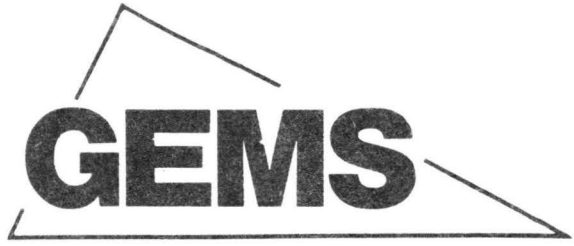
GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY



FIRST ANNUAL MEETING

PROGRAMS AND ABSTRACTS

October 14, 1983
Governor's Inn
Research Triangle Park, North Carolina
8:00 a.m. to 5:30 p.m.

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

Elected Officials for Terms Beginning October, 1983

President (2 years)	– Larry Claxton (EPA)
Vice President (2 years)	– Andrew Kligerman (CIIT)
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1 Year	– T. K. Rao (EHRT)
	– Thomas Skopek (CIIT)
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(January 1, 1983 to October 14, 1983)

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Avram Gold (UNC-CH)
Douglas Keller (Duke University)
Donald Clive (Burroughs Wellcome)

Genotoxicity and Environmental Mutagen Society

On behalf of the officers and the Board of Directors, welcome to the first annual meeting of the Genotoxicity and Environmental Mutagen Society. Thank you for your attendance, support, and participation; for it is you, the member of GEMS, who will make this a successful scientific society. Knowing that there are a large number of toxicologists in the southeast and recognizing that many investigators need an additional forum for the exchange of information concerning genotoxicity and mutagenicity, scientists from industry, government, and universities have joined together to initiate GEMS. As a society GEMS welcomes the participation of students, bench scientists, scientific managers, and others interested in these areas of research. The society has committed itself to be of service in the following ways:

To provide for an annual meeting that allows not only senior scientists but also others to present their work and accomplishments to their peers.

To provide a newsletter that regularly gives the news of the society, listings of regional seminars and meetings, and other news important to the members of GEMS.

To sponsor a GEMS seminar series presenting topics of broad interest or of exciting new advances.

To cooperate with other societies and organizations in areas of common interest in order to be of service to those organizations and the scientific community. We hope that organizations of broader interest will welcome this supplemental effort of GEMS.

As a member of a new society, you have the opportunity to help to mold the character of the society. Your suggestions, comments, and ideas, therefore, are eagerly sought. What do you want the character of the society to be? How can the society be of help to you and your organization? How can you be of help to GEMS? Please discuss these questions with the society officers and the Board of Directors. Fill out the questionnaires that you will receive. Participate as much as you can so that GEMS can serve you.

Again, we offer a sincere welcome, since GEMS is here to support the needs and efforts of each member. Sustaining members provide a variety of services and make meetings, such as this one, financially possible; we also give you our sincere thanks and welcome. We hope that everyone will enjoy and benefit from this meeting.

Sincerely yours,

Larry Claxton

Larry Claxton, Ph.D.
President-elect

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Representatives: Mr. Larry Sivik, Mr. Bob Weingard (312-357-3720)

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2 QUEUE SYSTEMS, P.O. Box 1901, Parkersburg, WV 26102. Representatives: Mr. Alan Miller (919-688-1490), ~~Dr. William B. White~~ (304-464-5400)

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PROGRAM
GEMS FIRST ANNUAL MEETING
Friday, October 14, 1983
Governors Inn, Research Triangle Park, North Carolina
8:00 a.m. - 5:30 p.m.

8:00–8:30 a.m. Registration, exhibit and poster set-up (2nd floor)

8:30–9:15 a.m. MUTAGENIC ANALYSIS OF NITROSAMINES. *W. Lijinksy*, Keynote Speaker. Frederick Cancer Research Institute, Frederick, MD

9:15–9:30 a.m. MUTAGENICITY OF ELLIPTICINES IN STRAINS TA97 AND TA102 OF SALMONELLA. *D. M. DeMarini*, Oak Ridge Associated Universities, Oak Ridge, TN 37830

9:30-9:45 a.m. METABOLISM AND BIOLOGICAL ACTIVITY OF CYCLOPENTA-FUSED PAH. *R. Sangaiah*,[a] A. Gold,[a] S. H. Toney,[b] R. Easterling,[c] L. D. Claxton[c] and S. Nesnow.[c] [a]University of North Carolina, Chapel Hill, NC 27514; [b]Northrop Services, Inc., Research Triangle Park, NC 27709; [c]US EPA, Research Triangle Park, NC 27711.

9:45–10:00 a.m. A MUTATION IN THE *Hbb*^{s} GENE INDUCED IN A FEMALE BY ETHYLNITROSUREA. *Susan E. Lewis*[a], F. M. Johnson,[b] L. C. Skow,[b] Lois B. Barnett[a] and R. A. Popp[c] [a]Research Triangle Institute, Research Triangle Park, NC; [b]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [c]Oak Ridge National Laboratory, Oak Ridge, TN

10:00–10:30 a.m. Coffee Break

10:30–11:15 a.m. COMPARATIVE BIOLOGICAL STUDIES WITH COMPLEX MATERIALS FROM COAL CONVERSION PROCESSES. *T. K. Rao*, Keynote Speaker. Environmental Health Research and Testing Laboratory, Research Triangle Park, NC

11:15–11:30 a.m. MAMMALIAN METABOLISM AND ACTIVATION OF 1-NITROPYRENE. *L. M. Ball* and J. Lewtas. GBB, GTD, HERL, U.S. Environmental Protection Agency, Research Triangle Park, NC

11:30–11:45 a.m. SOMATIC MUTAGEN SENSITIVITY, EXCISION DEFICIENCY AND GERMINAL HYPERMUTABILITY CHARACTERIZE THE *MUS(2)201* MUTANT OF *DROSOPHILA MELANOGASTER*. *P. Dennis Smith* and Ruth L. Dusenbery, Department of Biology, Emory University, Atlanta, GA 30322

11:45–12 noon DNA METABOLISM DURING MEIOSIS IN *RAD50* AND *RAD52* MUTANTS OF *SACCHAROMYCES CEREVISIAE*. *J. Nitiss*[a,b] and M. A. Resnick.[a] [a]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [b]Illinois Institute of Technology, Chicago, IL

12:00–1:30 p.m. Lunch (3rd floor)

1:30–2:00 p.m. GEMS Business Meeting. Open to all members. Membership and financial report, installation of elected officers, and address by President.

2:00–2:45 p.m. GENETIC TOXICOLOGY OF SOME KNOWN HUMAN CARCINOGENS. *M. Waters*, Keynote Speaker. Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, NC

2:45–3:00 p.m. METAL-INDUCED MUTAGENESIS IN THE *lacI* GENE OF *ESCHERICHIA COLI*. *Richard A. Zakour* and Barry W. Glickman, NIEHS, Laboratory of Genetics, Research Triangle Park, NC 27709

3:00–3:15 p.m. STRUCTURE–ACTIVITY RELATIONSHIPS IN THE SALMONELLA MUTAGENESIS ASSAY WITH NITRO AROMATIC HYDROCARBONS. *T. K. Rao*,[a] D. Daston,[a] D. Walsh[a] and L. Claxton[b] [a]Environmental Health Research and Testing, Inc.; [b]Genetic Toxicology Division, Health Effects Research Laboratory, Research Triangle Park, NC 27709

3:15–3:30 p.m. RAT AND MOUSE LYMPHOCYTE CULTURES FOR *IN VIVO* CYTOGENETIC INVESTIGATIONS. *A. D. Kligerman*, G. L. Erexson and J. L. Wilmer, Chemical Industry Institute of Technology, Research Triangle Park, NC 27709

3:30–5:30 p.m. Poster Sessions and Vendor Exhibits
Sponsored Social

5:30 p.m. Meeting Adjourns

Poster Session A

Pure Compounds

A1 MUTAGENICITY OF 1-NITROPYRENE METABOLITES ISOLATED FROM LUNG CELLS AND RESPIRATORY TISSUES. *L. C. King*, L. M. Ball, M. J. Kohan and Joellen Lewtas, EPA, RTP, NC 27711

A2 MUTAGENICITY OF PYRENE IN SALMONELLA. *Z. Matijasevic* and E. Zeiger, Cellular and Genetic Toxicology Branch, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709

A3 THE MUTAGENIC STABILITY OF CHEMICALS STORED IN SOLUTION. *D. A. Pagano* and E. Zeiger, Cellular and Genetic Toxicology Branch, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709

A4 DESIGN OF AN INTERLABORATORY STUDY FOR THE *SALMONELLA* PLATE ASSAY. Larry Claxton, EPA, Research Triangle Park, NC 27711; Thomas J. Hughes, Jim Murphy, Lawrence Myers and *Nancy Sexton*, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709.

