GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

SECOND ANNUAL MEETING

PROGRAMS AND ABSTRACTS

October 19, 1984
Sheraton University Center
Durham, North Carolina
8:00 a.m. to 5:00 p.m.

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

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Genotoxicity and Environmental Mutagen Society

On behalf of the officers and the Board of Directors, welcome to the Second Annual Meeting of the Genotoxicity and Environmental Mutagen Society. We hope that this meeting will be beneficial to each attendee, and we encourage your full participation. As a society GEMS welcomes the participation of students, bench scientists, scientific managers, and others interested in these exciting and critical areas of toxicology. Local societies, such as GEMS, provide an additional forum for both informal and formal exchanges of information, and we hope that all members will avail themselves of this opportunity.

This year GEMS focuses its annual meeting upon the critical issue of assessing the exposure and risk to humans of enviromental genotoxicants. DR. BARBARA HULKA, chairperson of the Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, will discuss THE USE OF GENOTOXICITY ASSAYS IN EPIDEMIOLOGICAL STUDIES. DR. JOELLEN LEWTAS, chief of the Genetic Bioassay Branch, U. S. Environmental Protection Agency, Research Triangle Park, will discuss THE USE OF COMPARATIVE RISK ASSESSMENT METHODS. We thank these two outstanding scientists for their contributions to this year's meeting.

GEMS is excited that this year we will begin to give to a junior scientist a cash award for the BEST ORAL PRESENTATION. GEMS believes that local scientists should encourage and aid young scientists in the presenting of scientific information, and we look forward to this year's efforts by several of our young scientists.

SUSTAINING MEMBERS provide a variety of services to GEMS and its members, and we encourage our members to visit these exhibits and thank the representatives. This meeting would not be financially possible without their aid. I also hope that you will join with me in thanking the other officers and board members for their time and effort in planning this meeting.

As a member of GEMS, you have the opportunity to help mold the character of the society. Your suggestions, comments, and ideas are eagerly sought. Please give any suggestions to one of the officers or board members.

Again WELCOME to the Second Annual Meeting of GEMS.

Sincerely,

Larry D. Claxton

Larry Claxton, President

Post Office Box 13475, Research Triangle Park, North Carolina 27709

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PROGRAM
GEMS SECOND ANNUAL MEETING
October 19, 1984

Sheraton University Center, Durham, North Carolina

8:00 a.m. - 5:00 p.m.

8:00 a.m.-8:50 a.m. Registration, exhibit and poster set-up

8:50-9:00 a.m. Welcoming remarks. L. Claxton, President, GEMS

9:00-9:15 a.m. SISTER CHROMATID EXCHANGE [SCE] INDUCTION IN PERIPHERAL BLOOD LYMPHOCYTES [PBLs] OF PATIENTS TREATED WITH 2,5-DIAZIRIDINYL-3,6-BIS (CARBOETHOXYAMINO)-1,4-BENZOQUINONE[AZQ]OR 1,3-BIS-2-CHLOROETHYL)-3-NITROSOUREA [BCNU]. *A. D. Kligerman*,[1] G. L. Erexson,[1] J. L. Wilmer,[1] and S. C. Schold, Jr.[2] [1]CIIT, RTP, NC; [2]Duke Univ. Med. Ctr., Durham, NC

9:15-9:30 a.m. *ENHANCED ABILITY OF HUMAN BRONCHIAL EPITHELIAL CELLS TO SURVIVE IN CULTURE PRODUCED BY TREATMENT WITH NICKEL SULFATE. *D.K. Beeman*,[1] D.H. Pollard,[1] J. M. Siegfried,[1] and M. J. Mass.[2] [1]EHRT, RTP, NC; [2]US EPA, RTP, NC

9:30-9:45 EVALUATION OF THE MOUSE LYMPHOMA L5178Y FORWARD MUTATION ASSAY. *W. Caspary*,[1] D. S. Daston,[1] A. Mitchell,[2] C. Rudd,[2] B. Myhr.[3] [1]NIEHS, RTP, NC; [2]SRI Int'l., Menlo Park, CA; [3]LBI, Kensington, MD

9:45-10:00 a.m. METABOLISM AND MUTAGENICITY OF ACEANTHRYLENE. *A. Gold*,[1] R. Sangaiah,[1] L. M. Ball,[1] M. Kohan,[2] B.J. Bryant,[2] K. Rudo,[2] L. Claxton,[2] and S. Nesnow.[2] [1]UNC-CH, Chapel Hill, NC; [2]US EPA, RTP, NC

10:00-10:30 a.m. Coffee Break [cosponsored by COSTAR]

10:30-10:45 a.m. INDIVIDUAL VARIATION IN RESPONSES OF HUMAN BRONCHIAL EPITHELIAL CELLS TO CHEMICAL CARCINOGENS. *J.M. Siegfried*,[1] D.H. Pollard,[1] D. Beeman,[1] M. Mass,[2] K. Rudo,[1] and B. J. Bryant.[1] [1]EHRT, RTP, NC; [2]US EPA, RTP, NC

10:45-11:00 a.m. *STRAND BREAKS AND RECOMBINATION DURING MEIOSIS IN REPAIR MUTANTS OF YEAST. *J. Nitiss*,[1,2] and M. Resnick.[1] [1]NIEHS, RTP, NC; [2]IIT, Chicago, IL

11:00-11:15 a.m. *ATMOSPHERIC TRANSFORMATIONS OF PARTICLE BOUND WOOD SMOKE MUTAGENS. *D. A. Bell* and R. M. Kamens, UNC-CH, Chapel Hill, NC

11:15-noon THE USE OF COMPARATIVE RISK ASSESSMENT METHODS. Keynote address by *J. Lewtas*, US EPA, RTP, NC

12:00-1:30 p.m. Lunch, Membership and Financial Reports, Installation of Elected Officers

1:30-1:45 p.m. A COLLABORATIVE STUDY OF A STANDARDIZED AMES TEST PROTOCOL. *N. Adams*,[1] T. J. Hughes,[1] L. Myers,[1] and L. Claxton.[2] [1]RTI, RTP, NC; [2]US EPA, RTP, NC

1:45-2:00 p.m. *EVALUATION OF THE EFFECT OF AGAR ON PLATING EFFICIENCY IN THE L5178Y MOUSE LYMPHOMA ASSAY. *M. Meyer*,[1] K. Brock,[1] K. Lawrence,[2] and M. M. Moore.[2] [1]EHRT, RTP, NC; [2]US EPA, RTP, NC

2:00-2:15 p.m. *TEDLAR® BAGS AS A TECHNIQUE TO MEASURE MUTAGENIC POTENCY OF VAPOR-PHASE COMPOUNDS. *D. M. Simmons*,[1] L. G. Monteith,[1] S. E. Frazier,[1] T. J. Hughes,[1] and L. Claxton.[2] [1]RTI, RTP, NC; [2]US EPA, RTP, NC

2:15-2:30 p.m. DOMINANT VISIBLE AND ELECTROPHORETIC VARIANTS AMONG PROGENY OF MALE MICE TREATED WITH ETHYLENE OXIDE BY INHALATION. *S. E. Lewis*,[1] C. Felton,[1] L. B. Barnett,[1] L. C. Skow,[2] F. M. Johnson,[2] and M. D. Shelby.[2] [1]RTI, RTP, NC; [2]NIEHS, RTP, NC

2:30-3:30 p.m. USE OF GENOTOXICITY ASSAYS IN EPIDEMIOLOGICAL STUDIES, Keynote Address by B. Hulka, UNC-CH, Chapel Hill, NC

