GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

FIFTH ANNUAL MEETING

PROGRAM AND ABSTRACTS

October 22, 1987
Sheraton Imperial
Research Triangle Park, North Carolina
7:45 a.m. to 6:00 p.m.

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

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Genotoxicity and Environmental Mutagen Society

Dear GEMS Member:

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

GEMS is pleased to have **Dr. David Brusick,** of Hazleton Biotechnologies Corporation as the keynote speaker. He will speak on, "Genetic Toxicology: Past, Present, and Future." We will also have two other featured talks. **Dr. Vernon Steele** from Northrop Services will discuss "In Vivo Teratology Testing," and **Dr. Joseph Haseman** from N.I.E.H.S. will talk on "Comparative Results of Animal Carcinogenicity Studies." In addition, scientists will be competing for the Third Annual GEMS Travel Award—to attend the Environmental Mutagen Society Meeting in Charleston, SC, in March 1988—and the award for best presentation by a junior scientist.

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

I thank **Dr. Gene Elmore** for organizing this Annual Meeting for the second year in a row; I also thank **Dr. Louise Ball, Ms. Diane Daston, Mr. Tom Hughes, Mr. Dennis Pagano, Ms. Virginia Houk,** and **Dr. Gene Elmore** for the time and effort they put into the Society during the last year. I congratulate the new officers and members of the board, and thank the retiring members for their service to GEMS. This is my last official day as President of GEMS, and I would like to thank all the members and sponsors for their support. I hope the Society will continue to grow and remain a vital force in the field of genetic toxicology and mutagenicity.

Welcome to the Fifth Annual Meeting of GEMS.

Sincerely,

Andrew D. Kligerman
President

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

SUN BROKERS, INC. and ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC., are cosponsoring the coffee and danish at 9:45 a.m.

INTEGRATED LABORATORY SYSTEMS is cosponsoring, with GEMS, the social hour at 4:50 p.m.

BURROUGHS WELLCOME COMPANY is sponsoring the Best Talk by a Junior Scientist award.

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PROGRAM
GEMS FIFTH ANNUAL MEETING
October 22, 1987
7:45 a.m. to 6:00 p.m.

Sheraton Imperial, Research Triangle Park, NC

7:45 a.m.	REGISTRATION OPENS
8:15 a.m.	Welcoming Remarks, A. Kligerman, President, GEMS
8:18 a.m.	**T1** USE OF WATERS SEPPAK C-18 CARTRIDGES FOR DETECTION OF GROWTH FACTORS IN CONDITIONED MEDIUM. *V. Y. Lawrence* and J.M. Siegfried. EHRT, RTP, NC 27709.
8:30 a.m.	**T2** *THE PERSISTENCE OF SISTER CHROMATID EXCHANGES IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING IN VIVO EXPOSURE TO DIAZIQUONE. M. F. Bryant,* G. L. Erexson, and A. D. Kligerman. EHRT, RTP, NC 27709.
8:42 a.m.	**T3** HETEROZYGOUS EFFECTS OF MULTILOCUS DELETION MUTATIONS AT THE *AD-3A* LOCUS IN *NEUROSPORA CRASSA. L. K. Overton* and F. J. de Serres. RTI, RTP, NC 27709.
8:54 a.m.	**T4** MUTAGENICITY EVALUATION OF NOVEL AMINOAZO DYES AND THEIR REDUCTIVE CLEAVAGE PRODUCTS IN *SALMONELLA TYPHIMURIUM. J. F. Esancy,*[2] L. D. Claxton,[2] H. S. Freeman[1] and M. K. Esancy.[1] [1]NCSU, RALEIGH, NC 27695; [2]US EPA, RTP, NC 27711.
9:06 a.m.	**T5** MOLECULAR ANALYSIS OF *HIS-3* MUTANTS OF *NEUROSPORA CRASSA:* CORRELATION BETWEEN BIOCHEMICAL AND GENETIC DATA. *J. S. Dubins,* L. K. Overton, R. R. Cobb and F. J. de Serres. RTI, RTP, NC 27709.
9:18 a.m.	**T6** ISOLATION AND CHARACTERIZATION OF THE DNA ADDUCT N-(DEOXYGUANOSIN-8-YL)-3-AMINOFLUORANTHENE. *A. M. Dietrich,* L. M. Ball, A. Gold. UNC, CHAPEL HILL, NC 27599.
9:30 a.m.	**T7** MOLECULAR FRAGMENT ANALYSIS OF RETINOIDS. *S. W. Woods,*[1] M. J. Maas.[2] [1]EHRT, RTP, NC 27709' [2]US EPA, RTP, NC 27711.
9:42 a.m.	**T8** SOUTHERN BLOT ANALYSIS OF THE *TK* LOCUS IN LARGE AND SMALL COLONY MOUSE LYMPHOMA CELL MUTANTS. *J. Martin,* E. Korytynski, T. Hughes, L. Monteigh, and R. R. Cobb. RTI, RTP, NC 27709.