A5 ALTERNATIVE ROUTE TO HYPERVALENT IRON IN BIOMIMETIC OXIDATIONS MEDIATED BY PORPHINATOIRON COMPLEXES. *A. Gold*, G. E. Toney, R. Sangaiah, J. E. Savrin, ESE, University of North Carolina, Chapel Hill, NC 27514

Poster Session B

Complex Mixtures

B1 DIRECT APPLICATION OF THE *SALMONELLA* ASSAY TO TLC PLATES FOR SCREENING COMPLEX HAZARDOUS WASTE STREAMS. *V. Houk*, UNC, Chapel Hill, NC 27514; and L. Claxton, US EPA, RTP, NC 27711

B2 DEVELOPMENT OF PROCEDURES FOR MUTAGENIC ANALYSIS OF AMBIENT AIR VAPORS. T. J. Hughes, *D. M. Simmons*, L. G. Monteith, RTI, RTP, NC 27709; and L. Claxton, US EPA, RTP, NC 27711

B3 MUTAGENICITY OF DILUTE PEAT SMOKE PARTICULATE EXTRACTS UNDER SIMULATED ATMOSPHERIC CONDITIONS: A PRELIMINARY STUDY. *D. A. Bell*, R. M. Kamens, ESE, SPH, UNC, Chapel Hill, NC 27514

B4 THE POSSIBLE ROLE OF ALCOHOL IN INFLAMMATORY CELL DIFFERENTIATIONS. C. Sachs, B. Thompson, P. Pratt and *W. S. Lynn*, Duke University Medical Center, Durham, NC 27710

Poster Session C

Mammalian Assays

C1 PRELIMINARY EXPERIMENTS INVESTIGATING THE ABILITY OF TRIFLUOROTHYMIDINE-RESISTANT MUTANTS OF L5178Y MOUSE LYMPHOMA CELLS TO RE-EXPRESS KINASE ACTIVITY. M. M. Moore, EPA, RTP, NC 27711; K. Loud, R. K. Templeton, and *K. Lawrence*, Northrop Services, Inc., RTP, NC 27711; J. C. Hozier, Florida Institute of Technology, Melbourne, FL 32901

C2 HISTORICAL CONTROL VALUES AND THEIR STATISTICAL CONSEQUENCES FOR THE L5178Y/TK$^{+/-}$ → TK$^{-/-}$ MOUSE LYMPHOMA MUTAGENICITY ASSAY. *D. Clive* and Nancy T. Turner, Wellcome Research Laboratories, RTP, NC

C3 A COMPARISON OF CLASTOGENIC AND MUTAGENIC ACTIVITIES OF THREE MUTAGENS AND A NON-MUTAGEN IN THE L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA SYSTEM. *J. Eyre*, D. Clive, R. Krehl, and N. Turner, Wellcome Research Laboratories, RTP, NC; J. C. Hozier and J. Sawyer, Florida Institute of Technology, Melbourne, FL

C4 TYPES OF CYTOGENETIC DAMAGE DETECTED IN THE L5178Y/TK$^{+/-}$ → TK$^{-/-}$ MOUSE LYMPHOMA ASSAY BY BANDED KARYOTYPE ANALYSIS. *Roberta Krehl* and D. Clive, Wellcome Research Laboratories, RTP, NC; and J. C. Hozier and J. Sawyer, Florida Institute of Technology, Melbourne, FL

C5 COMPARATIVE MUTAGENICITY STUDIES ON METHAPYRILENE AND PYRILAMINE IN THE L5178Y/TK$^{+/-}$ → TK$^{-/-}$ MOUSE LYMPHOMA ASSAY. *Nancy T. Turner*, J. Woolley, and D. Clive, Wellcome Research Laboratories, RTP, NC

C6 SISTER CHROMATID EXCHANGE INDUCTION IN MOUSE B AND T LYMPHOCYTES EXPOSED TO CYCLOPHOSPHAMIDE *IN VITRO* AND *IN VIVO*. *James L. Wilmer*, Gregory L. Erexson, and Andrew D. Kligerman, Dept. of Genetic Toxicology, Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, NC 27709

C7 SISTER CHROMATID EXCHANGE INDUCTION IN CULTURED PERIPHERAL BLOOD LEUKOCYTES OF A COLDWATER MARINE FISH. *Helen R. Zakour*, Marsha L. Landolt, and Richard M. Kocan, School of Fisheries, University of Washington, Seattle, WA 91895

C8 MODIFICATIONS OF GENE EXPRESSION AND DIFFERENTIATION IN RAT TRACHEAL EPITHELIAL CELLS EXPOSED TO A TUMOR PROMOTER. *V. E. Steele*, Northrop Services, Inc.; and R. Wu, W. Alton Jones Cell Science Center, Lake Placid, NY 12946

C9 EVALUATION OF GENOTOXIC EFFECTS IN HUMAN POPULATIONS. J. W. Allen, L. D. Claxton, N. E. Garrett, S. L. Huang, M. M. Moore, Y. Sharief, G. H. S-Strauss and *M. D. Waters*. US EPA, Research Triangle Park, NC 27711; and Northrop Services, Inc., Research Triangle Park, NC 27709

Poster Session D

Hepatocytes/DNA Repair

D1 COMPARISON OF RAT AND HAMSTER HEPATOCYTE DNA REPAIR GENOTOXICITY ASSAYS. D. J. Kornbrust, *M. E. Hall*, and T. R. Barfknecht, Research Triangle Institute, Research Triangle Park, NC 27709

D2 METABOLISM OF BENZO(a)PYRENE TO ACTIVE AND DETOXIFIED PRODUCTS IN PRIMARY CULTURES OF HUMAN HEPATOCYTES. *Stephen Strom*, Alan Novotny, Randy Jirtle, and George Michalopoulos, Duke Medical Center, Durham, NC 27710

D3 RESPONSE OF PRIMARY CULTURES OF PROLIFERATING HEPATOCYTES TO CARCINOGENS. *Deborah L. Novicki*, Mark R. Rosenberg, and George Michalopoulos, Departments of Pathology and Radiology, Duke University Medical Center, Durham, NC 27710

Poster Session E

Quality Assurance

E1 QUALITY ASSURANCE FOR MUTAGENICITY STUDIES. *R. S. DeWoskin*, B. M. Sadler, and Nancy H. Adams, Research Triangle Institute, Research Triangle Park, NC 27709

ABSTRACTS

T1

MUTAGENICITY OF ELLIPTICINES IN STRAINS TA97 AND TA102 OF SALMONELLA. *D. M. DeMarini*, Oak Ridge Associated Universities, Oak Ridge, TN 37830

The mutagenicity of five antitumor compounds (ellipticines) was investigated in strains TA97 and TA102 of Salmonella. The compounds tested were ellipticine, 9-methoxyellipticine, 9-hydroxyellipticine, 9-aminoellipticine, and 2-methyl-9-hydroxyellipticinium. The first four had been shown previously to be mutagenic in TA1537, but only the first three were positive in TA1977, which is the *uvr*B$^+$ homologue of TA1537. In the present study, the first four compounds were mutagenic in TA102. It is interesting that 9-aminoellipticine is positive in TA102, which is *uvr*B$^+$, because the presence of the *uvr*B$^+$ allele causes this compound to be negative in TA1977. TA97 detected only two (ellipticine and 9-aminoellipticine) of the four ellipticines that were mutagenic in TA1537. Although Ames has suggested that TA97 is more sensitive than TA1537 and that TA97 should be used instead of TA1537 for general screening purposes, these results suggest that TA97 is insensitive to some polycyclic hydrocarbons such as the ellipticines, which are detected easily by TA1537. In addition, the spontaneous reversion frequency of TA97 is reduced substantially by the addition of 0.1 to 0.2 ml of DMSO per plate.