3:30-5:00 p.m. Poster sessions, Vendor Exhibits, and Social

5:00 p.m. Meeting Adjourns

*Eligible for Best Oral Presentation Award

Poster Group A

Mechanistic Studies

A1 STATISTICAL ANALYSIS OF MUTATIONAL SPECTRA. *W. T. Adams* and T. R. Skopek, CIIT, RTP, NC

A2 ACROLEIN SCAVENGING BY 2-MERCAPTOETHANESULFONIC ACID (MESNA) DURING CYCLOPHOSPHAMIDE (CP) METABOLISM LOWERS THE SISTER CHROMATID EXCHANGE FREQUENCY IN CULTURED HUMAN LYMPHOCYTES. *J. L. Wilmer*, G. L. Erexson, and A. D. Kligerman, CIIT, RTP, NC

A3 EARLY PREMALIGNANT CHANGES IN PRIMARY TRACHEAL EPITHELIAL CELL CULTURES EXPOSED TO DIFFERENT CLASSES OF CARCINOGENS. *V. E. Steele*, J. T. Arnold, and F. R. Nelson, Northrop Services, RTP, NC

A4 STRUCTURE ACTIVITY RELATIONSHIPS FOR A SET OF NITROGEN CONTAINING CYCLIC COMPOUNDS TESTED IN SALMONELLA ASSAYS FOR MUTAGENICITY. *D. Walsh*,[1] T. K. Rao,[1] M. Kohan,[2] and L. Claxton.[2] [1]EHRT, RTP, NC; [2]US EPA, RTP, NC

A5 THE EFFECT OF HYDROXYLATION UPON THE BACTERIAL MUTAGENICITY OF 1-NITROPYRENE. *M. Kohan*,[1] L. Claxton,[1] and L. Ball[2] [1]US EPA, RTP, NC; [2]UNC-CH, Chapel Hill, NC

A6 INDUCTION OF METALLOTHIONEIN IN CHINESE HAMSTER OVARY CELLS. *C. F. Kuo*, S. Curtis, and K. S. McCarty, Duke Univ. Med. Ctr., Durham, NC

Poster Group B

Methodology

B1 MUTAGENS IN HUMAN URINE: A REVIEW OF MEDICAL, LIFESTYLE, AND OCCUPATIONAL EXPOSURES ASSOCIATED WITH HIGH LEVELS OF MUTAGEN EXCRETION AND EVIDENCE FOR LOW LEVELS OF MUTAGEN EXCRETION BY A LARGE PROPORTION OF NON-OCCUPATIONALLY EXPOSED NONSMOKERS. *R. B. Everson*, NIEHS, RTP, NC

B2 COMPARISON OF MASS AND MUTAGENICITY RECOVERY OF DIESEL EXTRACT SAMPLE PREPARATION TECHNIQUES. *R. Williams*,[1] E. Perry,[1] and J. Lewtas.[2] [1]EHRT, RTP, NC; [2]US EPA, RTP, NC

B3 THE EVALUATION OF CHEMICAL AND BIOLOGICAL METHODS FOR THE IDENTIFICATION OF MUTAGENIC AND CYTOTOXIC HAZARDOUS WASTE SAMPLES. *B. Andon*,[1] M. A. Jackson,[1] and V. Houk,[2] and L. Claxton.[3] [1]EHRT, RTP, NC; [2]UNC-CH, Chapel Hill, NC; [3]US EPA, RTP, NC

B4 A RADIOLABELED PROCEDURE FOR DIFFERENTIATING CHO HGPRT$^-$ CELLS FROM HGPRT$^+$ CELLS. *M. A. Jackson* and N. E. Garrett, EHRT, RTP, NC

B5 EFFECTS OF PRETREATMENT WITH INDUCERS OF LIVER MONOOXYGENASE ACTIVITY ON PRIMARY RAT HEPATOCYTE DNA REPAIR INDUCED BY GENOTOXIC CHEMICALS. D. J. Kornbrust,[1] M. E. Hall,[2] and *D. D. Dietz*.[2] [1]NIEHS, RTP, NC; [2]RTI, RTP, NC

Poster Group C

Mutagenicity and Testing

C1 INDUCTION OF SISTER CHROMATID EXCHANGES AND MICRONUCLEI IN MALE DBA/2 MICE AFTER INHALATION OF BENZENE. *G. L. Erexson*, J. L. Wilmer, C. D. Auman, and A. D. Kligerman, CIIT, RTP, NC

C2 MUTAGENICITY OF TOBACCO AND MARIJUANA SMOKE CONDENSATES. *T. J. Hughes*, D. M. Simmons, L. G. Monteith, B. M. Sadler, K. H. Davis, S. E. Frazier, and C. M. Sparacino, RTI, RTP, NC

C3 MUTAGENICITY OF LICHEN CONSTITUENTS. *C. I. Wei*[1] and T. Shibamoto.[2] [1]U Fla., Gainesville; [2]U Calif., Davis

C4 EVALUATION OF THREE "DIFFICULT-TO-TEST" CARCINOGENS IN THE CHINESE HAMSTER V-79 INHIBITION OF METABOLIC COOPERATION ASSAY. *E. Elmore*, E. A. Korytynski, and M. P. Smith, Northrop Services, RTP, NC

C5 DIFFERENTIAL CYTOTOXIC RESPONSE OF XERODERMA PIGMENTOSUM FIBROBLASTS AND NORMAL HUMAN FIBROBLAST TO VARIOUS PROMUTAGENS AFTER ACTIVATION BY CO-CULTIVATED RAT HEPATOCYTES. *L. L. Yang*, R. A. Lubet, P. M. Conklin, J. W. Cameron, and R. D. Curren, Microbiological Associates, Bethesda, MD

ABSTRACTS

T1 SISTER CHROMATID EXCHANGE [SCE] INDUCTION IN PERIPHERAL BLOOD LYMPHOCYTES [PBLs] OF PATIENTS TREATED WITH 2,5-DIAZIRIDINYL-3,6-BIS(CARBOETHOXYAMINO)-1,4-BENZOQUINONE [AZQ] OR 1,3-BIS-(2-CHLOROETHYL)-3-NITROSOUREA [BCNU]. *A. D. Kligerman*,[1] G. L. Erexson,[1] and S. C. Schold, Jr.,[2] [1]CIIT, RTP, NC 27709; [2]Dept. Medicine, Duke Univ. Med. Ctr., Durham, NC 27710.

At Duke University Medical Center, patients with brain tumors (astrocytomas and glioblastomas) are routinely given approximately 6000 rads whole brain radiation treatment, followed by monthly administration of BCNU. AZQ, a recently developed experimental chemotherapeutic agent, is being tested in clinical trials to determine its efficacy against brain tumors compared to the standard BCNU treatment. The existence of this group of patients on single drug chemotherapy allows us to evaluate the effects of each agent on SCE induction *in vivo*. Eight weeks following the final radiation treatment, patients were randomized into two groups: 1) 200 mg BCNU/M^2, i.v., every 2 months; 2) 3 × 15 mg AZQ/M^2, i.v., every 2 months. Blood (5 to 10 ml) was taken by venipuncture before treatment, within 10 hr after treatment, and two months following treatment. The mononuclear lymphocytes were isolated on a Ficoll-Paque density gradient, and the PBLs were cultured with concanavalin A for 72 hr in the presence of 5 μM 5-bromo-2′-deoxyuridine for the final 48 hr for SCE analysis [Wilmer et al. (1983)]. Eight weeks after irradiation but before chemotherapy, patients' PBLs had about 10 SCEs/metaphase. The baseline SCE frequency was increased 3- to 5-fold or 2.5- to 3-fold, respectively, following treatment with either BCNU or AZQ. Two months after BCNU treatment, there was less than a 25% reduction in SCE frequency apparently due to the release of new undamaged lymphocytes into the peripheral blood. These data show that cytogenetic damage leading to SCEs in human PBLs is relatively long-lived. Furthermore, on a mg/M^2 basis, AZQ is a much more potent SCE-inducer than BCNU.