9:54 a.m.	**Coffee, Posters, Exhibits**
11:12 a.m.	**T9** POTENTIAL USE OF HUMAN AMNIOTIC FLUID TO DETECT TRANSPLACENTAL MUTAGENS. *C. Fomous*, Georgetown Univ. Hosp., Washington, DC 20007-2197.
11:24 a.m.	**T10** USE OF SOLID PHASE EXTRACTION IN THE RECOVERY OF MUTAGENS FROM CIGARETTE SMOKERS' URINE. *R. Williams,*[1] R. Watts,[2] J. Inman,[2] T. Pasley,[1] J. Fitzgerald,[2] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.
11:36 a.m.	**T11** APPLICATION OF THE "COMPUTERIZED LABORATORY NOTEBOOK" CONCEPT TO THE TCGF RESPONSE AND PRODUCTION ASSAY. G. H. S. Strauss,[1] *S. J. Berkowitz,*[2] and W. L. Stanford.[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.
11:48 a.m.	**T12** DEVELOPMENT OF METHODS TO EVALUATE IMMUNOTOXIC EFFECTS OF ENVIRONMENTAL MUTAGENS: INDIVIDUAL CAPACITY TO PRODUCE AND RESPOND TO TCGF. G. H. S. Strauss,[1] S. J. Berkowitz,[2] and *W. L. Stanford.*[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.
Noon - 1:30 p.m.	**Lunch, Business Meeting, Recognition of Exhibitors and Sponsors, Installation of New Officers and Board of Directors Members.**
1:30 p.m.	**Keynote Address: D. Brusick. Genetic Toxicology Testing: Past, Present, and Future.**
2:30 p.m.	**T13** INTRODUCTION OF A FUNCTIONAL RAT P-450b cDNA INTO THE MOUSE C3H10T1/2CL8 CELL LINE PRODUCES INCREASED 2-AAF METABOLISM. *S. K. Hansen,*[1] J. A. Ross,[2] J. M. Siegfried,[1] S. Leavitt,[2] K. Rudo,[3] R. Langenbach,[3] and S. Nesnow.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711; [3]NIEHS, RTP, NC 27709.
2:42 p.m.	**T14** MUTAGENESIS RESEARCHERS' VIEWS ON THE SIGNIFICANCE AND PROPER USE OF MUTAGENICITY TESTS APPLIED TO CLINICAL SPECIMENS. *D. Busch,*[1] D. Easterling,[2] H. Leventhal,[2] and G. T. Bryan.[3] [1]AFIP, Washington, DC; [2] UPA, Philadelphia, PA 19104; [3]U WI, Madison.
2:54 p.m.	**T15** SYNAPTONEMAL COMPLEX DAMAGE IN RELATION TO MEIOTIC METAPHASE CHROMOSOME ABERRATIONS AFTER EXPOSURE OF MALE MICE TO CYCLOPHOSPHAMIDE. *L. Backer*[1], J. Gibson[2], M. Moses[2], D. Howard[1], and J. Allen[3]. [1]EHRT, RTP, NC 27709; [2]Duke University., Durham, NC; [3]US EPA, RTP, NC 27711.

3:06 p.m.	**T16** DOSE-DEPENDENT NEUTROPHIL MYELOPER-OXIDASE-DEFICIENCY AND ONSET OF MYELO-GENOUS LEUKEMIA IN γ-IRRADIATED BEAGLE DOGS: AN OPPORTUNITY TO LINK SUBTLE EFFECTS OF MUTAGEN EXPOSURE WITH DISEASE CONSE-QUENCES? *G. H. S. Strauss*[1] and J. S. Hanker[2]. [1]US EPA, RTP, NC 27711; [2]UNC, Chapel Hill, NC 27599.
3:18 p.m.	**Coffee, Posters, Exhibits**
3:45 p.m.	**Featured Talk: V. E. Steele: In Vitro Teratology Testing**
4:15 p.m.	**Featured Talk: J. K. Haseman: Interspecies correlations in Longterm Carcinogenicity Studies**
4:45 p.m.	**Awards: Best Talk, Travel**
4:50 p.m.	**Social: cheese, fruits, beverages**
6:00 p.m.	**Adjourn**

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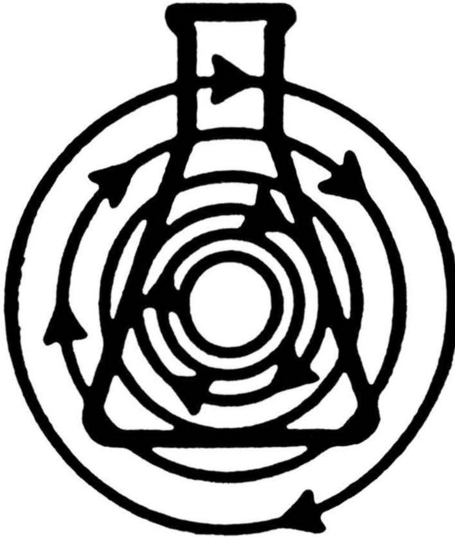

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POSTERS

P1 THE DETECTION OF GENOTOXIC ACTIVITY IN HAZARDOUS WASTE SAMPLES USING THE MICROSCREEN PHAGE-INDUCTION ASSAY. *V.S. Houk* and D.M. DeMarini. US EPA, RTP, NC 27711.

P2 APPLICATION OF ^{32}P POSTLABELING ASSAY IN THE ANALYSIS OF DNA ADDUCTS FOLLOWING EXPOSURE TO COMPLEX MIXTURES. *M. George,*[1] J. Gallagher,[2] I. Robertson,[2] M. Jackson,[1] M. Kohan,[2] J. Scott[1] and J. Lewtas.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

P3 COMPARISON OF MICRONUCLEUS FREQUENCIES IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING EITHER IN VITRO OR IN VIVO EXPOSURE TO COBALT-60 GAMMA RADIATION. *G.L. Erexson,*[1] A.D. Kligerman,[1] J.W. Allen,[2] E.C. Halperin,[3] and G. Honoré.[3] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711; [3]Duke Univ., Durham, NC 27710.

P4 INDUCTION OF PROPHAGE LAMBDA BY CHLORINATED ORGANIC COMPOUNDS. *D.M. DeMarini,*[1] D.G. Parkes, Jr.,[1] and H.G. Brooks.[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

P5 CYTOTOXICITY OF THREE BUFFERS TO SALMONELLA STRAIN TA98 IN A MICROSUSPENSION REVERSE-MUTATION ASSAY AND THE EFFECT OF THESE BUFFERS ON THE MUTAGENIC POTENCIES OF COMPLEX MIXTURES AND PURE COMPOUNDS. *M.M. Dallas,*[1] L.R. Forehand,[1] and D.M. DeMarini.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

P6 MUTAGENICITY OF FOUR ACRYLATE COMPOUNDS IN L5178Y MOUSE LYMPHOMA CELLS AND CHINESE HAMSTER OVARY CELLS. *C.S. Millis,*[1] L. Parker,[1] K.H. Brock,[1] C.L. Doerr,[1] K.L. Dearfield,[2] and M.M. Moore.[3] [1]EHRT, RTP, NC 27709; [2]US EPA, Washington, DC 20460; [3]US EPA, RTP, NC 27711.

P7 EFFECTS OF α-NAPHTHOFLAVONE ON NUCLEOID SEDIMENTATION IN CHINESE HAMSTER OVARY CELLS AND IN LYMPHOCYTES FROM SMOKERS AND NONSMOKERS. *J.M. Goldring,*[1] G.W. Lucier,[2] and C.L. Thompson.[2] [1]UNC-CH, Chapel Hill, NC 27514; [2]NIEHS, RTP, NC 27709.