T2

METABOLISM AND BIOLOGICAL ACTIVITY OF CYCLOPENTA-FUSED PAH. *R. Sangaiah*[a], A. Gold[a], S. H. Toney[b], R. Easterling[c], L. D. Claxton[c] and S. Nesnow[c]. [a]University of North Carolina at Chapel Hill, Chapel Hill, NC 27514; [b]Northrop Services, Inc., Research Triangle Park, NC 27709; [c]US EPA, Research Triangle Park, NC 27711

A series of four isomeric PAH has been synthesized based on fusing a cyclopenta ring onto the benz(a)anthracene skeleton. The metabolism and biological activity of the series has been studied and can be interpreted in terms of possible mechanisms of bioactivation. Elucidation of metabolite profiles shows that for this series of PAH, electronic structure is an important determinant of mixed function oxidase regioselectivity, with sites bearing high electron density generally most susceptible to metabolic oxidation. Highly localized peripheral bonds, such as the cyclopenta- and k-regions, are initially epoxidized and subsequently hydrated to dihydrodiols. Oxidation of aromatic bonds yields a mixture of dihydrodiols and phenols. Epoxidation appears to be the pathway through which biologically active metabolites are generated. Activity in the Ames assay correlates well with resonance stabilization energy ($\Delta E_{deloc}/\beta$) of the most stable carbocation resulting from epoxide ring opening. The importance of steric factors, such as the bay region, appears to lie in blocking detoxification pathways; particularly by decreasing the affinity of epoxide hydrolase for the metabolite. Hence the molecular structures of the metabolites allow an assessment to be made regarding the roles of electronic and steric factors.

T3

A MUTATION IN THE *HBB*^{s} GENE INDUCED IN A FEMALE BY ETHYLNITROSOUREA. *Susan E. Lewis*[a], F. M. Johnson[b], L. C. Skow[b], Lois B. Barnett[a], and R. A. Popp[c]. [a]Research Triangle Institute, Research Triangle Park, NC; [b]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [c]Oak Ridge National Laboratory, Oak Ridge, TN

An individual with a variant hemoglobin was discovered during electrophoretic screening of blood taken from the F_1 (C57Bl/6J × DBA/2J) progeny of mothers treated with

ethylnitrosourea (ENU). The variant trait, which was expressed as an electrophoretically fast band, was inherited by the progeny of the original F_1 mutant, and hence was due to a germinal mutation. The F_1 carrier transmitted the normal *Hbb*^{s} allele, the mutant *Hbb*^{s} allele and *Hbb*^{d} in backcrosses to a DBA/2J male. However, the trait behaved as a simple Mendelian alternate at the *Hbb* locus when heterozygotes from the backcross generation were subsequently mated to each other. It was concluded that ENU had induced a mutation at the *Hbb* locus and that the original carrier was a mosaic. (Supported in part by Contract #N01-ES-2-5012 from the National Institute of Environmental Health Sciences).

T4

MAMMALIAN METABOLISM AND ACTIVATION OF 1-NITROPYRENE. *L. M. Ball*[1] and J. Lewtas, GBB, GTD, HERL, U.S. Environmental Protection Agency[2], Research Triangle Park, NC

Nitrated polycyclic aromatic hydrocarbons have been identified in combustion emissions. Many of these compounds, such as the nitropyrenes, are potent bacterial and mammalian mutagens, and may contribute substantially to the burden of genotoxicity associated with air pollution. The activation of nitroarenes in bacteria can be largely attributed to electrophilic arylnitrenium intermediates formed during nitroreduction. The mechanisms of activation in mammalian systems are more complex, and not yet extensively documented. 1-Nitro[^{14}C]pyrene (NP) has been used to study mammalian routes of activation of a model nitroarene both *in vivo* and *in vitro*. Mammalian metabolites of NP have been isolated, identified and characterized by HPLC, HRGC/MS and chemical synthesis. In the intact rat NP is excreted in the urine as phenols of 1-nitropyrene (1-NP-6/8- and -3-OH) and of N-acetyl-1-aminopyrene (NAAP-6- and -8-OH), all extensively conjugated with glucuronic acid. These metabolites were mutageic in the Ames *Salmonella* assay, and NAAP-6/8-OH in particular was five-fold more potent than the parent compound. Studies in germ-free rats demonstrated that metabolism by the gut flora and enterohepatic recirculation play a vital role in the production of the highly mutagenic NAAP-6/8-OH. *In vitro* NP was rapidly metabolized by rat hepatic subcellular fractions (S-9, cytosol and microsomes) and isolated hepatocytes to both oxidized and reduced products. Metabolism in the isolated hepatocytes was accompanied by extensive binding to endogenous protein and DNA. Binding to calf thymus DNA added to the subcellular fractions was largely oxygen- and NADPH-dependent. Oxidative and reductive pathways all function in the activation of NP, and bacterial metabolism may also be involved in the genotoxicity of this class of compound *in vivo*.

[1]National Research Council Resident Research Associate
[2]This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

T5

SOMATIC MUTAGEN SENSITIVITY, EXCISION DEFICIENCY AND GERMINAL HYPERMUTABILITY CHARACTERIZE THE *MUS(2)201* MUTANT OF *DROSOPHILA MELANOGASTER*. *P. Dennis Smith* and Ruth L. Dusenbery, Department of Biology, Emory University, Atlanta, Georgia 30322.

Somatic mutagen hypersensitivity has been employed to identify genes of *Drosophila melanogaster* which involve DNA repair functions. A locus on the second chromosome, *mus(2)201*, has been identified which exhibits hypersensitivity to ultraviolet light (UV) and a series of mono- and difunctional alkylating agents. Autoradiographic analysis has been employed to measure unscheduled DNA synthesis (UDS) in a mixed population of embryonic cells prepared either from mutant or control strains and treated with various mutagenic agents. The *mus(2)201* mutant exhibits no detectable ability to perform the resynthesis step of exci-

sion repair following treatment by UV, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), methyl nitrosourea (MNU), or diepoxybutane (DEB). The sex-linked recessive lethal (SLRL) test was employed as a simple and objective monitor of the effect of the *mus(2)201* locus on mutation induction. Repair-proficient males were treated with a series of monofunctional alkylating agents, mated to either repair-proficient (*mus*$^+$) or repair-deficient (*mus[2]201*) females and assayed for mutation induction. The *mus(2)201* mutant exhibited hypermutability for MMS, MNU, and EMS, but not ENU (ethyl nitrosourea). The relative increase in mutability parallels very closely the expected production of 3-alkyl adenine, suggesting that this lesion acts as a promutagenic DNA alteration. We hypothesize that the hypermutability is caused by the conversion of 3-alkyl adenine to an apurinic site and subsequent misincorporation of a base opposite the non-coding lesion.

T6

DNA METABOLISM DURING MEIOSIS IN *RAD50* AND *RAD52* MUTANTS OF *SACCHAROMYCES CEREVISIAE*. *J. Nitiss*[a,b] and M. A. Resnick[a]. [a]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [b]Illinois Institute of Technology, Chicago

We are studying the role of DNA repair genes in normal meiosis. Of particular interest is the *rad52* mutant which is deficient in double-strand break repair, meiotic recombination, and produces inviable ascospores. As previously shown with strains that undergo a relatively synchronous meiosis, single-strand interruptions (SSIs) accumulate in *rad52* starting at the time of premeiotic DNA replication. The timing corresponds to the onset of commitment to meiotic recombination in wild-type strains. The *rad50* mutation, which is also deficient in meiotic recombination and produces inviable ascospores, does not accumulate SSIs. We have found that in return to growth experiments, *rad52* mutants commit to lethality at the same time as the appearance of SSIs. By contrast, commitment to lethality in *rad50* is much later and occurs at about the time of chromosome segregation.

Since the SSIs that accumulate in *rad52* appear to be associated with recombination, we have begun to examine their molecular nature. A gentle DNA isolation procedure that does not introduce nicks has been used to isolate meiotic chromosomal DNA in order to determine its priming ability in an *in vitro* DNA synthesis assay. The incorporation of ^{32}P-labelled nucleotide triphosphates by *E. coli* polymerase I into DNA isolated form wild type or *rad50* cells does not change with time into meiosis. The results with DNA isolated from the *rad52* mutant are quite different in that beginning at a time when SSIs appear, the level of incorporation of ^{32}P acid precipitable material increases to a maximum of about ten times over that of the mitotic control DNA. In contrast, the level of incorporation with the large fragment of *E. coli* DNA polymerase I does not increase in either *rad50* or *rad52* strains. From this we conclude that SSIs are nicks rather than gaps. When the *rad52* meiotic DNA is run on agarose gels, we find that the pattern of ^{32}P incorporation is nonrandom, suggesting unique sites or regions of incorporation.