T2 ENHANCED ABILITY OF HUMAN BRONCHIAL EPITHELIAL CELLS TO SURVIVE IN CULTURE PRODUCED BY TREATMENT WITH NICKEL SULFATE. *D. K. Beeman*,[1] D. H. Pollard,[1] J. M. Siegfried,[1] and M. J. Mass.[2] [1]EHRT, RTP, NC 27711; [2]US EPA, RTP, NC 27711.

Effects of nickel sulfate on adult human bronchial epithelial cells were evaluated by exposure of bronchial tissue fragments to the metal in organ culture. Bronchial tissue from two resection patients and one autopsy case was used for exposures in organ culture. One resection case was for a nontumorous condition, and the other case was an osteosarcoma metastatic to the lung. Four weekly 24-hour exposures to 0, 1.0, or 10 μg/ml $NiSO_4$ were performed, after which tissue was placed in explant culture. Outgrowths from explants were evaluated for morphologic alterations and survival in culture during subsequent subculturing. Cells from outgrowths were also tested for ability to form colonies in the presence of fetal bovine serum, which induces squamous differentiation in normal adult bronchial epithelial cells. Exfoliated cells from explant cultures were collected onto slides and stained by the Papanicolaou method, to assess for morphological abnormalities. Toxicity of $NiSO_4$ was also assessed by performing colony-forming assays on inactivated 3T3 cells. Variation in

toxicity among different individuals was observed. Enhanced growth formation was observed after nickel sulfate treatment, and cells from these outgrowths survived for longer periods in culture in comparison to controls. Nickel sulfate-treated tissue fragments produced cells capable of forming colonies in 10% fetal bovine serum, while control cultures did not. A correlation was found between cultures capable of growth in serum and cultures with increased ability to survive after subculturing. Alterations suggestive of atypical metaplasia were observed in exfoliated cells from some nickel sulfate-treated explants, while control cultures produced exfoliated cells resembling regular metaplasia.

T3 EVALUATION OF THE MOUSE LYMPHOMA L5178Y FORWARD MUTATION ASSAY. *W. Caspary*,[1] D. S. Daston,[1] A. Mitchell,[2] C. Rudd,[2] B. Myhr.[3] [1]NIEHS, RTP, NC 27709; [2]SRI, Menlo Park, CA; [3]Litton Bionetics, Kensington, MD.

LBI and SRI followed a common protocol in testing 63 coded compounds in the mouse lymphoma (ML) assay. Each test consisted of replicate trials in the presence or absence of liver S9 from aroclor-induced Fischer 344 rats. Each trial consisted of 6 doses each in duplicate or triplicate, 4 solvent controls (SC) and 3 positive controls. Quality control (QC) criteria had to be met before an experiment was evaluated for a response. To formulate these criteria, we examined histograms of the SC mutant frequencies (MF) from over 800 trials. We also analyzed the effects of the toxicity parameters measured in this assay on the variability of the MF. Plots of the coefficient of variation (CV) of the MF against these toxicity parameters generally showed a flat response until high toxicities were attained, where sharp increases in the CV of the MF were observed. The toxicities at which these sharp increases were observed were used to generate the QC criteria. Response categories for trials were defined on the basis of the significance of the trend and on the significance of one of the highest three doses. If a positive response occurred only after precipitation, the response was considered negative and footnoted. On the test level, a positive trial had to be reproduced for the test to be considered positive. The test results between the two laboratories agreed in 90% of the tests. Many disagreements appeared to be caused by differences in the dose at which precipitation was observed. An analysis of histograms showed that SC MFs were slightly higher in one of the laboratories but that the positive controls were superimposable. The addition of S9 increased the median MF of the SCs about 1.6-fold in both laboratories. Histograms of the CEs of the SCs were also very similar in the two laboratories. A comparison of mutagenicity results from the ML assay with that from other systems suggests that this assay provides a unique profile of the mutagenic activity of chemicals that is not duplicated in other assays.

T4 METABOLISM AND MUTAGENICITY OF ACEANTHRYLENE. *A. Gold*,[1] R. Sangaiah,[1] L. M. Ball,[1] M. Kohan,[2] B. J. Bryant,[2] K. Rudo,[2] L. Claxton[2] and S. Nesnow.[2] [1]UNC-CH Chapel Hill, NC 27514; [2]US EPA, RTP, NC 27709.

Structure-activity relationships governing the mutagenicity of cyclopenta-fused PAH have been extended to aceanthrylene. Biological activity is predicted despite low molecular weight (202) and a four-ring structure lacking sterically encumbered peripheral indentations. The basis for the predicted activity is the large resonance energy $\Delta E_{deloc}/\beta = 0.931$) of the carbonium ion derived from the expected cyclopenta-epoxide metabolite. Aceanthrylene

proved to be an active mutagen in the Ames assay with S9 activation (35 rev/nmole, TA100). The profile of activity over tester strains TA98, TA100, TA1538, TA1537, and TA1535 indicated that aceanthrylene is a frame-shift mutagen like other PAH. The protein dependence of optimum mutagenic response in TA98 is similar to that observed for cyclopenta (cd) pyrene and the benzaceanthrylene isomers, which are thought to be activated by expoxidation of the cyclopenta ring. The major metabolites are four dihydrodiols, which have been isolated and characterized. The predominant metabolite is the cyclopenta dihydrodiol, presumably arising from the corresponding epoxide. The three remaining diols result from oxidation of the accessible positions on the terminal benzo rings. This profile is consistent with the protein-dependence of mutagenicity and with the hypothesis that the cyclopenta ring is primarily responsible for activity.

T5 INDIVIDUAL VARIATION IN RESPONSES OF HUMAN BRONCHIAL EPITHELIAL CELLS TO CHEMICAL CARCINOGENS. *J. M. Siegfried*,[1] D. H. Pollard,[1] D. Beeman,[1] M. Mass,[2] K. Rudo,[1] and B. J. Bryant.[1] [1]EHRT, RTP, NC 27711; [2]US EPA, RTP, NC 27711.

Human bronchial epithelial cells have been obtained from explant culture of tissue fragments from over 30 individuals. Epithelial cells have been repeatedly obtained from the same tissue piece for up to 18 months. These cells display epithelial characteristics as demonstrated by presence of desmosomes and intermediate filaments by electron microscopy, and histochemical markers such as binding of antibody to keratin, presence of PAS-Alcian Blue positive material, and presence of enzymes found in frozen sections of bronchial epithelium. A predominantly diploid chromosome number was found in bronchial cultures. Cytotoxicity of N-methyl-N′-nitro-N-nitrosoguanidine, benzo(a)pyrene (BP), nickel sulfate, 7,12-dimethylbenz(a)anthracene, and potassium chromate was measured in a colony-forming assay. Conditions for the assay were optimized by monitoring colony-forming efficiency. Variation was found in sensitivity to agents requiring activation but not to direct-acting agents. Metabolic profiles of BP were obtained from 10 individuals. Percent BP metabolized ranged from 4.2–26.9; phenols and quinones made up the principal metabolites. These studies serve as an indicator of the potential of chemical agents to induce genotoxic damage in human cells.

T6 STRAND BREAKS AND RECOMBINATION DURING MEIOSIS IN REPAIR MUTANTS OF YEAST. *J. Nitiss*,[1,2] and M. Resnick.[1] [1]NIEHS, RTP, NC 27709; [2]IIT, Chicago, IL 60616.

The *RAD52* gene of *Saccharomyces cerevisiae* is important in the repair of DNA damage due to ionizing radiation and alkylating agents and is essential in ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS) induced mutagenesis and proper chromosomal disjunction. Mutants in this gene are deficient in both damage-induced and spontaneous mitotic and meiotic recombination. Though *rad52* strains are able to undergo meiosis and sporulate, the ascospores produced are inviable. At the molecular level, mitotic *rad52* cells are unable to repair DNA double-strand breaks due, presumably, to the lack of some step in recombination.

