds with 70% being halogenated. The Salmonella mutagenicity of a group of pyridine compounds predicted in each of the data bases will be compared to the actual experimental results. [This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.]

P11

SPONTANEOUS ad-3 MUTANTS RECOVERED FROM A TWO-COMPONENT HETEROKARYON (H-12) OF NEUROSPORA CRASSA CONSIST OF GENE/POINT MUTATIONS AND MULTILOCUS DELETIONS. Frederick J. de Serres, Center for Life Sciences and Toxicology, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709

X ray-induced specific locus mutations in the ad-3 region of two-component heterokaryons consist of gene/point mutations and multilocus deletions (MLDs). (Webber and de Serres, Proc. Natl. Acad. Sci. U.S.A. 53, 430-437, 1965). Chemicals can induce predominantly or exclusively gene/point mutations or both classes, with the frequency of MLDs being mutagen-dependent (de Serres, Banbury Report 28: Mammalian Cell Mutagenesis 1987). The frequency of spontaneous ad-3 mutants recovered with the two-component heterokaryon 12 from experiments performed at the Oak Ridge National Laboratory is 0.39 X 10^{-6}. A total of 172 spontaneous mutants were collected and have been subjected to genetic analyses; of these, 141/172 (81.9%) were gene/point mutants, $(ad\text{-}3)^{R}$, 172 (14.5%) were MLDs $(ad\text{-}3)^{IR}$ and 6/172 (3.5%) grew too rapidly on minimal medium alone to enable us to characterize them genetically $(ad\text{-}3)^{UNKN}$. Among the gene/point mutants, 41/101 (23.8%) were $(ad\text{-}3A)^{R}$ and 100/172 (58.1%) were $(ad\text{-}3B)^{R}$. Among the MLDs, 5/172 (2.9%) were $ad\text{-}3A^{IR}$, 15/172 (8.7%) were $ad\text{-}3B^{IR}$, 4/172 (2.3%) were $(ad\text{-}3A\ ad\text{-}3B)^{IR}$ and 1/172 (0.6%) was $(ad\text{-}3A^{IR}\ ad\text{-}3B^{R})$. No large MLDs of genotypes $(ad\text{-}3A\ ad\text{-}3B\ nic\text{-}2)^{IR}$ or $(ad\text{-}3B\ nic\text{-}2)^{IR}$ were found in this sample. Studies of allelic complementation among the $ad\text{-}3B^{R}$ mutants showed that 41/101 (40.6%) were nonpolarized complementing $(ad\text{-}3B)^{R\text{-}NP}$, 12/101 (11.9%) were polarized complementing $(ad\text{-}3B)^{R\text{-}P}$ and 47/101 (46.5%) were noncomplementing $(ad\text{-}3B)^{R\text{-}NC}$. (Research sponsored by Dept. of Energy).

P12

MOST X RAY-INDUCED ad-3 IRREPARABLE MUTANTS IN NEUROSPORA CRASSA MAP AS A SERIES OF OVERLAPPING MULTILOCUS DELETIONS BUT AN UNEXPECTEDLY HIGH NUMBER RESULT FROM CLOSELY LINKED INDEPENDENT SITES OF GENETIC DAMAGE. Frederick J. de Serres, Center for Life Sciences and Toxicology, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709

Genetic analysis of the extent and type of functional inactivation in X ray-induced ad-3 irreparable (ad-3IR) mutants (Webber and de Serres, Proc. Natl. Acad. Sci. U.S.A. 53, 430-437, 1965) has been performed using a series of markers in the immediately adjacent genetic regions. Mutants analyzed were: 97 single locus mutants, 33 ad-3AIR, 64 ad-3BIR, and 102 multilocus mutants, 89 (ad-3A ad-3B)IR, 9 (ad-3A ad-3B nic-2)IR, and 4 (ad-3B nic-2)IR. Because ad-3IR mutations increased as the square of the x-ray dose and did not grow as homokaryons on adenine supplemented medium, the assumption was made that they might result from multilocus deletions (MLDs). In MLDs, other closely linked loci could show simultaneous inactivation in mutants scored as ad-3IR. Homology tests, with a series of strains carrying closely-linked markers, were performed on individual ad-3IR mutants. Most single locus ad-3IR mutants map as series of overlapping MLDs that cover other known loci (lys-4, his-3, and nic-2). However, 6/97 are actually point mutations with a separate and closely linked site of recessive lethal damage (ad-3R + RL/CL). Most multilocus ad-3IR mutants also map as a series of overlapping MLDs, but 17/102 are actually MLDs with a separate, closely-linked point mutation (ad-3IR + ad-3R) involving the closely-linked loci lys-4, his-3, ad-3A, ad-3B, and nic-2. (Research sponsored by Natl. Institute of Environmental Health Sciences and Dept. of Engery).