T7

METAL-INDUCED MUTAGENESIS IN THE *lacI* GENE OF *ESCHERICHIA COLI*. *Richard A. Zakour* and Barry W. Glickman, NIEHS, Laboratory of Genetics, MD-E301, RTP, NC 27709

Mutagenesis in the *lacI* gene of *Escherichia coli* has been examined in cells grown in the presence of beryllium, manganese or chromium compounds, metals with suspected mutagenic or carcinogenic potential. Two- to three-fold increases in mutation frequency were produced by $BeCl_2$, $MnCl_2$ and $K_2Cr_2O_7$. Among the cells grown in the presence of Be^{2+}, the frequen-

cy of amber and ochre mutants was three-fold higher than the spontaneous background, suggesting that at least part of the increased mutagenicity was due to base-substitution mutations. The specificity of base-substitution mutations induced by Be^{2+} and Mn^{2+} in the *lacI* gene was analyzed. Among the amber mutations induced in cells grown in the presence of Be^{2+}, an increase in G:C → A:T transitions was detected. In contrast, following growth in Mn^{2+}, no increase in amber and ochre mutation frequencies was observed and the mutational spectrum resembled that obtained spontaneously indicating that mutations induced by Mn^{2+} in the *lacI* gene involve changes that do not yield nonsense mutations. These results suggest that metals may exert a number of different mutagenic effects and that these effects vary for each metal.

78

STRUCTURE–ACTIVITY RELATIONSHIPS IN THE SALMONELLA MUTAGENESIS ASSAY WITH NITRO AROMATIC HYROCARBONS.[1] *T. K. Rao*[a], D. Daston[a], D. Walsh[a], and L. Claxton[b]. [a]Environmental Health Research and Testing, Inc. and [b]Genetic Toxicology Division, Health Effects Research Laboratory, Research Triangle Park, NC 27709.

Complex environmental samples such as diesel particulates, fly ash, photocopy toners and combustion products have often exhibited mutagenic activity in the Salmonella histidine reversion assay even in the absence of exogenous metabolic activation. Extensive chemical and biological characterization of these samples has suggested nitro substituted aromatic compounds as the suspect mutagens. We generated a mutagenicity data base in the Salmonella assay with a group of commercially available nitro aromatic hydrocarbons. This data base has been adopted for a pattern recognition technique (ADAPT), to predict mutagenicity of unknown compounds. Certain broad conclusions can be drawn based on mutagenicity data: Mutagenic activity depended on the size of fused ring structures. The smaller molecular weight compounds were less mutagenic than the larger fused ring compounds. Substitution by nitro groups increased the mutagenic activity, the dinitro compounds being more mutagenic than the mononitro compounds. Heteroatom (nitrogen) substitution contributed significantly towards the mutagenic activity. The mutagenicity data obtained in the Salmonella assay was analyzed for structure activity relationships using a pattern recognition technique (ADAPT).

[1]Portions of the work were done under Contract No. 68-02-2566 (Northrop Services Inc.) and Contract No. 68-02-4031 (Environmental Health Research and Testing, Inc.) with the U.S. Environmental Protection Agency.
This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

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RAT AND MOUSE LYMPHOCYTE CULTURES FOR *IN VIVO* CYTOGENETIC INVESTIGATIONS. *A. D. Kligerman*, G. L. Erexson, and J. L. Wilmer, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

Lymphocytes offer many advantages for *in vivo* cytogenetic studies. They can be removed nonlethally from the animal allowing a subject to serve as its own control and permitting the analysis of cytogenetic damage over time. Because peripheral lymphocytes do not normally divide once they have matured, they have the potential to accumulate DNA lesions during chronic exposures to genotoxicants. In addition, lymphocyte culture is at present the only practical means to study cytogenetic damage in human populations. With the goal of determining the sensitivity of lymphocyte culture systems, we developed standard methodologies for the whole blood culture of rat and mouse peripheral lymphocytes. The protocols yield reproducible results with relatively high mitotic indices, stable baseline sister chromatid exchange (SCE) frequencies, and ample numbers of the first and second division metaphases for scoring chromosome aberrations and SCE. Inhalation studies have been conducted to determine if ethylene oxide, formaldehyde, and nitrobenzene would lead to detectable levels of

cytogenetic damage in peripheral lymphocytes of rats. The results indicate that only ethylene oxide induced significant increases in SCE at the concentrations examined, and none of the compounds caused statistically significant increases in chromosome aberration frequencies. Results with these and other more potent genotoxicants indicate that the screening of human populations for increased incidences of chromosome aberrations following suspected exposure to low doses of chemical genotoxicants may not be a sensitive enough indicator of DNA damage. Thus, we propose that preliminary *in vivo* studies with rodents should be done first to determine if even high concentrations of the suspected genotoxicant would cause cytogenetic damage in peripheral lymphocytes.

A1

MUTAGENICITY OF 1-NITROPYRENE METABOLITES ISOLATED FROM LUNG CELLS AND RESPIRATORY TISSUES. *L. C. King*, L. M. Ball, M. J. Kohan and Joellan Lewtas, EPA, MD68, RTP, NC 27711

The mutagenicity of 1-nitropyrene metabolites from lung S9 incubates, cultures of rabbit alveolar macrophages, and respiratory tissues were evaluated using the Ames *Salmonella typhimurium* plate incorporation assay with strain TA-98, with and without Aroclor induced rat liver S9. The following metabolites were isolated and quantitated by HPLC: solvent front conjugates (SF), polar unknowns A,B and C, 1-nitropyrene-4,5 or 9,10-dihydrodiol (K-DHD), unknown D, N-acetyl-1-aminopyrene (NAAP), 1-aminopyrene (1-AMP), 10-hydroxy-1-nitropyrene (Phenols 1), 4-,5-,6-,8- or 9-hyroxy-1-nitropyrene (Phenols 2), 3-hydroxy-1-nitropyrene (Phenols 3) and unknown Z. All of the metabolites exhibited direct acting mutagenicity; however three of the metabolites (unknown A, Phenols 3 and unknown B) were more potent (2-4x) than the unmetabolized 1-nitropyrene. The mutagenicity of three of the metabolites (NAAP, Phenols 1 and 2) were enhanced by S9 while most of the other metabolites were less mutagenic in the presence of S9. These results indicate that lung cells and respiratory tissues are capable of both oxidative and reductive metabolism which produced mutagenic metabolites, several of which were more potent than the parent compound 1-NP.

This abstract does not necessarily reflect EPA policy.

A2

MUTAGENICITY OF PYRENE IN SALMONELLA. *Z. Matijasevic* and E. Zeiger, Cellular and Genetic Toxicology Branch, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709

Mutagenicity of pyrene has been examined in the standard Salmonella/microsome plate test with strains of TA1537, TA100, TA98 and TA97. In addition, the plate toxicity test (Waleh et al., 1982) with strains NS1137 and NS137 (*his*D and *his*$^+$ derivatives of strain TA1537) was performed. Aroclor-pretreated rat liver S-9 fraction was used for metabolic activation.

Pyrene was mutagenic in all four strains within the same dose range (approximately 1-5 μg/ml), although the magnitudes of the responses were different. The most responsive strain was TA97, and the number of revertants per plate began to decrease at the dose which produced toxicity on the plate and as measured by NS1137 and NS137. At higher doses, however, the toxic response did not increase and the mutagenicity plates exhibited a background lawn that was normal in appearance. This was probably due to precipitation of the pyrene in the agar and may explain many of the inconsistencies in the literature regarding the mutagenicity of pyrene in the plate test, where much higher doses of pyrene were tested.

A3

THE MUTAGENIC STABILITY OF CHEMICALS STORED IN SOLUTION. *D. A. Pagano* and E. Zeiger, Cellular and Genetic Toxicology Branch, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709

Using the *Salmonella*/microsome assay, we have looked at the stability of mutagenic responses of stored chemicals over a period of 18 months. Each of the standard mutagens was prepared in January 1982, and aliquots were stored at −20°C and at −80°C. Sodium azide (NaN_3) was dissolved in water; 4-nitro-o-phenylenediamine (4NOP), 4-nitroquinoline-N-oxide (4NQO), benzo(a)pyrene (BAP) and 2-aminoanthracene (2-AA) were dissolved in DMSO, all at 100 μg/ml. At various times, aliquots were removed, thawed and tested in parallel with freshly weighed and prepared mutagen samples using strain TA100 in a standard plate test and freshly prepared Aroclor 1254-induced rat liver S-9 mix where needed. NaN_3 0.1-0.8 μg/plate, 4NOP 2-10 μg/plate, 4NQO 0.01-0.10 μg/plate, BAP 0.5-2.5 μg/plate, and 2-AA 0.25-2.0 μg/plate showed no differences between the freshly-prepared solutions and the solutions stored at −20°C and −80°C. Variations in dose-response curves were seen for all samples when results from different days were compared.