P8 SCREENING OF CODED TERATOGENS IN TWO IN VITRO ASSAYS. *B.P. Wilkinson,*[1] E.L. Elmore,[1] R.E. Morrissey,[2] J.C. Lamb IV,[2] D.G. Rocha,[1] D.D. Murphy,[1] and V.E. Steele.[1] [1]Northrop Services, RTP, NC 27709; [2]NIEHS, RTP, NC 27709.

P9 GENETIC STUDIES OF A BEHAVIORAL MUTATION INDUCED BY ETHYLENE OXIDE IN THE MOUSE. *L.B. Barnett,*[1] S.E. Lewis,[1] M. Davisson.[2] [1]RTI, RTP, NC 27709; [2]Jackson Lab, Bar Harbor, ME 04609.

P10 ON DNA ADDUCTS USING A ^{32}P-POSTLABELING TECHNIQUE. *S.L. Huang.* EHRT, RTP, NC 27709.

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ABSTRACTS
TALKS

T1 USE OF WATERS SEPPAK C-18 CARTRIDGES FOR DETECTION OF GROWTH FACTORS IN CONDITIONED MEDIUM. *V.Y. Lawrence* and J.M. Siegfried. EHRT, RTP, NC 27709.

Our laboratory has been studying the secretion of Transforming Growth Factor a (TGHα) and Insulin-like Growth Factor-1 (IGF-1) by established lung tumor cell lines. We investigated the use of Waters Seppak C-18 columns in the isolation of these growth factors from conditioned medium. To check the recovery of growth factors from Seppak C-18 columns, Epidermal Growth Factor (EGF) added to Ham's F-12 serum-free medium was applied to columns that had been prewet with acetonitrile and rinsed with water. In half of the samples, BSA (1mg/ml) was added with EGF to simulate protein content of medium conditioned by tumor cells. These columns were then eluted with 80%, 60%, or 40% acetonitrile and the eluant was concentrated using a Speed Vac concentrator. The resulting material was reconstituted in binding buffer and used in an assay to determine competition for binding to EGF receptors. The results showed that even in the presence of high protein levels, EGF was recovered. The best recovery of EGF, 93%, was obtained with an elution of 40% acetonitrile. IGF-1 was also recovered at an efficiency of 67-100% from Seppak columns. Medium conditioned by A549-1, Calu 6, and 1000T cells (3 established lung tumor lines) was collected and applied to Seppak columns. The protein was eluted with 40% acetonitrile and the amount of TGF-α and IGF-1 as assayed by binding to EGF receptor or radioimmunoassay.

This research was supported by contract 68-02-4456 from the U.S. Environmental Protection Agency. This is an abstract of a research presentation and does necessarily reflect US EPA policy.

T2 THE PERSISTENCE OF SISTER CHROMATID EXCHANGES IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING IN VIVO EXPOSURE TO DIAZIQUONE. *M.F. Bryant,* G.L. Erexson, and A.D. Kligerman. EHRT, RTP, NC 27709.

Previous studies in our laboratory have shown diaziquone (AZQ) to be an efficient inducer of sister chromatid exchange (SCE) in vivo and in vitro in mouse peripheral blood lymphocytes (PBLs). AZQ was used to treat mouse PBLs in vivo to determine the persistence of SCEs. Animals were exposed to either 0, 1.25, or 5.0 mg AZQ/kg by i.p. injection in RPMI 1640 plus 10% DMSO. Blood was removed by cardiac puncture by 1, 3, 7, 14, 21, 28, 77, and 99 days after treatment. Mononuclear leucocytes were isolated using Lymphocyte Separation Medium® and cultured for 50 h according to the method of Erexson and Kligerman (*Environ. Molec. Mutagen.*, in press), using RPMI 1640 medium supplemented with phytohemagglutinin (5 µg/ml). Two animals/dose and 50 cells/animal were scored for SCE. The highest induced SCE frequency was observed at day 1 post-treatment. The SCE frequencies were 11.33 and 44.55 for the low and high doses of AZQ, respectively. By day 28, the SCE frequencies approached control level for both doses although a slight elevation (1-2 SCEs/cell) was still apparent. AZQ caused a slowing of the cell cycle at day 1 but caused an increase in replicative index in the treated PBLs at days 3 and 7. This may be attributed to the rapid cycling necessary for the replacement of damaged cells. The cell cycle appears to stabilize after day 28, possibly due to removal of AZQ or its reactive metabolite(s).

T3 HETEROZYGOUS EFFECTS OF MULTILOCUS DELETION MUTATIONS AT THE *AD-3A* LOCUS IN *NEUROSPORA CRASSA*. *L.K. Overton* and F.J. de Serres, RTI, RTP, NC 27709.

Previous studies on *ad3A* ^{IR} and *ad-3B* ^{IR} mutants (irreparable mutants in the *ad-3* region and presumptive multilocus deletions) demonstrated that they have heterozygous effects alone and more marked effects in various pairwise combinations (de Serres, in *Genes and Chromosomes*, Natl. Cancer Inst. Monogr. No. 18: 33-52, 1965). *Ad-3* ^{IR} mutants increased as the square of the dose after x-ray treatment (Webber and de Serres, *P.N.A.S.* (USA) 53: 430-37, 1965) and a 10-fold reduction in frequency was detected when dose rate was reduced from 1000r/min to 10r/min (de Serres, Malling, and Webber, *Brookhaven Symp. Biol.* 20: 56-76, 1967). These data support the hypothesis that these mutants resulted from multilocus deletions. New experiments have been performed to develop a screening method to evaluate *ad-3* ^{IR} mutants for heterozygous effects in terms of reduced growth rates as compared with a "wild-type" control. A group of 33 x-ray-induced *ad-3A* ^{IR} mutants (Webber and de Serres, 1965) have been tested in combination with an *ad-3B* ^{R} or with an *ad-3B* ^{IR} mutant. The level of heterozygous effect was expressed as the percentage of the growth rate of the "wild-type" control (*ad-3A* ^{R} + *ad-3B* ^{R}). In accord with our earlier data (de Serres, 1965), many *ad-3A* ^{IR} + *ad-3B* ^{R} combinations showed heterozygous effects that were more pronounced when the same *ad-3A* ^{IR} mutants were combined with an *ad-3B* ^{IR} mutant.