P13

MOLECULAR AND GENETIC CHARACTERIZATION OF X RAY-INDUCED SPECIFIC LOCUS MUTATIONS IN THE ad-3 REGION OF Neurospora crassa. Frederick J. de Serres, Laurie K. Overton and Jeffrey S. Dubins, Center for Life Sciences and Toxicology, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709

Genetic analysis of X ray-induced specific locus mutations in the ad-3 region has shown that they result from gene/point mutations and functional multilocus deletions (de Serres, Genetics 58: 79-83, 1968). Genetic analysis of the single locus (ad-3A or ad-3B) and multiple locus (ad-3A ad-3B, ad-3A ad-3B nic-2, and ad-3B nic-2) mutants has shown that they map as a series of overlapping deletions that also include other genetic markers in the immediately adjacent regions (de Serres, Environ. Mutagen. 11: 28, 1988). Some multilocus deletions at the ad-3A locus show heterozygous effects in combination with a gene/point mutation at the ad-3B locus, and even more pronounced heterozygous effects in combination with a multilocus deletion covering the ad-3B locus (Overton and de Serres, Environ. Mutagen. 11: 80, 1988). Heterozygous effects of multilocus deletions are expressed as reduced linear growth rates when compared with the wild-type control. In contrast, X ray-induced gene/point mutations at the ad-3A locus do not show heterozygous effects (they are completely recessive and grow at wild-type rate). In this study, no correlation was found between the level of the heterozygous effects shown by individual ad-3A mutants and either map position or radiation dose. With the use of a probe for his-3 (located 0.5 map units distal to the ad-3 region), studies are being made to determine the nature of the genetic damage in individual mutants classified as functional multilocus deletions. These molecular data may provide some understanding of the fact that some ad-3A^{IR} mutants show heterozygous effects and others do not.

P14

ANALYSIS OF THE ABILITY OF THE L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA ASSAY TO DETECT AND QUANTITATE INDUCED GENETIC EVENTS. M.M. Moore[1], K. Brock[2], C. Doerr[2], M. Applegate[3], J.C. Hozier[3]. [1]U.S. Environmental Protection Agency, Research Triangle Park, NC 27711; [2]Environmental Health Research and Testing, Inc., Research Triangle Park, NC 27709; [3]Florida State University, Tallahassee, FL 32306.

Research to date indicates that L5178Y/TK$^{+/-}$ -3.7.2C mouse lymphoma cells permit the measurement of forward mutation affecting the heterozygous tk locus. Furthermore, the quantitated TK-mutant frequency can be expressed by a small-colony and large-colony mutant frequency. Small colonies appear to be associated with chromosomal events affecting the expression of multiple loci including tk and thus appear related to the clastogenicity of the test chemical. Large colonies appear to result from events affecting the tk locus. Southern blot analysis of DNA isolated from mutants indicates that the entire tk locus may be deleted. Densitometric scanning of the Southern blots as well as measurement of TK-specific mRNA coded by the TK^{-} allele of the mutant cell line indicate the amplification of the TK^{-} allele in some mutants. These studies indicate that heterozygous loci may be particularly suited for the study of genetic events involving large deletions or for events requiring the presence of an homologous chromosome. A growing number of reports cite loss of heterozygosity and mitotic recombination associated with the development of malignancy in humans.

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

P15

SYNAPTONEMAL COMPLEX DAMAGE AND ITS RELATIONSHIP TO GERM CELL CHROMOSOME ABERRATIONS. L.C. Backer[a] J.B. Gibson[b] D.R. Howard[a], G. Honore[b], M.J. Moses[b], and J.W. Allen[c]. [a]EHRT, RTP, NC; [b]Duke Univ. Med. Ctr., Durham, NC; [c]U.S. EPA, NC.

The synaptonemal complex (SC) comprises the proteinaceous axes of paired homologous chromosomes at meiotic prophase and is intimately involved in the events of homologous chromosome pairing, crossing over, and segregation. Using mice treated with alkylating agents and ionizing radiation, we have evaluated damage induced in prophase SCs in relation to chromosome aberrations evident at metaphase 1 and 2. Light and electron microscopic analyses of SCs revealed structural aberrations and synapsis irregularities in mice treated with 100 mg/kg cyclophosphamide(CP) or ^{60}Co gamma irradiation (2 or 4 Gy). In the study using CP, mice were also injected with 200 uCi tritiated thymidine, and tritium-labeled cells were analyzed to better define the qualitative and quantitative relationships between respective SC and metaphase chromosome analyses. Correlation coefficients were calculated among SC and metaphase chromosome endpoints. Statistically significant correlations were found, for example, between SC breakage and both metaphase 1 isochromatid breakage and M1 chromosomal rearrangements following CP treatment. These correlations are interpreted in the context of theoretical relationships between chromosomes and component parts of the SC.