To further understand the importance of the *RAD52* gene in normal meiotic recombination processes, we have used strains that sporulate efficiently and synchronously to determine the extent and nature of residual recombination in *rad52* mutants. Recombination during meiosis in *rad52* mutants is considerably reduced compared to wild-type strains; however, there is a substantial increase relative to the mitotic controls when meiosis is interrupted prior to the first meiotic division. The recombination is not due to leakiness or allele specific effects. It appears that the recombination is not completed in meiosis but instead relies on a mitotic system acting in cells that have initiated events during meiosis.

Strains carrying the *rad52* mutation accumulate single strand breaks during meiosis; no double strand breaks are detected.[1] Based on their frequency, timing, and nonrandom distribution, they appear to be involved in the recombination that is initiated in *rad52* cells during meiosis. Since recombinants can be recovered, we propose that meiotic recombinational events need not be mediated by double-strand breaks and that the single-strand breaks are part of unique recombinational structures that do not require the *RAD52* gene product for resolution.

[1]Resnick, Chow, Nitiss, and Game, Cold Spring Harbor Symp. Quant. Biol. 1984, in press.

T7 ATMOSPHERIC TRANSFORMATIONS OF PARTICLE BOUND WOOD SMOKE MUTAGENS. *D. A. Bell* and R. M. Kamens, UNC-CH, Chapel Hill, NC 27514.

Residential wood heating has become a significant source of atmospheric pollution. However, little is known about how atmospheric processes affect the mutagens found on wood smoke particles. Dilute wood smoke from a residential wood stove was added to 25 m^3 outdoor Teflon chambers and allowed to age under ambient light conditions, with or without combustion generated NO_x, or in the presence of additional NO_2 and O_3. Inorganic gas concentrations, PAH concentrations, and the mutagenicity of particle extracts were determined in relation to residence time in the chamber.

The addition of sub ppm concentrations of NO_2 and O_3 produced 2- to 10-fold increases in TA 98, −S9 mutagenicity. HPLC fractionation indicated that the NO_2 + O_3 reaction decreased the mutagenic contribution of the PAH fraction while increasing the contribution of the nitro-PAH fraction. Wood smoke aged in bright sunlight showed significant decay in indirect mutagenicity, and concentrations of PAH were also observed to decline under these conditions. These results suggest that wood smoke particles emitted into a polluted atmosphere may become more genotoxic under clear sunlight conditions. Photodegradation of indirect-acting mutagens appears to decrease the genotoxicity of wood smoke particles.

T8 A COLLABORATIVE STUDY OF A STANDARDIZED AMES TEST PROTOCOL. *N. Adams*,[1] T. J. Hughes,[1] L. Myers,[1] and L. Claxton.[2] [1]RTI, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

Seven laboratories participated in a collaborative study to evaluate a proposed EPA standard protocol for the Ames Test. The study used TA98 and TA100 and three S9 levels (0, 2, and 10%). Six pure chemicals and two complex mixtures were tested as coded unknowns. The

three primary study goals were to evaluate the protocol, to determine if this protocol produced qualitative results that agreed with published qualitative results, and to determine the qualitative and quantitative reproducibility of results. Regarding the first goal, most of the participants agreed that this was a technically acceptable although labor-intensive protocol. The second and third goals of the study are interrelated. Ability to obtain qualitative results that agreed with published data was less than that reported in an earlier study by de Serres and Ashby (1981) in which each laboratory used its own protocol. Comparing the study results with the consensus outcome for each pure chemical, the EPA protocol had a disagreement rate of 15.3%, whereas the de Serres study disagreement rate was 5.6%. Retesting of equivocal results was not performed, and this might have reduced the error rate. The conclusion from analysis of the quantitative data from this interlaboratory Ames study was that both intralaboratory and interlaboratory variation were substantial. Results for the same substance varied by an order of magnitude or more when the mutagenic response was measured as a function of dose-response slope. Based on the assumption that logarithms of slopes are normally distributed with constant within-laboratory variance, one chemical must be about ten times as mutagenic as another (i.e., ratio of the slopes = 10) to have only an even chance finding a statistically significant difference at the 5% level. Taking interlaboratory variance into account, an even chance of finding a statistically significant difference ($\alpha = .05$) required a 25-fold difference in mutagenicity.

T9 EVALUATION OF THE EFFECT OF AGAR ON PLATING EFFICIENCY IN THE L5178Y MOUSE LYMPHOMA ASSAY.* *M. Meyer*,[1] K. Brock,[1] K. Lawrence,[2] and M. M. Moore.[2] [1]EHRT, RTP, NC 27710; [2]US EPA, RTP, NC 27711.

The L5178Y TK$^{+/-}$ Mouse Lymphoma Assay is widely utilized in mutagenicity testing. Trifluorothymidine-resistant (TFT^r) mutants are quantitated following growth in agar supplemented cloning medium. In an attempt to evaluate the effect of agar on plating efficiency, we tested several lots of Difco Noble agar (cat. no 0142-01-8; normally utilized in this assay) and compared it with Baltimore Biological Laboratory (BBL) agar (cat. no. 11849). We find that BBL agar gives a higher and less variable plating efficiency than any of the Noble lots tested. Colonies plated in BBL agar tend to appear significantly earlier on the plates than those cloned in Noble agar. The induced mutant frequency quantitated from a treated culture is generally higher in BBL compared to Noble agar. To determine if this higher frequency is due to increased mutant recovery rather than sneak through of nonmutant cells, we isolated 97 mutants from treated cultures (44 large colonies and 53 small colonies) and 69 mutants from untreated cultures (24 large colonies and 45 small colonies) and tested them for TFT-resistance. All but one (a large colony from an untreated culture) were found to be TFT^r. The spontaneous mutant frequency has been quantitated for 122 untreated cultures. Showing little variation within and between experiments, the frequency yielded a mean of 57.7, with a standard deviation of 14.4. We have found BBL agar to be more reliable and thus better for use in cloning L5178Y mouse lymphoma cells.

*This is an abstract of a paper presentation and does not necessarily reflect EPA policy. Any mention of trade names does not constitute an endorsement.

T10 TEDLAR® BAGS AS A TECHNIQUE TO MEASURE MUTAGENIC POTENCY OF VAPOR-PHASE COMPOUNDS. *L. G. Monteith*,[1] D. M. Simmons,[1] S. E. Frazier,[1] T. Hughes,[1] and L. Claxton.[2] [1]RTI, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

The overall objective of this research was to develop an Ames/Salmonella technique for detection of mutagenic vapors in ambient air. A technique to overcome the problem of the dilution factor in ambient air mutagenicity testing by keeping vapors in closer contact with the bacteria was evaluated. Resealable 500 ml Tedlar® plastic bags with a GC septum were used to test ethylene oxide and propylene oxide in the Ames/Salmonella assay. These results were compared to results with the preincubation technique. Three methods of dosing the bacteria were employed: 1) dilution of liquid chemical with solvent and preincubation of diluted chemical and bacteria for 10 min with rotating at 37°C before plating; 2) bacterial mix without chemical plated, plates sealed into bags and undiluted liquid chemical introduced through septum; 3) bacterial mix without chemical plated, and plates sealed into bags. Chemical as a gas at STP was placed in a glass gas bulb and gas withdrawn and introduced through septum in bag. All three methods used TA100, with and without Aroclor-induced liver S9 and incubation of plates at 37°C for 48 hr. Results suggested that for these vapor phase mutagens the Tedlar® gas bag technique was more effective in detecting mutagenic activity than the liquid preincubation technique. For ethylene oxide at 2000 μg/plate, there were twice as many revertants with the Tedlar® bag technique when comparison was made with preincubation at the same dose.

Research sponsored by EPA Contract No. 68-02-3170-097.