T4 MUTAGENICITY EVALUATION OF NOVEL AMINOAZO DYES AND THEIR REDUCTIVE CLEAVAGE PRODUCTS IN *SALMONELLA TYPHIMURIUM*. *J.F. Esancy*,[1] L.D. Claxton,[2] H.S. Freeman,[1] and M.K. Esancy.[1] [1]NCSU, Raleigh, NC 27695; [2]US EPA, RTP, NC 27711.

The scientific literature contains a large number of papers that describe the genotoxicity of synthetic azo dyes. These reports led to the banning of many dyes that had been used in food, drug, cosmetic, and textile products. Although mutagenicity and carcinogenicity data have been used as a basis for the removal of certain synthetic dyes from the marketplace, the literature contains little about the use of that same information to design new dyes that are void of such toxicity. In this research, the authors take advantage of published work which suggests that the incorporation of large alkyl or alkoxy groups into certain aromatic amines significantly lowers or removes their mutagenic properties. Fifteen aminoazo dyes and seven of their reductive cleavage products were synthesized and subjected to a battery of Ames tests. Dyes tested included five derivatives of 4-aminoazobenzene with different substituents at the 3 position (3 = OMe, OEt, OPr, OBu, or OCH_2CH_2OH) as well as five derivatives each of 4-[(4-amino)azo]N,N-diethylaniline and 4-[(4-amino)azo]N,N-bis(2-hydroxyethyl)aniline with the same substitutions ortho to the primary amino group. All 15 dyes were inactive without microsomal activation. In general, as the size of the substituent ortho to the primary amino group of the dyes was increased, mutagenicity decreased. A similar trend was observed for the reductive cleavage products. The results of this study suggest that the incorporation of a bulky group into azo dyes is a viable approach to the removal of mutagenicity.

T5 MOLECULAR ANALYSIS OF *HIS-3* MUTANTS OF *NEUROSPORA CRASSA*: CORRELATION BETWEEN BIOCHEMICAL AND GENETIC DATA, *J.S. Dubins*, L.K. Overton, R.R. Cobb, and F.J. de Serres. RTI, RTP, NC 27709.

Previous studies of *his-3* mutants of *Neurospora crassa* have demonstrated a correlation between the biochemical characteristics and the complementation pattern (B.B. Webber, *Genetics* 51 (2): 263-73, 1965). Three distinct biochemical groups (histidinol accumulators, nonaccumulators that can complement accumulator mutants, and nonaccumulators that cannot complement accumulator mutants) were shown to correspond to the three complementation groups (nonpolarized covering one or more of complons 9-14, nonpolarized covering one or more of complons 1-8, and polarized covering from at least complon 9 to complon 14, respectively). The present study was initiated to determine whether or not this correlation could be extended to the molecular level, i.e., are nonpolarized mutants due to point mutations and polarized and noncomplementing mutants due to deletions or insertions, as has been suggested by work done with *ad-3B* mutants of *Neurospora crassa* (de Serres, *Genetics* 48: 351-60, 1964). Restriction digest and Southern blot analyses of 29 *his-3* mutants covering the three biochemical and complementation groups were used to determine any correlation between the complementation pattern and genetic alteration at the molecular level. It will also be of interest to see whether the mutant order on the complementation map is the same as on the genetic map. The results of these experiments will be discussed.

T6 ISOLATION AND CHARACTERIZATION OF THE DNA ADDUCT N-(DEOXYGUANOSIN-8-YL)-3-AMINOFLUORANTHENE, *A.M. Dietrich*, L.M. Ball, A. Gold, UNC, CH, NC 27599.

3-Nitrofluoranthene has been detected in the ambient air and is highly mutagenic. It has also been shown to be carcinogenic in laboratory animals. The mutagenicity of 3-nitrofluoranthene is related to its ability to be reduced and form an electrophilic nitrenium ion that can covalently bind to DNA. Similar to other nitroaromatics (such as 1-nitropyrene) and aminoaromatics, the major DNA adduct formed by 3-nitrofluoranthene is expected to be guanosine modified at the C-8 position. In our research, DNA was modified by reaction with both chemically and enzymatically reduced 3-nitrofluoranthene. The chemical reaction consisted of forming N-hydroxy-3-aminofluoranthene by reduction with hydrazine monohydrate in the presence of palladium on activated carbon, and then reacting with calf thymus DNA. The enzymatic system utilized the mammalian enzyme xanthine oxidase to reduce 3-nitrofluoranthene to its reactive intermediate, which then was allowed to react with calf thymus DNA. The modified DNA was isolated and enzymatically digested to nucleosides. The modified nucleosides were isolated by a series of solvent extractions and chromatographic separations. The isolated adduct was characterized by several spectroscopic and chromatographic methods, including UV-VIS, fluorescence, NMR, and mass spectrometry. Interpretation of the data indicate that the major adduct synthesized and isolated was N-(deoxyguanosin-8-yl)-3-aminofluoranthene.

This work was supported by Environmental Protection Agency Cooperative Agreement #CR811817, and by grant #BRSG 34127 from the School of Public Health at the University of North Carolina.

T7 MOLECULAR FRAGMENT ANALYSIS OF RETINOIDS. *S.W. Woods,*[1] M.J. Mass.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

The *CASE* program was utilized to analyze a data set of 141 retinoids for the presence of molecular fragments that could be correlated with retinoidal activity. Retinoids are potent cancer chemopreventive agents. In this study, the ability to reverse squamous metaplasia and keratinization in hamster tracheal organ cultures was the biological basis that defined retinoid activity. The *CASE* program dissects molecules into their component fragments (i.e., CH_3-CH_2-R, R-COOH, etc.), and then determines the distribution and statistical significance of all fragments with respect to activity. The ideal goal is to find fragments in the group of active molecules that are not present, or are less well represented, in the set of inactive molecules. In this retinoid data set *CASE* discerned 3 fragments that were distributed in the group of active molecules but were significantly less frequent in the inactive group. The first fragment, a 10-membered substituent of the alkenyl side chain (R-C-C=CH-CH=C-CH=CH-CH-CH=C(CH_3)-R) was present in 24.5% of inactives, in 58.1% of compounds with marginal activity, and in 72.1% of active retinoids. The second, R=C-CO-R, had a distribution of 2% presence in the inactives, 12.7% in marginals, and 37.7% in actives. The third fragment, R=C-NH-R appeared in 2.0, 3.2, 14.7% of inactives, marginals, and actives, respectively. A fourth fragment, identified by an alternate application of *CASE*, was present in 98% of actives. We believe these fragments may represent important determinants of retinoid activity, and some may have specific significance as receptor recognition sites. Other determinants of retinoid activity will be discussed, as will the predictive utility of this data set and the design of novel retinoids suggested by *CASE*.