A4

DESIGN OF AN INTERLABORATORY STUDY FOR THE *SALMONELLA* PLATE ASSAY. Larry Claxton, EPA, MD68, Research Triangle Park, NC 27711; Thomas J. Hughes, Jim Murphy, Lawrence Myers and *Nancy Sexton*, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709

An EPA-sponsored interlaboratory study to investigate the repeatability and reproducibility of results from the *Salmonella*/mutagenicity plate incorporation assay will be described. The study involves three rounds of assays of six samples by each of thirteen laboratories. Problems of data handling and interpretation will be discussed.

A5

ALTERNATIVE ROUTE TO HYPERVALENT IRON IN BIOMIMETIC OXIDATIONS MEDIATED BY PORPHINATOIRON COMPLEXES. *A. Gold*, G. E. Toney, R. Sangaiah, J. E. Savrin, ESE, University of North Carolina, Chapel Hill, NC 27514

Stable products have been isolated and characterized form the reaction of tetraarylporphinatoiron (III) complexes and excess t-butyl hydroperoxide. The compounds have been identified as isoporphyrins in which a t-butyl peroxy anion has been added to a *meso* position of the porphyrin ring. The structure of the isoporphyrins has been established by the distinctive electronic spectrum having strong bands in the near-IR, by high field ^1H NMR and fast atom bombardment mass spectrometry. The presence of the peroxy linkage has been confirmed by redox titration, IR and determination of elemental composition by high resolution mass spectrometry. t-Butyl peroxy substitution of the porphyrin ring was confirmed by demonstrating that the peroxy functionality remains after treatment of the isoporphyrin with hydrogen chloride. The isoporphyrin can oxidize both allylic and aromatic carbons. Similar results were obtained with m-chloroperoxybenzoic acid. Since a number of model mixed function oxidase systems also utilize tetraarylporphinatoiron (III) complexes as catalysts in the presence of excess hydroperoxide or peroxyacid, the compounds identified in this study represent possible intermediates in these model reactions. The peroxy-substituted complexes therefore represent a possible alternative to the high-valent oxoiron species currently assumed to be involved and imply that the "peroxide shunt" may not be an important pathway for organic hydroperoxide oxidations catalyzed by porphinatoiron (III) systems.

B1

DIRECT APPLICATIONS OF THE *SALMONELLA* ASSAY TO TLC PLATES FOR SCREENING COMPLEX HAZARDOUS WASTE STREAMS. *V. Houk*, UNC, Chapel Hill, NC 27514; and L. Claxton, US EPA, MD68, RTP, NC 27711

Investigators are becoming increasingly reliant on bioassay-directed fractionation for characterization of complex environmental mixtures. The present investigation examines the feasibility of coupling the fractionation capabilities of thin layer chromatography (TLC) with the *Salmonella* assay for the detection of mutagens in hazardous waste streams. Neat waste samples were spotted on commercially available cellulose and silica HPTLC plates. Plates were developed and examined for fluorescent activity or any other distinguishable chromatographic pattern. Mutagenic activity was determined by a method that mimics the *Salmonella* spot test. A mix incorporating agar, tester strain, and the metabolic activation system is applied onto the developed side of a chromatogram, permitting the bacteria to come in direct contact with the segregated components on the plate. The subsequent appearance of localized clusters of revertant colonies indicates the presence of a mutagenic constituent. Toxicity, in principle, should interfere less with mutagenic expression because toxic and mutagenic compounds have been separated on the plate. This method eliminates the need for chromatogram sectioning or elution of constituents from the plate. Validation of this approach to genotoxic analysis has been carried out with 15 known mutagens of various polarities and 12 hazardous waste samples.

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

B2

DEVELOPMENT OF PROCEDURES FOR MUTAGENIC ANALYSIS OF AMBIENT AIR VAPORS. T. J. Hughes, *D. M. Simmons*, L. G. Monteith, RTI (Building 3), RTP, NC 27709 and L. Claxton, EPA (MD68), RTP, NC 27711

The overall objective of this research was to further develop an Ames/*Salmonella* preincubation system for detection of mutagenic vapors in ambient air. Parameters for detection of vapors in the Ames/*Salmonella* mutagenicity assay were investigated and 12 vapor-phase compounds were tested for mutagenic potential in the developed protocol with strains TA97, TA98 and TA100, both with and without Aroclor-induced hamster and rat liver S9. The 12 vapor-phase compounds tested were: methylene chloride, formaldehyde, trichloroethylene, ethylene dichloride, epichlorohydrin, nitropropane, nitrobenzene, hydrogen sulfide, butene, propylene, acetylene, and ethylene oxide. Results suggested that: (1) the preferred method for detection of vapor-phase mutagens is to use log phase cells and a preincubation technique, (2) the maximum response for one known mutagen was detected under these conditions: 10 minutes of preincubation, in full vials, with no shaking during the preincubation phase, (3) solvent type and metabolic activation did affect the mutagenic potential of vapors, and (4) ethylene oxide, epichlorohydrin, ethylene dichloride, and methylene chloride were shown to have a positive mutagenic potential.

Research was sponsored by EPA Contract No. 68-21-3170-082.

B3

MUTAGENICITY OF DILUTE PEAT SMOKE PARTICULATE EXTRACTS UNDER SIMULATED ATMOSPHERIC CONDITIONS: A PRELIMINARY STUDY. *D. A. Bell*, R. M. Kamens, ESE, SPH, UNC, Chapel Hill, NC 27514

Dilute peat smoke was added to a 25 m^3 outdoor chamber after combustion in a residential wood stove. The peat smoke was permitted to age by itself or react with sub-ppm concentra-

tions of O_3 and NO_2, both in the dark and under natural sunlight conditions. Mutagenic assays were performed on filter sample particulate extracts using TA 98. Filter extracts of peat smoke aged in the dark were mutagenic both with and without S9 activation but no mutagenic changes were observed. Peat smoke aged in the day under photochemical smog conditions had a comparatively higher −S9 mutagenicity and a lower +S9 mutagenicity. Peat smoke reacted in the dark with 0.261 ppm O_3 and 0.471 ppm NO_2 showed a 3 fold increase for direct acting mutagenicity and a slight increase for indirect acting mutagenicity.

B4

THE POSSIBLE ROLE OF ALCOHOL IN INFLAMMATORY CELL DIFFERENTIATIONS. C. Sachs, B. Thompson, P. Pratt and *W. S. Lynn*, Duke University Medical Center, Durham, NC 27710

Consumption of ethanol by man has been noted to decrease the prevalence and severity of emphysema in cigarette users (Pratt *et al.*, Aspen Lung Conf., Aspen, Colo., June 1983). Cigarette smoke is thought to produce emphysema by activating pulmonary inflammatory cells to secrete elastase and/or by inactivating α-1 trypsin inhibitor. Since studies of the effects of alcohol on differentiated inflammatory cells have yielded inconsistent results, we have evaluated the role of ethanol in inflammatory cell differentiation using cultured HL-60 myeloblasts. At physiological concentrations, ethanol was found to cause complete differentiation of HL-60 cells to neutrophils within 6 days of culture. Differentiation was assessed by (1) decrease in growth rate, (2) disappearance of mitochondria, (3) altered plasma membrane potential, (4) appearance of cytochrome b_{-245} (as assessed at 559 and 428 mμ, (5) production of $O_2^{\cdot}$ (when the cells are activated by F•met-leu-phe or digitonin or phorbol esters), (6) increased content of myeloperoxidase. In contrast to other differentiation agents, e.g. retinoic acid, ethanol activated the NADPH oxidase system $O_2^{\cdot}$ (24 hrs) before the appearance of cytochrome b_{-245} or the disappearance of mitochondria (72 hrs). These data suggest that ethanol may exert its protective effect on lung alveoli *in vivo* by altering inflammatory cell differentiation or maturation in such a manner that neutrophils, rather than pulmonary macrophages, are produced from the alcoholic's precursor cells. The known decrease in circulating leukocytes in alcohol users also suggests that a defect in leukocyte differentiation exists.