T11 DOMINANT VISIBLE AND ELECTROPHORETIC VARIANTS AMONG PROGENY OF MALE MICE TREATED WITH ETHYLENE OXIDE BY INHALATION. *S. E. Lewis*,[1] C. Felton,[1] L. B. Barnett,[1] L. C. Skow,[2] F. M. Johnson,[2] and M. D. Shelby.[2] [1]RTI, RP, NC 27709; [2]NIEHS, RTP, NC 27709.

Male DBA/2J mice were treated by inhalation with 200 ppm ethylene oxide (EtO) for periods up to 6 months. Males were mated during the exposure period to untreated C57Bl/6J females. A total of 1,986 progeny were recovered from the treated group in two experiments. All F_1 progeny were examined for morphological abnormalities. Blood and kidney samples were screened for electrophoretic variation at 32 loci. Two coat color mutants were recovered that were shown in heritability tests to be due to dominant mutations. A runted blind animal with various skeletal defects did not breed. However, in collaboration with Dr. Walderico Generoso and Nestor Cachiero of the Oak Ridge National Laboratory, the variant mouse was shown to carry a 12/18 translocation. Two other morphological variants have been mated to determine heritability. Four electrophoretic variant mice are also currently undergoing heritability testing. No dominant mutations or variants with a possible origin in the parental generation have been found among 579 progeny from males mated following the exposure period. No variants, morphological or electrophoretic, were obtained from 1461 progeny of control males.

Supported by Contract #N01-ES-2-5012 from the National Toxicology Program.

A1 STATISTICAL ANALYSIS OF MUTATIONAL SPECTRA. *W. T. Adams* and T. R. Skopek, CIIT, RT, NC 27709.

Complex and detailed information on the specific types of mutations occurring in *E. coli* have become available via the characterization of nonsense mutations in the *lacI* gene (Coulondre and Miller, *J. Mol. Biol. 117*:577–606) and by direct sequence analysis of the *cI* gene (Skopek and Hutchinson, *J. Mol. Biol. 159*:19–33). A sample of mutants induced by a mutagenic agent, when analyzed to determine the sites and types of modifications to the DNA sequence, yields information on the probability of each type of mutation at each potential site. Comparison of spectral samples generated under different conditions can provide insight into the specificity and mechanism of induced mutations. We present a statistical test for the hypothesis that two or more spectral samples represent mutations drawn from the same population. Monte Carlo methods (Agresti et al., *Psychometrika 44*:75–83) are used to estimate the P-value of the conditional test described by Freeman and Halton (*Biometrika 38*:141–149).

A2 ACROLEIN SCAVENGING BY 2-MERCAPTOETHANESULFONIC ACID (MESNA) DURING CYCLOPHOSPHAMIDE (CP) METABOLISM LOWERS THE SISTER CHROMATID EXCHANGE (SCE) FREQUENCY IN CULTURED HUMAN LYMPHOCYTES. *J. L. Wilmer,* G. L. Erexson, and A. D. Kligerman, CIIT, RTP, NC 27709.

CP is metabolized to the reactive intermediates, phosphoramide mustard (PAM) and acrolein (AC). PAM is a potent bifunctional alkylating agent and inducer of SCE. AC binds to nucleophiles primarily through addition reactions and has been reported to be comparable to PAM in the induction of SCE. MESNA has been used to alleviate hemorrhagic cystitis caused by CP chemotherapy, exhibited anticarcinogenic effects in rats exposed to CP during a longterm bioassay, and acts presumably by binding selectively to AC. The aim of this study was to assess the efficacy of MESNA in lowering the SCE frequency of human lymphocytes cultured in the presence of CP and to estimate the contribution of AC to the genotoxicity of CP. The ability of MESNA to attenuate the genotoxicity of AC was also determined. Mononuclear leukocytes were separated on a Ficoll-Hypaque density gradient and cultured in medium composed of RPMI 1640, 10% fetal bovine serum, 1% antibiotics, 2 mM L-glutamine, and 4 μg concanavalin A/ml. MESNA (1, 5, or 10 mM), CP (0.5, 1, or 2 mM), and bromodeoxyuridine (5 μM) were added at 24 hr. Cultures were harvested at 72 hr after a 4 hr exposure to 1.35 μM demecolcine. The SCE frequency of triplicate cultures at each treatment were as follows: control, 8.5 $\pm$ 0.5; 10 mM MESNA, 8.5 $\pm$ 0.6; 2 mM CP, 25.6 $\pm$ 1.4; and 2 mM CP plus 10 mM MESNA, 18.0 $\pm$ 1.4. Similar decreases in the CP-induced SCE frequency were observed at lower MESNA and CP concentrations. AC (0.001-40.0 μM) induced significant concentration-related increases in SCE of up to 1.6 times control frequency at 20 μM. 1 mM MESNA protected completely against SCE induction, inhibition of cell cycle progression, and depressed cell proliferation by AC concentrations up to 40 μM. These results are consistent with the clinical findings that MESNA scavenges AC and suggest that SCE induction and cytotoxicity may be critical events in AC toxicity in the whole animal.

A3 EARLY PREMALIGNANT CHANGES IN PRIMARY TRACHEAL EPITHELIAL CELL CULTURES EXPOSED TO DIFFERENT CLASSES OF CARCINOGENS. *V. E. Steele*, J. T. Arnold, and F. R. Nelson, Northrop Services, RTP, NC 27709.

The aim of these studies was to quantitate the various types of early premalignant changes that are observed in primary cultures of respiratory tract epithelial cells shortly after chemical treatment. Rat tracheal cells were exposed to various classes of carcinogens/noncarcinogens. Primary cultures of rat tracheal epithelial cells were prepared by plating 2 x 10^4 viable cells per 60 mm dish on a collagen film. The cultures were exposed beginning on day 1 of culture for either 24 or 120 hr to N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 4-nitroquinoline-N oxide (4NQO), benzo(a)pyrene (BaP), benzo(e)pyrene (BeP), cigarette smoke condensate (CSC), sodium chromate (Cr), or 4,4-methylene bis(2-chloroaniline)(MOCA). All cultures were fixed either on day 5 or 30 and scored under a dissecting microscope. For MNNG, 4NQO, CSC, Cr, and MOCA the colony formation at day 5 decreased as a function of dose. BaP and BeP at concentrations up to 30 μg/ml did not significantly lower the colony forming efficiency compared to controls. At day 30 epithelial foci were scored as Class I, II, or III, having 0 ± 100, 100 ± 400, or >400 cells/mm^2, respectively. Cultures exposed to BaP produced all three classes of foci in a dose-dependent manner and had the highest percentage of plates with Class III foci of any compound tested. MNNG and Cr produced a 3- to 5-fold increase in foci formation. BeP, 4NQO, CSC, and MOCA exposure produced less than a two-fold effect on foci production. The results suggest that the rat tracheal focus assay might provide a rapid quantitative assay for potential respiratory tract carcinogens.

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A4 STRUCTURE ACTIVITY RELATIONSHIPS FOR A SET OF NITROGEN CONTAINING CYCLIC COMPOUNDS TESTED IN SALMONELLA ASSAYS FOR MUTAGENICITY. *D. Walsh*,[1] T. K. Rao,[1] M. Kohan,[2] and L. Claxton.[2] [1]EHRT, RTP, NC 27711; [2]US EPA, RTP, NC 27711.

A computerized SAR system (ADAPT) was utilized to study the relationship between mutagenicity and molecular structure for two separate data sets of nitrogenous cyclic compounds. The mutagenic activity of the compounds were determined by testing in the five frequently used *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538. A compound was considered mutagenic if there was a positive response in any tester strain either with or without metabolic activation. The criteria for a compound to be classified as nonmutagenic was a negative response in all of the strains both with and without metabolic activation.