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

T8 SOUTHERN BLOT ANALYSIS OF THE *TK* LOCUS IN LARGE AND SMALL COLONY MOUSE LYMPHOMA CELL MUTANTS. *J. Martin*, E. Korytynski, T. Hughes, L. Monteith, and R.R. Cobb. RTI, RTP, NC 27709.

Mouse lymphoma cells of the L5178Y TK $^{+/-}$ 3.7 2C line were exposed to sidestream and mainstream cigarette smoke fractions in the presence of S9. Exposed cells were cultured on media containing trifluorothymidine (TFT) which allowed growth of colonies carrying the forward mutation of thymidine kinase $^{+/-}$ to thymidine kinase $^{-/-}$ ($TK^{+/-} \rightarrow TK^{-/-}$). Mutants that survived the TFT challenge fell into two classes: fast growing (large colony formers) and slow growing (small colony formers). Southern blot analysis of *Nco* I digested DNA from TFT cultured mutant colonies yielded two distinct restriction fragment patterns when probed with the cDNA clone mPtk4. One such pattern was composed of 4 bands at 6.4, 5.5, 4.7, and 2.1 kb and was identical to that of TK $^{+/-}$ controls. A second pattern differed from the first only in the absence of the 6.4 kb band. The majority of both large and small colonies derived from cells exposed to cigarette smoke fractions exhibited restriction digest fragment patterns lacking the 6.4 kb band. In colonies derived from DMSO and hycanthone treated cells (included as controls), most small colonies yielded the full complement of bands found in TK $^{+/-}$ Southern blots. It has been hypothesized that the absence of a 6.4 kb band in Southern blot analyses of mouse lymphoma TK $^{-/-}$ mutant DNA is related to chromosomal alterations giving rise to slow growing (small) colonies, while presence of the band in the DNA of these mutants is indicative of intragenic damage which results in large colony formation. However, our data showed that the bimodal distribution of colony sizes in this mouse lymphoma assay was not explicable in terms of the presence or absence of a 6.4 kb band on Southern blots of *Nco* I digested TK $^{-/-}$ DNA. We hypothesize that small colony formation was not well correlated with cnromosomal alterations at the *Tk* locus under our assay conditions.

(EPA Contract NO68-02-3992-60 and RTI 5311).

T9 POTENTIAL USE OF HUMAN AMNIOTIC FLUID TO DETECT TRANSPLACENTAL MUTAGENS. *C. Fomous*, Georgetown Univ. Hosp., Washington, DC 20007-2197.

Exposure of the fetus to mutagenic agents can lead to fetal death, malformation, or development of neoplasm later in life. Few screening tests detect transplacental mutagens and these have been applied only to rodent studies. This study lays the groundwork for using human amniotic fluid to detect transplacental mutagens. The in vitro short term assays used in this study include sister chromatid exchange (SCE), chromosome aberrations (CA), and unscheduled DNA synthesis (UDS). Preliminary investigations established that amniotic fluid does not effect the in vitro baseline or induced SCE frequency; statistical analysis of SCEs showed no variation from flask to flask or individual to individual, and an elevated response to mutagen can be observed up to a week after cultures were initially challenged. Still ongoing is the comparison of the response to mutagen among the cell types found in amniotic fluid. In general, three basic cell types are found in amniotic fluid cultures: fibroblast-like (F) cells, epithelial-like (E) cells, and amniotic fluid-like (AF) cells. The E cells, difficult to manipulate and rarely found in longterm cultures, will not be studied. Data on F cells have been collected and AF cell lines are being grown. The embryonically derived WI-38 cell line has also been studied to compare with amniotic fluid cells. The use of short term assays and amniotic fluid to monitor at-risk pregnancies will be briefly discussed.

T10 USE OF SOLID PHASE EXTRACTION IN THE RECOVERY OF MUTAGENS FROM CIGARETTE SMOKERS' URINE. *R. Williams*, [1] R. Watts,[1] J. Inmon,[2] T. Pasley,[1] J. Fitzgerald,[2] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

Cigarette smoker's urine was prepared for mutagenic testing by extracting mutagens with solid phase extraction columns. Concentrates collected from various trials using octadecyl, cyclohexyl, and cyanopropyl bonded silicas were assayed using a *Salmonella typhimurium* micro reversion assay. Results indicate that the cyanopropyl adsorbent gives the best recovery of mutagenicity with the fewest interferences from bacterial toxicity. Urinary pH was found to only influence recovery of mutagens using the cyanopropyl adsorbent with basic urine samples. Storage of frozen urine at -80°C, with subsequent thawing and mutagen extraction, was not found to be a factor for storage periods up to 170 days.

T11 APPLICATION OF THE "COMPUTERIZED LABORATORY NOTEBOOK" CONCEPT TO THE TCGF RESPONSE AND PRODUCTION ASSAY. G.H.S. Strauss,[1] *S.J. Berkowitz,*[2] and W.L. Stanford.[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

The computer must be viewed, in genetic toxicology, as a very useful laboratory tool for performing bioassays. The computer should aid routine laboratory work rather than hinder it by requiring a large expenditure of time in learning to use the computer. Just as a blastogenesis assay would be impossible without a scintillation counter, the Battery of Lymphocyte Tests (BLT) is dependent on the use of a computer. The BLT was designed to evaluate blood specimens for toxic, immunotoxic, and genotoxic effects after in vivo exposure to putative mutagens. This talk will address the application of the "Computerized Laboratory Notebook" concept to the T-Cell Growth Factor (TCGF) response and production assay portions of the BLT. The actual input screens seen by the technicians will be shown to illustrate better the ease of use of the system. The data received from a scintillation counter are grouped according to the information given by the technicians. The data are then statistically analyzed in a spreadsheet and stored in a central data base. The data are output into a preset graph definition which allows easy comparison to other samples. The central data base can be used to compare an individual to a group of normal people, as well as to earlier tests of the same individual. This approach might locate subtle immunotoxic effects in healthy individuals. Some general computer concepts will be discussed which can be used to automate any bioassay and overcome the obstacle of personnel inexperienced with a computer.