This abstract does not necessarily relect U.S. EPA policy.

P16

SISTER CHROMATID EXCHANGE (SCE) INDUCTION AND DNA ADDUCT FORMATION IN RODENT PERIPHERAL BLOOD LYMPHOCYTES (PBLs). A.D. Kligerman[1], S. Nesnow[1], G.L. Erexson[2], and R. Gupta[3]. [1]U.S. EPA, Research Triangle Park, NC 27711; [2]EHRT, Inc., P.O. Box 12199, Research Triangle Park, NC 27709; [3]Baylor College of Medicine, Houston, TX 77030.

Experiments were undertaken to investigate differences in interspecies sensitivity and the correlation between SCE induction and DNA adduct formation as measured by ^{32}P postlabeling. Both mice and rats were injected i.p. with benzo(a)pyrene (BP) suspended in corn oil at doses ranging from 10 to 100 mg/kg. Controls received corn oil only. Twenty-four hours after injection, blood was removed by cardiac puncture from each animal. Ficoll-Hypaque-separated mononuclear leucocytes from each rat or pooled for each group of mice were separated into two aliquots. One aliquot was used for DNA adduct determination and the other for SCE analysis after PBL culture according to published protocols. BP caused a dose-dependent increase in SCE frequency in PBLs of both rats and mice with little difference in magnitude of the response between the two species. However, the major DNA adduct (BPDEI-dGuo) found in the isolated cells was approximately tenfold greater (attomol/μg DNA) in the mouse than in the rat. Thus, the shapes of the dose-response curves for SCE and DNA adduct formation were dissimilar, especially at the higher doses.

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

P17

The Chinese Hamster V79 Metabolic Cooperation Assay: Applications in Toxicology. G.P. Wyatt and E. Elmore, NSI Technology Services Corporation, P.O. Box 12313, Research Triangle Park, NC 27709

Elucidating the mechanisms of chemical induced alterations of cellular regulation is critical to our understanding of chemical toxicology. Intercellular communication is known to have an important role in regulating normal cellular differentiation and function. The responses of over 200 chemicals that have been characterized for their ability to inhibit metabolic cooperation, a major form of intercellular communication, have been compiled. We have included both unpublished and published data from our laboratory and published data from other laboratories. We have compared the responses of these chemicals to their reported activity as teratogens/reproductive toxicants, carcinogens, mutagens, or neurotoxicants. The predictive accuracy of metabolic cooperation for teratogenicity or reproductive toxicity, 76% for 103 chemicals; carcinogenicity, 65% for 85 chemicals; and mutagenicity, 56% for 100 chemicals. Due to the lack of known non-neurotoxic agents, only the sensitivity for predicting known neurotoxic agents, 73% for 80 chemicals, could be determined. The data suggest that the Chinese hamster V79 metabolic cooperation assay be used to prescreening chemicals for teratogenicity/reproductive toxicity, carcinogenicity, and neurotoxicity.

P18

ACTIVITIES OF SELECTED CYCLIC HYDROCARBONS OF KNOWN CARCINOGENICITY IN THE V79 METABOLIC COOPERATION ASSAY. E. Elmore[a], H.A. Milman[b], B.P. Wilkinson[a], and G.P. Wyatt[a]. [a]NSI Technology Services Corporation, Research Triangle Park, NC 27709 and [b]U.S. EPA, Washington, DC 20460.

Structurally similar cyclic hydrocarbons were evaluated in the V79 metabolic cooperation assay and their responses correlated to their known carcinogenicity. The carcinogens evaluated were: 2,4-diaminotoluene, 2,5-diaminotoluene (suspect carcinogen), 2,4-dinitrotoluene, o-phenylenediamine, 4-chloro-o-phenylenediamine, 4-chloro-m-phenylenediamine, 2-nitro-p-phenylenediamine, 2,6-dichloro-p-phenylenediamine, 4,4'-methylenedianiline, 2-chlorobiphenyl (suspect carcinogen), and 2-phenoxyethanol (carcinogenicity unknown). The noncarcinogens evaluated were: aniline (human), m-phenylenediamine, p-phenylenediamine, 2-chloro-p-phenylenediamine sulfate and 4-nitro-o-phenylenediamine. The V79 metabolic cooperation assay is based on the recovery of 6TG-resistant mutants from mixed cultures of wild-type and mutant cells. Inhibition of cell-cell communication results in the enhanced recovery of mutant cells. For a compound to be considered positive, it must significantly enhance the recovery of mutant cells ($p = 0.005$, Dunnett's test) at least for two concentrations in two separate experiments or at three consecutive concentrations in one experiment. The sensitivity of the V79 metabolic cooperation assay to detect known carcinogens was 90% (the suspect carcinogens were included in this calculation). The specificity of the assay was 80%, and the accuracy--the ability to correctly identify both carcinogens and noncarcinogens--was 86.6%. The responses of the structurally related cyclic hydrocarbons in the V79 metabolic cooperation assay show a high correlation with their reported in vivo carcinogenicity.