A data set of 36 nitrogenous cyclic compounds (20 mutagens, 16 nonmutagens) was correctly classified by three molecular structure descriptors. Then, using a holdcut technique, 20 training and prediction subsets were randomly generated from the linearly separable data set. The internal consistency of the data was evaluated by this holdout method, and the average correct predictive ability was 93%. The weight vectors generated by the three descriptors were used to predict the activity of 12 compounds not included in the original analysis. All 12 compounds were predicted correctly by the ADAPT system. The second data

set was considerably larger. A group of 114 nitrogenous cyclic compounds (64 mutagens, 50 nonmutagens) was completely separated by 17 molecular descriptors. Utilizing the same holdout technique, 60 training and prediction subsets were randomly generated and the average correct predictive ability was 91.2%. This work demonstrated the practical utility of a computerized structure activity program for evaluating compounds for potential genotoxicity.

This work was performed under Contract No. 68-02-4031 (Environmental Health Research and Testing, Inc.) with the U.S. Environmental Protection Agency. This abstract does not necessarily reflect EPA policy.

A5 THE EFFECT OF HYDROXYLATION UPON THE BACTERIAL MUTAGENICITY OF 1-NITROPYRENE. *M. Kohan*,[1] L. Claxton[1] and L. Ball.[2] [1]US EPA, RTP, NC 27711; [2]UNC-CH, Chapel Hill, NC 27514.

Recent studies have shown that the bacterial mutagen, 1-nitropyrene, is metabolized in the rat. While isolating the principal metabolites of 1-nitropyrene in rat urine, Ball et al. identified several monohydroxylated derivatives. Seven monohydroxylated-1-nitropyrenes were assayed for mutagenic activity in the Ames plate incorporation assay. The seven phenolic 1-nitropyrenes (3-hydroxy-1-nitropyrene, 4-hydroxy-1-nitropyrene, 5-hydroxy-1-nitropyrene, 6-hydroxy-1-nitropyrene, 8-hydroxy-1-nitropyrene, 9-hydroxy-1-nitropyrene, 10-hydroxy-1-nitropyrene) were synthesized at Midwest Research Institute and were separated and quantified by HPLC in our laboratory. Except for the 3- 6- and 8-monohydroxylated-1-nitropyrenes, the mutagenic activity of the hydroxylated derivatives was greater than 1-nitropyrene when tested without activation using TA98. With activation only the 3- and 8-hydroxy-1-nitropyrene had less mutagenic activity than 1-nitropyrene. It appears that hydroxylation generally increases the mutagenicity of 1-nitropyrene, and the position of the hydroxyl group plays an important part in determining the mutagenic potency.

A6 INDUCTION OF METALLOTHIONEIN IN CHINESE HAMSTER OVARY CELLS. *C. F. Kuo*, S. Curtis, and K. S. McCarty, Duke Univ. Med. Ctr., Durham, NC 27710.

A cadmium resistant clone CHO-R40F cell was derived from wild type CHO-K1 cells by stepwise selection in increasing concentrations of cadmium. Characterization of this 200μM cadmium-resistant R40F clone revealed a 200-fold increase in metallothionein production, a 500-fold increase in mRNA levels, and a 40-fold amplification of the genes. In collaboration with Dr. J. Biedler, *in situ* hybridization studies utilizing tritiated mouse metallothionein-I cDNA probe obtained from Dr. Richard Palmiter localized the metallothionein gene on chromosome *X*. This region of the chromosome *X* is characterized as a homogeneous staining region. Since metallothionein genes have been assigned to chromosome *III*, (Dr. Brian Crawford) it is suggested that the amplified gene in the CHO-R40F cell represents a translocation. Although two genes for metallothionein are present in the Chinese hamster ovary cells (metallothionein I and II), only the native gene responds to both glucocorticoid and divalent cations, whereas the amplified gene fails to respond to glucocorticoid. We are developing techniques to select for glucocorticoid responsive genes for metallothionein.

This project is supported in part by NIH 1 R01 ES03203-01.

B1 MUTAGENS IN HUMAN URINE: A REVIEW OF MEDICAL, LIFESTYLE, AND OCCUPATIONAL EXPOSURES ASSOCIATED WITH HIGH LEVELS OF MUTAGEN EXCRETION AND EVIDENCE FOR LOW LEVELS OF MUTAGEN EXCRETION BY A LARGE PROPORTION OF NONOCCUPATIONALLY EXPOSED NONSMOKERS. *R.B. Everson*, NIEHS, RTP, NC 27709.

A review of literature on mutagenesis studies of human urine will be presented. These studies reported increased mutagenicity in body fluids of individuals exposed to lifestyle and dietary factors, including smoking and ingestion of cured meat products; medications, including metronidazole, nitrofurantoin, and several agents used for cancer chemotherapy; and workplace exposures, including exposure to drugs used for cancer chemotherapy, coke oven and foundry work, and several other chemical manufacturing processes. In addition data from several studies suggest positive findings for specimens from nonsmoking, nonoccupationally-exposed control subjects. We investigated whether these small increases might be attributable to an artifact involving promotion of the growth of histidine-requiring bacteria used to indicate mutagenesis by employing a previously reported simple method for quantitating growth of the test bacteria on plates used for mutagenesis studies (Cancer 51:371–377, 1983). This method was applied to extracts of urine from 17 subjects being tested for mutagenicity by a modification of the procedure described by Yamasaki et al. (Proc. Natl. Acad. Sci. 74:3555–3559, 1977). The mean and standard error for bacterial revertant counts was 64 ± 4.4 using *Salmonella typhimurium* tester strain TA98. This was significantly increased over values for assays of solvent controls which averaged 35 ± 1.2 ($p < 0.0001$ by analysis for variance of log transformed data, controlling for experiment). There was no evidence of increased growth of test bacteria: ratios of bacterial lawn growth on test divided by control plates averaged 1.1 ± 0.04, and growth on test and control plates did not differ significantly by analysis of variance ($p = 0.16$). Assaying distilled water instead of urine suggested no comparable increase in revertants. Data from other studies with similar trends will be presented.

B2 COMPARISON OF MASS AND MUTAGENICITY RECOVERY OF DIESEL EXTRACT SAMPLE PREPARATION TECHNIQUES. *R. Williams*,[1] E. Perry,[1] and J. Lewtas.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

Particulate samples from diesel exhaust were used to compare several sample preparation techniques for the bioassay. Sample extraction, concentration and solvent exchange procedures that might have a bearing on the recovery of mutagenic activity in the *Salmonella* histidine reversion assay (Ames et al. 1975) were compared. Techniques were also investigated for a reproducible recovery of the extracts. Of several solvent systems utilized (methylene chloride, acetone, cyclohexane, isopropanol, acetonitrile and dimethylsulfoxide), methylene chloride was found to yield maximum mutagenic activity. For large-volume extract concentration, the differences between rotary evaporation and Kuderna-Danish techniques were statistically insignificant. Vortex Evaporation was more efficient in mass recovery than a micro-Kuderna-Danish procedure for small scale solvent reduction. Solvent-exchanged samples in comparison to samples taken totally to dryness before bioassay gave higher mutagenic yields, although a statistical difference was not evident among various techniques. Dry mass determination methods yielded inconsistent results when not fully optimized. Of the optimized methods compared, desiccation drying techniques were superior.

B3 THE EVALUATION OF CHEMICAL AND BIOLOGICAL METHODS FOR THE IDENTIFICATION OF MUTAGENIC AND CYTOTOXIC HAZARDOUS WASTE SAMPLES. *B. Andon*,[1] M. Jackson,[1] V. Houk,[2] and L. Claxton.[3] [1]EHRT, RTP, NC 27709; [2]UNC-CH, Chapel Hill, NC 27514; [3]US EPA, RTP, NC 27711.

To assist in the development of methods for identifying potentially hazardous wastes, we have conducted studies on the extraction of toxicants from several solid waste samples. The extracts were subsequently bioassayed for toxicity in the Chinese hamster ovary (CHO) cytotoxicity test and for mutagenic potential Salmonella histidine reversion assay. A new technique, which measures the mutagenicity of neat waste sample by coupling thin layer chromatography (TLC) with the Salmonella histidine reversion assay, has also been employed.