C1

PRELIMINARY EXPERIMENTS INVESTIGATING THE ABILITY OF TRIFLORUOTHYMIDINE-RESISTANT MUTANTS OF L5178Y MOUSE LYMPHOMA CELLS TO RE-EXPRESS KINASE ACTIVITY. M. M. Moore, US EPA (MD-68) Research Triangle Park, NC 27711; K. Loud, R. K. Templeton, *K. Lawrence*, Northrop Services, Inc., Research Triangle Park, NC 27711; J. C. Hozier, Florida Institute of Technology, Melbourne, FL 32901

Trifluorothymidine (TFT) is an effective selective agent for thymidine kinase (TK)-deficient mutants of L5178Y mouse lymphoma cells. Mutants can be divided by colony size into (1) small colonies (σ) (many of which show readily observable chromosome abnormalities associated with chromosome 11 – the location of the TK gene) and (2) large colonies (λ) (which may represent events limited to the TK gene). The precise nature of the induced damage causing the loss of the TK enzyme activity for both mutant types is not known. In some cell types, it has been shown that 5-Azacytidine treatment of TK (BrdU-resistant) mutants causes a massive conversion to HAT-resistance (enzyme competency). This presumably results form 5-Azacytidine mediated hypomethylation of the DNA causing the re-expression of previously suppressed but not genetically altered genes. We have begun and are continuing to investigate the mechanisms of TFT-resistance in L5178Y cells. Of particular interest are those σ mutants showing translocations to one chromosome 11. The possibility exists that some of these may

be TK-deficient due to the presence of the translocation (position effect) rather than actual damage within the TK locus itself. Preliminary experiments have been completed for 11 mutants (9 σ, 2 λ). None of these 11 mutants shows a high conversion to TK-competency following 5-Azacytidine treatment.

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

C2

HISTORICAL CONTROL VALUES AND THEIR STATISTICAL CONSEQUENCES FOR THE L5178Y/TK$^{+/-}$ → TK$^{-/-}$ MOUSE LYMPHOMA MUTAGENICITY ASSAY. *D. Clive* and Nancy T. Turner, Wellcome Research Laboratories, RTP, NC

The L5178Y/TK$^{+/-}$ → TK$^{-/-}$ mouse lymphoma assay (MLA) detects both single gene and some classes of viable chromosomal mutations affecting total loss of thymidine kinase (TK) gene activity. Further, these two genetic classes of mutants are distinguishable on the basis of colony diameter, large colony (λ) mutants involving no discernible chromosome defects at the 300-band level of resolution and small colony (σ) mutants usually characterized by a 2-band-or-greater alteration to chromosome 11b, the deduced site of the TK^{+} gene in TK$^{+/-}$ parental cells. This dual endpoint requires careful monitoring through appropriate solvent and positive controls. We present historical data for the most recent 3½ years' use of the MLA in our laboratory on the following parameters: solvent control mutant frequency, f^{b} ($(56 \pm 16) \times 10^{-6}$, N = 225); solvent control plating efficiency (86 ± 10%, N = 225); positive control (hycanthone, without S9; 2-AAF with S9) mutant frequency and σ/λ ratio. Consequences of severe deviations from these ranges are explored. In addition to these historical control data, intra-experimental variation in mutant frequency in the MLA have been assessed in 3 dodecuplicate (i.e., N = 12) experiments: for no treatment, $f^{b} \pm \sigma = (44 \pm 5) \times 10^{-6}$, range = $(33-52) \times 10^{-6}$; for treatment with 8% DMSO + S9 ("worst case" condition of toxicity – 18% survival – with little or no mutagenicity), $f \pm \sigma = (81 \pm 11) \times 10^{-6}$, range = $(66-102) \times 10^{-6}$; for treatment with hycanthone (10 μg/ml; 22% survival), $f \pm \sigma = (666 \pm 129) \times 10^{-6}$, range = $(531-1031) \times 10^{-6}$. These data support the use of a "doubling" rule in assessing statistical significance in the MLA.

C3

A COMPARISON OF CLASTOGENIC AND MUTAGENIC ACTIVITIES OF THREE MUTAGENS AND A NON-MUTAGEN IN THE L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA SYSTEM. *J. Eyre*[a], D. Clive[a], R. Krehl[a], N. Turner[a], J. C. Hozier[b] and J. Sawyer[b]. [a]Wellcome Research Laboratories, RTP, NC; [b]Florida Institute of Technology, Melbourne, FL

Using a micro-well cytogenetic technique with the L5178Y/TK$^{+/-}$ mouse lymphoma system we were able to study simultaneously the clastogenic and mutagenic properties of methapyrilene, methotrexate, caffeine and colchicine. From samples of the same treated cell populations following exposure to a chemical agent, we showed: (1) concentrations of methapyrilene and methotrexate which produced meaningful cytotoxicity (10-90% lethality), induced mutant frequencies at the TK locus which were 4- to 10-fold over background, and also produced increases in chomosome breakage that were 10- to 19-fold over background; (2) 4 hr exposure at 37°C to caffeine was neither mutagenic nor clastogenic; under all other treatment conditions studied (25°C, 4 hr or 24 hr exposure; 37°C, 24 hr exposure) caffeine was both mutagenic (8.5-fold over background) and clastogenic (12-fold over background); (3) concentrations of colchicine which produced meaningful cytotoxicity did not induce an increase in either mutant frequency at the TK locus or chromosome breakage; (however, colchicine was very effective in producing polyploidy); (4) *in situ* techniques for banded karyotyp-

ing (300-band level of resolution) of cells from small (σ) $TK^{-/-}$ mutant colonies induced by methapyrilene, methotrexate and caffeine revealed the presence of chromosome abnormalities involving chromosome 11b (which is presumed to carry the TK^{+} gene in the parental $TK^{+/-}$ cells); (5) the three clastogens produced high proportions of small (σ) mutant colonies (methapyrilene 72%, caffeine 80%, methotrexate 40%), while the spindle poison colchicine was ineffective in inducing σ or λ (large) mutant colonies; (6) when σ and λ mutant frequencies were plotted separately against the % aberrant metaphases in the same caffeine treated cultures, a linear relationship existed between the two for σ $TK^{-/-}$ mutants but not for λ $TK^{-/-}$ mutants. These facts underscore the ability of the L5178Y/$TK^{+/-}$ mouse lymphoma system to detect both gene and viable chromosome damage by differentiating between the two colony size classes.

C4

TYPES OF CYTOGENETIC DAMAGE DETECTED IN THE L5178Y/$TK^{+/-}$ → $TK^{-/-}$ MOUSE LYMPHOMA ASSAY BY BANDED KARYOTYPE ANALYSIS. *Roberta Krehl*[a], D. Clive[a], J. C. Hozier[b] and J. Sawyer[b]. [a]Wellcome Research Laboratories, RTP, NC; [b]Florida Institute of Technology, Melbourne, FL

At the 300-band level of resolution, small colony (σ) $TK^{-/-}$ mutants induced by a variety of mutagens demonstrate a spectrum of damage to chromosome 11b, the deduced location of the single functional thymidine kinase (TK) gene in the parental $TK^{+/-}$ cells; large colony (λ) $TK^{-/-}$ mutants are all karyotypically normal. Quantitatively, this damage varies from less than the 1–2 band limit of resolution (i.e., apparently normal chromosome 11b) up to a whole chromosome 2 translocated to the distal end of 11b. Qualitatively, there is variation in the type of chromosome 11b damage arising from 2 sources – the mutagen used and how many cell generations have elapsed between mutant induction and karyotype analysis. For classical mutagens such as MMS, EMS, and hycanthone, late karyotype analysis (e.g. 20–30 generations) reveals mostly large translocations to the distal end of chromosome 11b. The antifolates methotrexate and pyrimethamine and the antiviral drug acyclovir produce primarily lesser damage, ranging from ca. 2 band insertions into or deletions from chromosome 11b to apparently normal karyotypes at this late stage of analysis. Earlier karyotyping (e.g., ca. 10 generations) reveals both a higher proportion of aberrant 11b's (88% vs 59% at 20–30 generations) as well as a high proportion (27%) of dicentrics involving chromosome 11b. These dicentrics are usually evolving toward normal chromosome 11b's through random breakage over the other chromosome's length. Trisomy 11 has been observed at 20–30 generations in 1 acyclovir-induced mutant and in 1 pyrimethamine-induced mutant while tetrasomy 11 has been seen at earlier analysis in a methapyrilene-induced mutant. Thus insertions, deletions, dicentrics, translocations and aneuploidy events involving the selected chromosome 11 appear to be responsible for various mutagen-induced σ $TK^{-/-}$ mutants. At the present, however, it is not possible to estimate how quantitative the recoveries are of these various classes of chromosomal events.