P19

INHIBITION OF BENZO(a)PYRENE INDUCED MALIGNANT TRANSFORMATION IN CULTURED TRACHEAL EPITHELIAL CELLS BY RETINOIC ACID. B. Wilkinson, J. Arnold and V. Steele, Cellular and Molecular Toxicology Program, NSI Technology Services Corporation, Research Triangle Park, NC 27709

To understand the mechanisms of cancer inhibition and to screen chemicals for chemopreventive potential a culture system was developed using respiratory epithelial cells. Tracheal epithelial cells were isolated from Fischer 344 rats and plated onto collagen coated dishes. On Day 1 cultures were exposed to either 10 ug benzo(a)pyrene [B(a)P] per ml, 0.1% dimethylsulfoxide (solvent control), or culture media. Twenty-four hr later the cultures were rinsed, and refed with either 100 nM retinoic acid (RA) or media alone. Neither B(a)P nor RA were cytotoxic at the concentrations tested. Fresh RA was added with each biweekly media change. After 30 days the cultures were stained and scored for the appearance of morphologically transformed cell colonies. Cultures exposed to B(a)P contained 10.4% transformed colonies/ surviving cell. Cultures exposed to B(a)P followed by RA contained 0.07% transformed colonies/surviving cell. Cultures treated with RA alone, solvent control, or media control contained no transformed colonies. These results suggest that this system will be useful to study the inhibition of transformation in respiratory epithelial cells and to identify chemopreventive agents. (Work supported by NCI Contract #NO1-CN55503).

P20

APPLICATION OF WHEAT SEEDLING ASSAY FOR DETECTING CHEMICALLY INDUCED ANEUPLOIDY. Shahbeg S. Sandhu[1], J.S. Dhesi[2] and G.P. Redei[3]. [1]U.S. EPA (MD-68), RTP, NC 27711; [2]EHRT, P.O. Box 12199, RTP, NC 27709; [3]University of Missouri, Columbia, MO 65211

We have developed a simple short-term assay using Chinese spring wheat (2n=6x=42) for detecting environmentally induced clastogenicity and/or aneuploidy (Redei and Sandhu, 1988). This strain carries a pair of recessive alleles (v_1) on the short arm of chromosome 3B which in the homozygous condition and under low temperature interferes with normal chlorophyll development resulting in seedlings with cream color. In single dose this allele is ineffective in inhibiting chlorophyll development. As a result of nondisjunction, deletion, or duplication, the cell lineage with functional monosomy gives rise to green strips on virescent leaves, whereas, trisomy produces white sectors. We have challenged this assay system with two physical and seven chemical agents that are known to induce aneuploidy and/or clastogenicity in mammalian and some nonmammalian assays. The physical and chemical agents tested in the wheat seedling assay in parallel studies in two laboratories over 3 years yielded a reproducible dose-related positive response. We consider this assay to have potential utility for evaluating genotoxicity of environmental agents and for in situ monitoring the genetic hazard from industrial waste sites.

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

P21

DIRECT DNA SCREENING FOR SPONTANEOUS AND INDUCED MUTATIONS. E. Korytynski, J. Dubins, S.E. Lewis, L.B. Barnett, and R.R. Cobb. Center for Life Sciences and Toxicology, Research Triangle Institute, Research Triangle Park, NC 27711.

Studies on mutation induction in vivo have been performed by detecting both morphological and biochemical specific-locus mutations. These studies rely on phenotypic changes or alterations in protein and enzyme electrophoretic properties. With the discovery of different cloned DNA sequences, the possibility to screen DNA directly for induced or spontaneous alterations is becoming a reality. Three cloned DNA probes are currently being used in our laboratory and are being integrated into the mouse germ-line screening program. One probe is a long terminal repeat (LTR) clone of an endogenous murine retrovirus. Using this as a probe against EcoRI-digested DNA from a variety of mutants yields a complicated electrophoretic variant possessing an altered restriction fragment banding pattern when compared to normal progenitor strains. The other molecular probes are the human hypervariable "minisatellite" clones 33.15 and 33.6 (Jeffreys et al., 1985) that cross-hydridize to mouse DNA and detect multiple variable loci. The restriction enzyme HinfI is being used to investigate the induction of unique restriction fragment banding patterns induced by various mutagens.

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