The wastes selected for study were coke plant waste, herbicide manufacturing acetone-water effluent, and oil refining waste. Three extraction solvents, ethanol (ETOH), dichloromethane (DCM), and dimethylsulfoxide (DMSO), were chosen based on solvent properties and compatibility and bioassay procedures. Each sample was divided into three parts and extracted with each of the three solvents separately.

All extracts were evaluated in the Salmonella assay at five dose levels with the five Ames tester strains in the presence and in the absence of an exogenous metabolizing system. DMSO and DCM extracts were utilized for CHO cytotoxicity evaluations. Selected neat waste samples and extracts were assayed with the TLC technique. In addition to the biological assessments, the gross chemical parameters for each sample were determined.

Results show that coke plant waste and herbicide manufacturing acetone-water were mutagenic to *S. typhimurium* with the standard plate test. With the TLC technique, the neat coke plant waste was mutagenic and oil refining waste was toxic. Oil refining waste was also toxic to CHO cells. Results of the gross chemistry determinations showed the three samples to have a wide range of solid content, total organic content, and extractables. Evaluation of the chemical extraction methods demonstrates few differences in extraction capabilities with respect to mutagenic activity.

B4 A RADIOLABELED PROCEDURE FOR DIFFERENTIATING CHO HGPRT$^-$ CELLS FROM HGPRT$^+$ CELLS. *M. A. Jackson* and N. E. Garrett, EHRT, RTP, NC 27709.

A procedure for enumerating HGPRT$^-$ cell number in the presence of HGPRT$^+$ cells without the use of genotoxic agents or drug resistance is described. Purine nucleotides in HGPRT$^-$ and HGPRT$^+$ cells were labeled with [^{14}C]glycine via *de novo* synthesis. The label was ultimately collected in the cellular nucleic acid pool and assayed by acid precipitation onto glass-fiber filters after glycine-labeled protein was digested with pronase. However, in the presence of 500 μM of exogenous hypoxanthine, incorporation of ^{14}C from glycine into the nucleic acid of HGPRT$^+$ cells was almost totally inhibited. The extent to which this inhibition occurred was exponentially proportional to the concentration of hypoxanthine from 0.1 to 25 μM. Conversely, HGPRT$^-$ cells rapidly incorporated the label into nucleic acid in the presence of exogenous hypoxanthine. This difference in the abilities of HGPRT$^+$ and HGPRT$^-$ cells to label nucleic acid in the presence of exogenous hypoxanthine allows HGPRT$^-$ cell numbers to be enumerated. The amount of label incorporated is directly proportional to the number of HGPRT$^-$ cells over a range of at least four orders of magnitude.

This work was done under Contract No. 68-02-4031 (Environmental Health Research and Testing, Inc) with the U.S. Environmental Protection Agency. This abstract does not necessarily reflect EPA policy.

B5 EFFECTS OF PRETREATMENT WITH INDUCERS OF LIVER MONOOXYGENASE ACTIVITY ON PRIMARY RAT HEPATOCYTE DNA REPAIR INDUCED BY GENOTOXIC CHEMICALS. D. J. Kornbrust,[1] M. E. Hall,[2] and *D. D. Dietz*.[2] [1]NIEHS, RTP, NC 27709; [2]RTI, RTP, NC 27709.

Inducers of mixed function oxidase (MFO) activities have profound effects on the genotoxic manifestation of chemicals that undergo metabolic activation and/or deactivation by the MFO system. The effects of phenobarbital (PB), DDT, 3-methylcholanthrene (3MC), β-naphthoflavone (βNF) and Aroclor 1254 pretreatment on the responses produced by various genotoxicants in the *in vitro* rat hepatocyte primary culture/DNA repair (HDC/DR) assay were characterized. The DNA repair response elicited by dimethylnitrosamine was unaffected by all pretreatments except 3MC, which produced a slight reduction in the response. By contrast, DNA repair induced by diethylnitrosamine was significantly increased by all pretreatments. Benzo(a)pyrene, o-aminoazotoluene, and 4-aminobiphenyl are reputed to be metabolized by cytochrome P-448 and showed elevated DNA repair responses in hepatocytes from 3MC, βNF and Arodor pretreated rats relative to control hepatocytes. Repair responses to 2-acetylaminofluorene, 7,12-dimethylbenzanthracene and benzidine were not significantly affected by any pretreatment. The response to 6-aminochrysene was decreased by pretreatment with 3MC and βNF. Aflatoxin β_1 elicited a greater response in 3MC, βNF and Aroclor hepatocytes while 2-naphthylamine was most active in hepatocytes derived from βNF pretreated rats. Responses to the direct-acting genotoxicants, methyl methanesulfonate and N-methyl-N-nitro-N-nitrosoguanidine were not increased by any pretreatment, suggesting that the pretreatment-related effects observed with the other chemicals were due to induction of MFOs or other metabolizing enzymes (i.e. Phase II metabolism) rather than to a direct effect on the DNA repair capacity of the hepatocytes.

C1 INDUCTION OF SISTER CHROMATID EXCHANGES AND MICRONUCLEI IN MALE DBA/2 MICE AFTER INHALATION OF BENZENE. *G. L. Erexson*, J. L. Wilmer, C. D. Auman and A. D. Kligerman. CIIT, RTP, NC 27709.

Analyses of either the frequencies of sister chromatid exchanges (SCEs) or micronuclei (MN) are considered to be sensitive indicators of the genotoxic potential of a chemical. Thus, experiments were designed to investigate both the induction of MN in bone marrow polychromatic erythrocytes (PCEs) and SCEs in peripheral blood lymphocytes (PBLs) of mice after inhalation of benzene. Male DBA/2 mice (17–19 weeks old) were exposed to target concentrations of 0, 10, 100 or 1000 ppm benzene for 6 hr. Blood was obtained by cardiac puncture 18 hr. after exposure and PBLs cultured in the presence of lipopolysaccharide (60 μg/ml) to stimulate blastogenesis of B-lymphocytes. 5-Bromo-2′-deoxyuridine (2 μM) was added 24 hr. after culture initiation for SCE analysis, and the cultures were harvested at 60 hr. following a final 4 hr. demecolcine (0.5 μg/ml) treatment. Benzene caused a significant, concentration-dependent increase in SCEs of 7.6 ± 0.2, 9.5 ± 0.2 or 13.8 ± 0.9/metaphase at 10, 100 or 1000 ppm, respectively (controls = 5.9 ± 0.2). PBLs showed significant, concentration-dependent decreases in mitotic indices; however, cell cycle kinetics were not affected. Femoral bone marrow smears were analyzed for MN in PCEs 18 hr. after benzene exposure. Benzene caused a significant, concentration-dependent increase in the number of MN (0.90 ± 0.06, 2.03 ± 0.07 or 2.81 ± 0.08% MN at 10, 100 or 1000 ppm, respectively; control = 0.21 ± 0.03). These results show a statistically significant genotoxic effect in mice after a 6 hr. exposure to 10, 100 or 1000 ppm benzene in both the SCE assay and the MN test. Thus, SCE induction in PBLs appears to be as sensitive a measure of the genotoxicity of benzene as MN induction in bone marrow PCEs.

C2 MUTAGENICITY OF TOBACCO AND MARIJUANA SMOKE CONDENSATES. *T. J. Hughes*, D. M. Simmons, L. G. Monteith, B. M. Sadler, K. H. Davis, S. E. Frazier and C. M. Sparacino, RTI, RTP, NC 27709.