C5

COMPARATIVE MUTAGENICITY STUDIES ON METHAPYRILENE AND PYRILAMINE IN THE L5178Y/$TK^{+/-}$ → $TK^{-/-}$ MOUSE LYMPHOMA ASSAY. *Nancy T. Turner*, J. Woolley and D. Clive, Wellcome Research laboratories, RTP, NC

Methapyrilene (MP), a sedating antihistamine, is a potent rat hepatocarcinogen (Lijinsky et al., Science, 209 (1980) 817–819). Previous studies (Environ. Mutagen. (Abstr) 5:420) have shown MP to be mutagenic in the L5178Y/$TK^{+/-}$ → $TK^{-/-}$ mouse lymphoma assay (MLA). Increases in $TK^{-/-}$ mutant frequency up to 5-fold over spontaneous have been observed at con-

centrations of 500-600 μg/ml in the presence of an Aroclor-induced rat liver S9 activation system. Primarily small colony mutants of previously demonstrated chromosomal origin (Hozier, *et al.*, Mutation Res. 84 (1981) 169-181) were induced, indicating that MP is clastogenic; this was confirmed by a standard breakage study in aliquots of MP-treated mouse lymphoma cells subsequently cloned for $TK^{-/-}$ mutations. More recently, the antihistamine pyrilamine (Pyr), a non- or weakly carcinogenic analog of MP (Lijinsky, personal communication), has been examined in this same assay. Pyr is non-mutagenic in the MLA in the absence of S9 over the concentration range of 100-700 μg/ml, as is MP. In the presence of S9, Pyr gave somewhat variable results with respect to both cytotoxicity and mutagenicity; these are believed to relate to the use of commercial S9, a phenomenon seen to a lesser extent in our earlier extensive studies with MP. Overall, Pyr appeared to be less mutagenic than MP at comparable concentrations and cytotoxicities. This difference, although not sufficient to explain the dramatic difference in carcinogenicity in the rat, is at least in the right direction, in marked contrast to results obtained in rat hepatocyte UDS assay (McQueen and Williams, J. Toxicol. Environ. Hlth., 8 (1981) 463-477).

C6

SISTER CHROMATID EXCHANGE INDUCTION IN MOUSE B AND T LYMPHOCYTES EXPOSED TO CYCLOPHOSPHAMIDE *IN VITRO* AND *IN VIVO*. *James L. Wilmer*, Gregory L. Erexson, and Andrew D. Kligerman. Dept. of Genetic Toxicology, Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, NC 27709

Cyclophosphamide (CPA) is known to exert greater toxic effects on B- than T-lymphocytes *in vivo*. Both *in vitro* and *in vivo* CPA treatments were used to assess the possible cytogenetic basis for these observations. First, male C57Bl/6 mouse lymphocytes were stimulated to divide *in vitro* with either phytohemagglutinin (T-cell mitogen) or lipopolysaccharide (B-cell mitogen) and then treated with CPA (0.05-1.0 mM) and 5-bromo-2-deoxyuridine (2 μM) at 24 hr. Cultures were harvested at 60 hr following a 4 hr treatment with demecolcine (1.35 μM). CPA caused concentration-related increases in SCE up to 3 times control frequencies; the resulting SCE induction curves for B- and T-cells were sigmoidal and equivalent. Second, mice were given a single i.p. injection of CPA (0.5, 1.0, or 5.0 mg/kg). Blood was removed 24 hr later and cultured without additional CPA as described above. Dose-related increases in SCE frequencies were seen for both T- and B-lymphocytes. CPA induced consistently 2.5 to 3.7 more SCEs in B-cells than in T-cells. Thus, B- and T-lymphocytes exhibited an equal sensitivity to CPA *in vitro*, but B-cells were more susceptible to the genotoxic effects *in vivo*.

C7

SISTER CHROMATID EXCHANGE INDUCTION IN CULTURED PERIPHERAL BLOOD LEUKOCYTES OF A COLDWATER MARINE FISH. *Helen R. Zakour*, Marsha L. Landolt, and Richard M. Kocan, School of Fisheries, WH-10, University of Washington, Seattle, WA 98195.

The major objective of this research was to develop an *in vitro* sister chromatid exchange (SCE) assay, which would utilize the peripheral blood leukocytes (PBLs) of a coldwater marine fish species. Use of PBLs in cytogenetic genotoxicity tests has several advantages, the major one being that the experimental fish need not be sacrificed for sample collection. This non-destructive method of tissue collection permits the investigator to take multiple samples from a single individual and thereby allows one to use an individual as its own control and to monitor its SCE frequency over time. The *in vitro* conditions necessary to provide a sufficient number of dividing cells for performance of the SCE assay were established in our laboratory for the PBLs of the Pacific staghorn sculpin *(Leptocottus armatus)*, a common bottom-dwelling Puget

Sound fish. The major components of this culture system are heparinized whole blood, fetal bovine serum-supplemented enriched tissue culture medium (RPMI-1640), a mitogen (purified protein derivative of tuberculin) and an incubation temperature of 13.5°C. This *in vitro* PBL culture system is unique because it contains cultured blood cells from a coldwater marine fish species. Using this culture method, SCE induction was investigated in Pacific staghorn sculpin PBLs which had been exposed *in vitro* to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a known direct-acting inducer of SCEs. Cultured cells exposed *in vitro* responded to MNNG in a dose-related manner in regard to SCE induction. This PBL/SCE assay may be a feasible assessment tool for detecting exposure of marine fish to genotoxic environmental contaminants in laboratory and field situations.

C8

MODIFICATIONS OF GENE EXPRESSION AND DIFFERENTIATION IN RAT TRACHEAL EPITHELIAL CELLS EXPOSED TO A TUMOR PROMOTER. *V. E. Steele*, Northrop Services, Inc., RTP, NC 27709; and R. Wu, W. Alton Jones Cell Science Center, Lake Placid, NY 12946.

Since TPA (12-O-tetradecanoylphorbol-13-acetate) has been shown to enhance carcinogenesis both *in vivo* and *in vitro* in rat tracheal epithelium, the same system was used to study alterations in gene expression and cellular differentiation following TPA exposure. Epithelial cells were isolated form Fisher F-344 male rat tracheas. The cells were plated onto collagen-coated dishes in medium containing 0, 1, 10 or 100 ng TPA/ml. At seven days a portion of the cultures were dissociated to determine cell number and a portion labeled with ^{14}C-amino acids to determine patterns of protein synthesis. To begin determining TPA-induced changes in cellular differentiation, keratin was extracted from the labeled cells and analyzed by polyacrylamide gel electrophoresis. An increase in cell number per culture was observed in cultures exposed to TPA. The 10 ng TPA/ml group showed a 22-fold increase as compared to controls. Gel electrophoresis studies using keratin protein extracts showed over a 3-fold increase in total keratin being synthesized and, in particular, increased synthesis in the 48–59K region. Quantitative analysis of the autoradiograms revealed several bands that showed dose-dependent increases in new synthesis. The results of these studies indicate that TPA stimulates cell proliferation and alters gene expression in cultured tracheal epithelial cells.

C9

EVALUATION OF GENOTOXIC EFFECTS IN HUMAN POPULATIONS. J. W. Allen[a], L. D. Claxton[a], N. E. Garrett[b], S. L. Huang[b], M. M. Moore[a], Y. Sharief[b], G. H. S-Strauss[a], and *M. D. Waters*[a]. [a]US EPA, Research Triangle Park, NC 27711; [b]Northrop Services Inc., Research Triangle Park, Nc 27709

There are demonstrable associations in experimental animals between DNA damage in somatic cells and the development of cancer, and between DNA damage in germ cells and the incidence of genetic disease in offspring. Thus, there is substantial evidence of the need to detect and to quantitate chemically-induced genetic damage in humans in order to assess the potential for cancer and genetic disease. Current methods for estimating human risk of cancer have been based on knowledge of human exposure and epidemiological data. An alternative approach would base such estimates of risk on knowledge of exposure and of damage to the DNA of human cells and tissues. The principal difficulty in performing such assessments lies in the uncertainty of translating information from tests for genetic damage in human cells and tissues into reliable estimates of risk for cancer or genetic disease. This poster concerns: (1) the development of mammalian cell methods that may be used ultimately to evaluate genotoxic effects in humans and (2) our initial attempts to implement a "parallelogram" approach whereby such methods can be used, collectively, to relate quantitative information on genetic damage

to quantitative estimates of risk for cancer or genetic disease. The parallelogram approach involves somatic and germ-cell monitoring techniques wherein quantitative dose-response relationships are developed among component test systems. Estimates of chemical (external) exposure and cellular and molecular (internal) dose provide the quantitative basis extrapolating data from whole animal systems to human populations. The use of animal and human cells *in vitro* enables direct comparisons of induced effects under controlled dosages. Such comparisons are based on measurements of total dose to the DNA or common molecular products in the DNA determined after exposure of the organism to the agent in question.