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

T12 DEVELOPMENT OF METHODS TO EVALUATE IMMUNOTOXIC EFFECTS OF ENVIRONMENTAL MUTAGENS: INDIVIDUAL CAPACITY TO PRODUCE AND RESPOND TO TCGF. G.H.S. Strauss,[1] S.J. Berkowitz,[2] and *W.L. Stanford.*[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

The Battery of Leucocyte Tests (BLT) was developed by our laboratory to evaluate blood samples for selected toxic, immunotoxic, and genotoxic effects of in vivo mutagen and carcinogen exposures. Our candidate method for estimating immunotoxic effects of environmental mutagens is based upon the determination of relative capacities of mononuclear cells from subjects to produce and to respond to T-Cell Growth Factor (TCGF). Through use of the TCGF production assay we found that each normal person responds individually to various mitogens at various doses. Cells from each subject are cultured with PHA, PWM, and Con A at five different doses. The supernatants (SN) are harvested and tested along with a reference TCGF using a standard primed TCGF responder cell that proliferates at a rate determined by the quality and quantity of TCGF available in the SN. The TCGF activation assay requires priming cells in culture with various mitogens and doses. After a suitable incubation period, supernatants are removed and replaced with a standard reference TCGF. In this case, differences in lymphocyte proliferation are determined by the ability of the specimen cells to become activated (primed) to respond to TCGF. We hypothesize that an individual exposed to mutagens or carcinogens will be affected by immune impairment which, though subtle, can be measured by the assays presented here. In addition, we discuss the fact that it is necessary to produce TCGF in large quantities for use as standards and in the Clonal Assay of Lymphocyte Mutagenesis (CALM). We used approximately 3 billion leucocytes collected from a leucocyte residue of platelet pheresis. The mitogen and dose determined for the individual pheresis residue by the TCGF production assay is used to produce potent TCGF.

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

T13 INTRODUCTION OF A FUNCTIONAL RAT P-450b cDNA INTO THE MOUSE C3H10T1/2C18 CELL LINE PRODUCES INCREASED 2-AAF METABOLISM.
S.K. Hansen,[1] J.A. Ross,[2] J.M. Siegfried,[1] S. Leavitt,[2] K. Rudo,[3] R. Langenbach,[3] and S. Nesnow.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711; [3]NIEHS, RTP, NC 27709.

The mixed-function oxidases serve as the primary drug and carcinogen metabolizing enzymes in mammalian cells. Among these oxidases, the rat cytochrome P-450b is the major phenobarbital-induced cytochrome. Using a cDNA clone of the rat P-450b gene, we have constructed a plasmid containing that gene driven by an SV40 promoter and adjacent to a selectable neomycin resistance marker. This plasmid (pJRSL100) was transfected into the mouse embryo fibroblast cell line C3H10T1/2C18, which has minimal ability to metabolize 2-acetylaminofluorene (2-AAF). Geneticin resistant clones were selected and tested for cytotoxicity to (2-AAF), which is minimally toxic to C3H10T1/2C18 cells. Two clones showed increased sensitivity to 2-AAF and were then evaluated for the presence of cytochrome P-450b messenger RNA. One clone, 19P450b-4, was positive for cytochrome P-450b mRNA and was further tested for production of 2-AAF metabolites. HPLC results of the metabolic studies showed a generalized increase in the metabolism of 2-AAF in the cells containing the transfected P-450b gene versus controls.

This is an abstract of a proposed presentation and does not necessarily reflect EPA Policy. Work was performed in support of US EPA contract No. 68-02-4456.

T14 MUTAGENESIS RESEARCHERS' VIEWS ON THE SIGNIFICANCE AND PROPER USE OF MUTAGENICITY TESTS APPLIED TO CLINICAL SPECIMENS.
D. Busch, [1] D. Easterling,[2] H. Leventhal,[3] and G.T. Bryan.[3] [1]AFIP, Washington, DC 20306-6000, [2]U PA, Philadelphia, PA 19104, [3]U WI, Madison, WI.

Seventy-one individuals interested or involved in mutagenesis research answered a questionnaire soliciting views on the health significance and proper use of mutagenesis assays that use clinical specimens such as urine or blood. All of 15 mutagenicity tests discussed in the questionnaire were regarded as neither totally experimental nor diagnostic of degree of mutagen exposure or genetic damage, but rather as being somewhere in between. Respondents were slightly to moderately concerned about upsetting subjects with results suggesting excessive mutagen exposure or genetic damage, but favored sharing test data with subjects when the subject requested the results or when the results were thought to indicate a health risk. Most perceived benefits from following up subjects who had abnormal test data, including enhancing study quality, identifying new sources of exposure, and identifying previously undiscovered carcinogens. A majority also endorsed asking subjects about possible exposure sources, visiting areas where suspected exposure occurred, analyzing specimens for specific chemicals, and notifying appropriate agencies as part of the followup. However, most did not feel that a mutagenicity test result could be correctly used to assign blame for an adverse health effect. Most felt it would be unethical to screen subjects for mutagen exposure, not disclose test results, and follow up subjects for later development of cancer to see what correlation existed between mutagenicity test result and cancer risk. Ninety per cent believed that mutagen exposure increases cancer risk.

T15 SYNAPTONEMAL COMPLEX DAMAGE IN RELATION TO MEIOTIC METAPHASE CHROMOSOME ABERRATIONS AFTER EXPOSURE OF MALE MICE TO CYCLOPHOSPHAMIDE. *L. Backer,*[1] J. Gibson,[2] M. Moses,[2] D. Howard,[1] and J. Allen.[3] [1]EHRT, RTP, NC 27709; [2]Duke Univ., Durham, NC; and [3]US EPA, RTP, NC 27711.