Two marijuana smoke condensates (MSC) and a tobacco smoke condensate (TSC) were evaluated for mutagenic potency in the Ames/Salmonella assay. The mutagenic potency of high THC-content (3.7% tetrahydrocannabinol) and low THC-content (1.3% THC) MSC were compared to Kentucky reference tobacco condensate (TSC) under puff mode and constant draft smoking conditions. The condensates were formed from 70 mm cigarettes on a 30-port smoking machine. The constant draft condensate rate was 1200 ml/min, and the puff mode rate was 40 ml/2 sec (1 puff/min). The condensates were collected in ethanol. Test parameters were Salmonella strains TA98 and TA100, with and without Aroclor-induced hamster liver S9, 5 dose levels (10–250 μg/plates), triplicate plates per dose, and preincubation at 37°C for 20 min with shaking. MSC and TSC were tested as crude condensates and chemically fractionated into acidic, basic and neutral fractions. Results suggested: 1) the basic fractions from the high THC/MSC were the most mutagenic fractions (slopes in revertants/μg = 7.16 for constant draft and 7.04 for puff mode; 2) the high THC/MSC basic fraction was more mutagenic than the low THC/MSC basic fraction, and the tobacco basic fraction was the least mutagenic (slopes = 7.16, 3.80 and 1.16 respectively); 3) constant draft produced a higher mutagenic basic fraction than did puff mode with low THC/MSC; 4) the crude and neutral fractions were weakly mutagenic; 5) the acidic fractions were not significantly mutagenic; 6) chemical fractionation detected mutagenicity not detected in the crude condensates; and 7) mutagenic activity required S9 addition and was primarily detected with TA98. These data have health implications because most street marijuana has high THC levels and is held in the lungs for extended periods. Research is presently being conducted to identify the mutagens in the basic and neutral fractions.

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C3 MUTAGENICITY OF LICHEN CONSTITUENTS, *C. I. Wei*[1] and T. Shibamoto.[2] [1]U Fla., Gainesville 32611; [2]U Calif., Davis 95616.

The position of phenolic OH groups has been shown to be an important structural determinant of the mutagenicity of many naturally occurring and synthetic flavonoid compounds by the Ames Salmonella/microsome assay. Lichens are important food sources for animals in arctic regions. Several human population groups also use lichens as a food source. Since some lichen constituents, such as depsidones, are similar to the above-mentioned natural plant constituents in their structures, a better evaluation of the toxicity of lichen constituents is recommended. In this study, the most common lichen depsidones, physodic and physodalic acids, and the most abundant lichen constituent, usnic acid, were tested for mutagenicity in both plate incorporation and preincubation assay systems, using *Salmonella typhimurium* strains TA 98 and TA 100 in the presence or absence of rat liver S9 mix. Only physodalic acid exhibited a clear dose-related mutagenic activity to strain TA 100 with or without S9 mix in both assay systems. The addition of S9 mix increased the number of revertants approximately threefold and fourfold in preincubation and plate incorporation assays, respectively. The major structural difference between physodalic acid and physodic acid is the presence of an aldehyde group on the aromatic ring of the first compound. The much larger alkyl/acyl ring substituents in physodic acid could account for the lack of mutagenicity.

C4 EVALUATION OF THREE "DIFFICULT-TO-TEST" CARCINOGENS IN THE CHINESE HAMSTER V-79 INHIBITION OF METABOLIC COOPERATION ASSAY. *E. Elmore*, E. A. Korytynski, and M. P. Smith. Northrop, RTP, NC 27709.

The development and validation of *in vitro* bioassays to detect toxic chemicals, which may act through epigenetic mechanisms, is of great importance since many environmentally important chemicals fall into this category. Chemicals that could potentially be detected by such assays include: cocarcinogens, tumor promoters, teratogens, toxins that alter reproductive maturation or function, neurotoxins, and some carcinogens. The Chinese hamster V-79 inhibition of metabolic cooperation assay has been shown to be responsive to many of the classes of compounds known to act through epigenetic mechanisms. We have evaluated several chemicals that have been shown to be "difficult-to-test" in that they produce false negative responses in *in vitro* genetic bioassays. Three chemicals, diethylhexylphthalate (DEHP), o-toluidine (TOL), and acrylonitrile (ACN), all known carcinogens in rodent bioassays, were evaluated in the Chinese hamster V-79 inhibition of metabolic cooperation assay. DEHP produced a strong positive, dose-related response, which was detectable at 5 to 200 μg/ml with a reproducible peak at 25 μg/ml. TOL produced a strong positive response at 5 to 20 μg/ml. ACN produced a dose related positive response at 20 to 50 μg/ml. These results suggest that the Chinese hamster V-79 inhibition of metabolic cooperation assay may be useful for screening difficult-to-test environmental carcinogens.

C5 DIFFERENTIAL CYTOTOXIC RESPONSE OF XERODERMA PIGMENTOSUM FIBROBLASTS AND NORMAL HUMAN FIBROBLASTS TO VARIOUS PROMUTAGENS AFTER ACTIVATION BY CO-CULTIVATED RAT HEPATOCYTES. *L. L. Yang*, R. A. Lubet, P. M. Conklin, J. W. Cameron, and R. D. Curren. Microbiological Associates, Bethesda, MD.

Skin fibroblasts from patients suffering the cancer-prone syndrome xeroderma pigmentosum (XP) are defective in the excision repair of DNA-damage caused by many direct-acting carcinogens/mutagens. This defect generally results in lower relative colony-forming ability for the XP fibroblasts than for normal fibroblasts (NF) after exposure to equal amounts of direct-acting genotoxins. In fact, a differential survival curve for normal and XP fibroblasts after exposure to the same agent is often taken as presumptive evidence of the ability of that chemical to interact with DNA. We examined whether similar differential survival curves would result after exposure of XP and NF cells to selected promutagens or procarcinogens. To activate these compounds, hepatocytes isolated from Aroclor 1254-induced rats were overlayed on a confluent monolayer of either XP or NF cells. After a 24 hr exposure, the cultures were trypsinized, appropriately diluted, and reseeded to measure the survival of cloning ability. Four of the compounds tested [benzo(a)pyrene, 2-acetylaminofluorene, aflatoxin B_1 and cyclophosphamide] cause significantly more killing of XP cells than normal cells. Equal killing of XP and NF cells was found when dimethylnitrosamine was used. These data are compatible with the current concept that the first four compounds cause assorted DNA adducts that can be repaired by an DNA-excision repair system, while lesions caused by the latter compound, a methylating agent, are repaired by other pathways. Our results indicate that human XP and NF cells can be combined with rat hepatocytes to form a rapid and sensitive system for detecting promutagenic/procarcinogenic chemicals.

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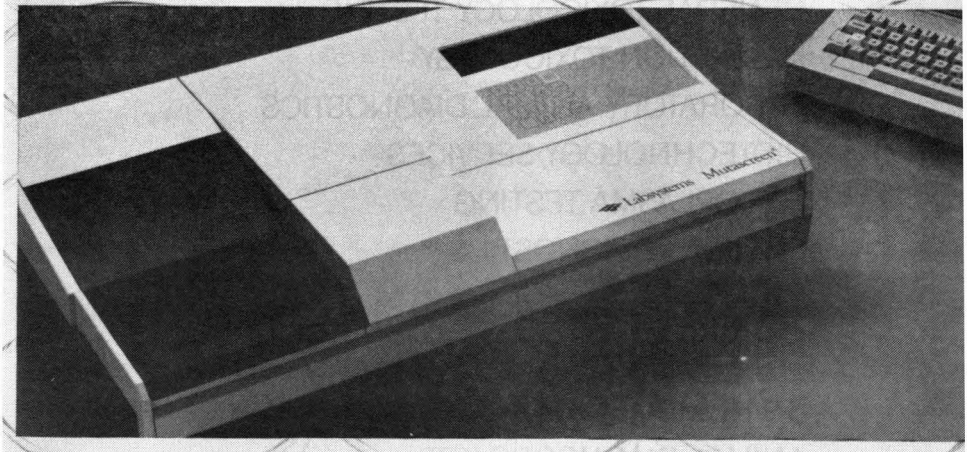

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