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

D1

COMPARISON OF RAT AND HAMSTER HEPATOCYTE DNA REPAIR GENOTOXICITY ASSAYS. D. J. Kornbrust, *M. E. Hall* and T. R. Barfknecht, Research Triangle Institute, Building 3, Research Triangle Park, NC 27709

Several studies have demonstrated significant differences in the capacity of cell-free homogenates and/or hepatocytes derived from rat and hamster livers to metabolize chemicals to genotoxic agents. However, few investigations of species differences in DNA repair capacity have been performed. Therefore, a comparison of the relative extent of DNA repair induced by various genotoxic chemicals in rat and hamster hepatocytes was conducted, employing the hepatocyte primary culture/DNA repair assay. Of the 18 chemicals tested, 10 were more potent in inducing DNA repair in hamster hepatocytes than in rat hepatocytes. Dimethylnitrosamine, diethylnitrosamine, 2-acetylaminofluorene, 9-aminoacridine, pararosaniline hydrochloride, 1-naphthylamine, 2-napthylamine, benzidine and 1,2:3,4-diepoxybutane were all active in hamster hepatocytes at an order of magnitude, lower concentration than in rat hepatocytes. The dye trypan blue was 3- to 4-fold more active in hamster hepatocytes. Compounds having similar DNA repair inducing activity in both hepatocyte types were methylmethane sulfonate, Congo Red, 2,5-diaminotoluene, o-toluidine, and N,N'-dimethyl-p-phenylenediamine. 1-Nitropyrene was found to be a more potent inducer of DNA repair in rat than hamster hepatocytes. Chemicals that elicited a negative response in both hepatocyte types were o-toluidine and the bacterial mutagen 3-(chloromethyl)pyridine hydrochloride. These studies demonstrate the utility of using the hamster as a source of hepatocytes for the hepatocyte/DNA repair assay. The data also suggest that hamster hepatocytes may prove to be useful for genotoxicity testing when the sample size of the test agent is limited.

D2

METABOLISM OF BENZO(a)PYRENE TO ACTIVE AND DETOXIFIED PRODUCTS IN PRIMARY CULTURES OF HUMAN HEPATOCYTES. *Stephen Strom*, Alan Novotny, Randy Jirtle, and George Michalopoulos. Duke Medical Center, Durham, NC 27710.

Primary cultures of isolated human hepatocytes were exposed to 0.1 μM Benzo(a)pyrene (BP) for 24 hr. The cultures were subsequently scraped and the hepatocytes were centrifuged into a pellet. Cell-free culture media was extracted 3 times with 2 volumes of ethyl acetate to remove organic solvent soluble BP metabolites. Water soluble metabolites were identified by release into the organic solvent soluble phase by treatment of the media with β-glucuronidase or aryl-sulfatase. BP-metabolites were separated and identified by high pressure liquid chromatography, using a 60% - 100% methanol: water gradient and a Zorbax ODS column. The major metabolites found in the organic phase extract of the culture media were identified as 3-OHBP, BP-7,8- and 9,10-dihydrodiol, and BP-quinones. Smaller and more variable amounts

of 9-OHBP, BP-4,5-dihydrodiol, and an unidentified metabolite migrating pre-4,5 dihydrodiol were observed. Large amounts of the 9,10-, 4,5-, and the pre-4,5-dihydrodiol metabolites generated by the cells were recovered in the media as glucuronic acid conjugates. The major BP metabolites recovered from the culture media as the sulfate conjugates were identified as 3-OHBP, and the unidentified pre-4,5 dihydrodiol metabolite. There was clear evidence of the generation of the (anti)diolepoxide metabolite of BP by the recovery of its principal hydrolysis product, BP-7,10/8,9-tetrol. These results indicate that human hepatocytes metabolize BP to its ultimately carcinogenic and mutagenic metabolite.

D3

RESPONSE OF PRIMARY CULTURES OF PROLIFERATING HEPATOCYTES TO CARCINOGENS. *Deborah L. Novicki*[a], Mark R. Rosenberg[a], George Michalopoulos[a,b]. Departments of Pathology[a] and Radiology[b], Duke University Medical Center, Durham, N.C. 27710

Rat hepatocytes in primary culture can be stimulated to replicate under the influence of rat serum and sparse plating conditions. Higher replication rates are induced by serum from ⅔ partially hepatectomized rats. The effects of carcinogens and noncarcinogens on the ability of hepatocytes to synthesize DNA was examined by measuring the incorporation of 3H-thymidine by liquid scintillation counting and autoradiography. Hepatocyte DNA synthesis was not decreased by ethanol or dimethylsulfoxide at concentrations less than 0.5%. No effect was observed when 0.1 mM ketamine, nembutal, phenobarbital, hypoxanthine, sucrose, ascorbic acid, or benzo(e)pyrene was added to cultures of replicating hepatocytes. Estrogen, testosterone, tryptophan, and vitamin E inhibited DNA synthesis by approximately 50% at 0.1 mM, a concentration at which toxicity was noticeable.

The direct-acting carcinogen, MNNG, and several carcinogens requiring metabolic activation interfered with DNA synthesis. Aflatoxin B1 inhibited DNA synthesis at concentrations as low as 1×10^{-8} M. AAF decreased the incorporation of 3H-thymidine at concentrations greater than 1×10^{-8} M. Benzo(a)pyrene and 3-methyl-DAB inhibited DNA synthesis at 1×10^{-5} M. Diethyl- and dimethylnitrosamine and 1- and 2-naphthylamine inhibition of DNA synthesis overlapped with concentrations that caused measurable toxicity.

The ability of hepatocytes to activate AAF to reactive intermediates capable of binding to DNA and inhibiting new DNA synthesis decreased as a function of time in culture. Gamma-glutamyltransferase-positive hepatocytes from DEN-treated rats were observed to be less sensitive to carcinogen inhibition of DNA synthesis.

El

QUALITY ASSURANCE FOR MUTAGENICITY STUDIES. *R. S. DeWoskin*, B. M. Sadler, and Nancy H. Adams, Research Triangle Institute, Research Triangle Park, NC 27709

Sponsors of health effects research, both government and corporate, have recently increased their demands for a quality assurance program to accompany the work they fund. The evaluation of the quality of a study is performed by comparing selected attributes of the study to a set of reference standards. Often the conclusions of quality evaluations are contradictory when different standards or different study attributes are used. To aid the quality assurance effort, the US Food and Drug Administration published a set of reference standards known as the Good Laboratory Practices Regulations (GLPs) for Nonclinical Laboratory Studies (Title 21 Vol. 48, Federal Register, December 22, 1978). Intended for a broad application, the GLPs were written in general language so that researchers could apply their expertise to the design of the specific quality control procedures applicable to their work. In practice, however, the design of the specific procedures and their relationship to the quality of the study have been cause for heated debate. This poster discusses one approach to the prioritization of specific practices for quality control and quality assurance monitoring in light of three criteria: End Use of Data; Effects of Assay Error on Data Error; and Cost of Additional QA/QC Information. The Ames assay is used as a test case to demonstrate the application of the above criteria to the design of the quality assurance program.

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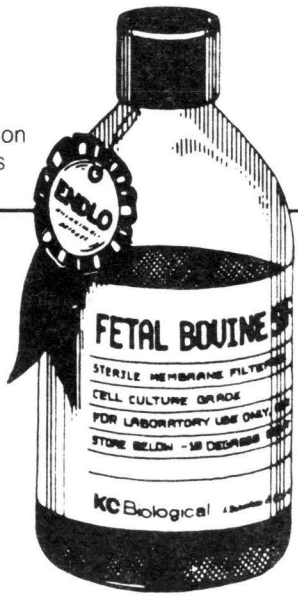

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