The synaptonemal complex (SC) comprises the proteinaceous axes of paired homologous chromosomes at meiotic prophase and is intimately involved in the events of homologous chromosome pairing, crossing over, and segregation. We have recently shown that the SC can serve as a new genotoxicity endpoint confirming chemical interaction with germ-line cells: light and electron microscope analyses of SCs revealed structural and functional damage in mice treated with alkylating agents. In the present study, mice were injected i.p. with 0 (saline controls) or 100 mg/kg cyclophosphamide and tritiated thymidine (200μCi). Both prophase SC and metaphase chromosome analyses were conducted. Harvest times, day 4 for SCs and day 11 plus 6 hours for chromosomes, were chosen to evaluate cells exposed during premeiotic DNA synthesis. Only tritium-labeled cells were analyzed to better define the qualitative and quantitative relationships between respective SC and chromosome endpoints. A Wilcoxan Rank test was used to establish whether specific types of damage in labeled cells were attributable to CP exposure. Correlation coefficients were calculated among SC and metaphase chromosome endpoints. Statistically significant correlations were found, for example, between SC breakage and both metaphase I (MI) isochromatid breakage and MI chromosomal rearrangements. These correlations are interesting because of the theoretical relationships between chromosomes and component parts of the SC.

This abstract does not necessarily reflect US EPA policy.

T16 DOSE-DEPENDENT NEUTROPHIL MYELOPEROXIDASE-DEFICIENCY AND ONSET OF MYELOGENOUS LEUKEMIA IN γ-IRRADIATED BEAGLE DOGS: AN OPPORTUNITY TO LINK SUBTLE EFFECTS OF MUTAGEN EXPOSURE WITH DISEASE CONSEQUENCES? *G.H.S. Strauss*[1] J.S. Hanker.[2] [1]US EPA, RTP, NC 27711; [2]UNC, Chapel Hill, NC 27599.

Our laboratories are developing a novel method for demonstrating specific abnormalities in the subcellular distribution of the hemoprotein enzyme myeloperoxidase (MP). These result from (1) abnormal aggregations of MP to form rods and phi bodies in the immature neutrophil series cells of acute myeloid leukemia (AML), and (2) a marked MP deficiency in the mature neutrophils from AML, myeloid metaplasic, and preleukemia. Continuous exposure to low-dose ionizing radiation (IR) causes a progression of effects leading to overt leukemia in beagle dogs. We studied blood smears from irradiated dogs to determine if our MP staining and scoring approach would track the gradual loss of neutrophil MP. Briefly, 7 controls and 14 irradiated beagles were scored for MP. The average score of controls was 282 (range = 225 – 357). The average score of treated dogs was 100 (range = 33—171). We describe our method for staining and scoring and provide dose-response data. We also discuss its utility for early diagnosis of leukemia in humans. To determine whether this technique can be used to monitor for effects of contact for leukemogens, such as IR and benzene, we are applying it to blood films taken from individuals knowingly at risk from therapeutic or occupational exposures. Further, we are performing other tests of the Battery of Leucocyte Tests (BLT) on blood samples from the same individuals in an effort to recognize results which are related through common causes.

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

ABSTRACTS
POSTERS

P1 THE DETECTION OF GENOTOXIC ACTIVITY IN HAZARDOUS WASTE SAMPLES USING THE MICROSCREEN PHAGE-INDUCTION ASSAY. *V.S Houk* and D.M. DeMarini. US EPA, RTP, NC 27711.

The microscreen phage-induction assay, which quantitatively measures the induction of prophage y in *Escherichia coli* WP2s (λ), was used to test 14 crude (unfractionated) hazardous industrial waste samples for genotoxic activity in the presence and absence of metabolic activation. Eleven of the 14 wastes induced prophage, and induction was observed at concentrations as low as 0.4 picograms per ml. A comparison of the ability of these waste samples to induce prophage and their mutagenicity in the Salmonella reverse mutation assay indicated that the phage-induction assay detected genotoxic activity in all but one of the wastes that were mutagenic in Salmonella. Moreover, the phage-induction assay detected as genotoxic 5 additional wastes that were not mutagenic in the Salmonella assay. Partial chemical characterizations of these wastes showed high concentrations of carcinogenic metals, solvents, and chlorinated compounds, most of which are detected poorly by the Salmonella assay but readily by the Microscreen assay, as recent studies indicate. This may explain the enhanced ability of the Microscreen phage-induction assay to detect the additional 5 waste samples. The applicability of the Microscreen phage-induction assay for screening hazardous wastes for genotoxic activity was examined, along with some of the problems associated with screening highly toxic wastes containing toxic volatile compounds.

P2 APPLICATION OF ^{32}P POSTLABELING ASSAY IN THE ANALYSIS OF DNA ADDUCTS FOLLOWING EXPOSURE TO COMPLEX MIXTURES, *M. George,*[1] J. Gallagher,[2] I. Robertson,[3] M. Jackson,[1] M. Kohan,[2] J. Scott,[1] and J. Lewtas.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

The ^{32}P-postlabeling assay has been shown to be an effective tool for the detection of adducts covalently bound to DNA. The detection of adducts by this method is not dependent on radiolabeled substrate. In addition, due to the assay's high degree of sensitivity, μg quantities of DNA can be analyzed. These advantages provide a means of determining the extent of interaction between DNA and reactive intermediates following the metabolism of toxicants. We have successfully used this technique to evaluate DNA from a variety of animal tissues and isolated cells following in vitro and in vivo treatments. Some of these DNA samples include: rabbit tracheal epithelial cells treated with three dinitropyrenes (1, 3; 1, 6; and 1, 8) whose adduct levels are reported at 0.17, 0.17, and 0.32 adducts per 10^8 normal nucleotides, respectively. Higher levels were detected in liver DNA following the treatment of rats with benzo(a)pyrene (B(a)P) and acetylaminoflorene (AAF). DNA was modified to the extent of 560 and 35 adducts per 10^8 nucleotides, respectively. Placenta DNA was extracted from women exposed to smoky coal emissions. Adduct levels were detected at approximately 12 adducts per 10^8 nucleotides. Cord blood pooled from these women revealed an adduct level that approximated 0.73 adducts per 10^8 normal nucleotides. In vitro experiments using calf thymus DNA treated with various complex mixtures (diesel exhaust and coke oven main extract) plus activating system had adduct levels detected at 4.1 and 7.0 adducts per 10^8 normal nucleotides, respectively.

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

P3 COMPARISON OF MICRONUCLEUS FREQUENCIES IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING EITHER IN VITRO OR IN VIVO EXPOSURE TO COBALT-60 GAMMA RADIATION. *G.L. Erexson,* [1] A.D. Kligerman,[1] J.W. Allen,[2] E.C. Halperin,[3] and G. Honoré.[3] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711; [3]Duke Univ., Durham, NC 27710.

Experiments were designed to investigate the induction of micronuclei (MN) in mouse peripheral blood lymphocytes (PBLs) after in vitro or in vivo exposure to cobalt-60 gamma radiation. Male C57B1/6 mice were bled by cardiac puncture, and the blood was pooled for in vitro studies. Mononuclear leucocytes (MNLs) were isolated from whole blood using Lymphocyte Separation Medium®. For the in vivo analysis of MN in PBLs, the whole animal was irradiated, blood was obtained by cardiac puncture, and MNLs were isolated for each mouse. Exposures were at a rate of 0.83 Gy/min to yield doses of 1, 2, 3, or 4 Gy. Isolated MNLs were cultured in RPMI 1640 medium containing phytohemagglutinin (PHA). At 21 h, the medium was removed and replaced with medium lacking PHA but containing 3 ug cytochalasin B/ml for MN analysis in binucleated PBLs. MN cultures were harvested at 48 to 50 h using a cytocentrifuge. In each experiment, MN were quantitated in 2000 binucleated PBLs/dose. The nucleation index (NI) was obtained for each dose by analyzing 400 consecutive PBLs. A significant exponential increase in MN was observed at all doses. The NI was significantly decreased at the 3 and 4 Gy dose in vivo and at the 4 Gy dose in vitro. In vivo, the MN frequency at 4 Gy was 2 times that seen in vitro. The large difference in MN response was apparently attributable to the PBLs being hypoxic in the in vitro experiments, thereby suppressing the formation of chromosome-damaging free radicals. Further details of this phenomenon will be discussed.

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

P4 INDUCTION OF PROPHAGE LAMBDA BY CHLORINATED ORGANIC COMPOUNDS. *D.M. DeMarini,* [1] D.G. Parkes, Jr.,[1] and H.G. Brooks.[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

Chlorinated organics represent an important class of environmental carcinogens. They are used as pesticides, herbicides, wood preservatives, bacteriocides, etc. They are present in drinking water, agricultural runoff, industrial effluents, incinerator effluents, body fat, and the urine of up to 80% of the U.S. population. Because of the prevalence of chlorinated organics in the environment, it is important to have bioassays that can reliably detect this class of carcinogen. Although the Salmonella assay is the bioassay used predominantly to examine the mutagenicity of environmental samples, it detects a relatively low percentage of carcinogenic chlorinated organics (Zeiger, *Cancer Res.* 47:1287, 1987). Consequently, we have tried to identify a short-term bioassay that might detect carcinogenic chlorinated organics more reliably than the Salmonella assay. Our preliminary investigation of 10 pesticides showed that the Microscreen prophage-induction assay developed by Rossman et al. (*Environ. Mutagen.* 6:59, 1984) detected all six of the Salmonella-positive carcinogens; in addition, the assay also detected two other carcinogenic compounds that were not detected by the Salmonella assay (Houk and DeMarini, *Mutat. Res.* 182:193, 1987). The present study extends these observations to 40 additional chlorinated organics of various types. Among these, certain chlorinated phenols, none of which are mutagenic in Salmonella, induced prophage in the presence of S9. Results of structure-activity studies with a set of chlorinated phenols will be presented. Some of the chlorinated phenols have been shown by the National Toxicology Program to be clastogenic in CHO cells. Studies by others indicate that chlorinated phenols are metabolized to hydroquinones that induce single-strand DNA breaks. Because prophage induction is known to occur in response to single-strand DNA

breaks, prophage-induction assays may be capable of detecting a subset of clastogenic compounds, such as chlorinated phenols, that might otherwise be detected only in more costly cytogenetic assays in eukaryotic organisms.

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

P5 CYTOTOXICITY OF THREE BUFFERS TO SALMONELLA STRAIN TA98 IN A MICROSUSPENSION OF REVERSE-MUTATION ASSAY AND THE EFFECT OF THESE BUFFERS ON THE MUTAGENIC POTENCIES OF COMPLEX MIXTURES AND PURE COMPOUNDS.
M.M. Dallas,[1] L.R. Forehand,[1] and D.M. DeMarini.[2] [1]EHRT, RTP, NC 27709; [2] US EPA, RTP, NC 27711.

We have investigated the cytotoxicity of three buffers, Vogel-Bonner (VB) minimal salts, and 0.15 and 0.015 M sodium phosphate, to Salmonella strain TA98 in a microsuspension assay developed by Kado et al. (*Mutat. Res.* 121:25, 1983). We also have examined the effect of these buffers on the mutagenic potencies of environmental tobacco smoke (ETS), cigarette smoke condensate (CSC), and pure compounds such as 1-nitropyrene (1-NP). Experiments were performed using a modification of the microsuspension assay that consisted of adding 50μl of a 5-X concentrate of an overnight culture of TA98; 50μl of S9 buffer or S9 mix; and 2 ul of DMSO or mutagen to a small vial or test tube. The suspension was incubated for 90 min at 37°C, top agar was added, and the contents of the tube were poured onto minimal medium. The cytotoxicities of the buffers were determined by comparing the cell counts obtained from control vials before the 90-min incubation to those obtained after the 90-min incubation. Dilutions were performed in the appropriate buffer, and cells were plated for survival onto V-B minimal medium supplemented with excess histidine and biotin. In the absence of S9, 78% survival was obtained with 0.015 M sodium phosphate buffer; however, only 23-35% survivals were obtained with 0.15 M phosphate or V-B minimal salts, respectively. The mutagenic potency of 1-NP was 2-3 times greater in 0.015 M phosphate buffer or V-B minimal salts than in 0.15M phosphate. Based on these studies, 0.015 M phosphate is less cytotoxic than the other two buffers, and it enables 1-NP to exhibit a mutagenic potency similar to that obtained in V-B minimal salts. The effects of these buffers on survival and mutagenic potency in the presence of S9 are currently under investigation. Complex mixtures being studied include ETS generated in the ACC Tavern in Durham, NC and in a small chamber at EHRT, Durham, NC. These samples are being tested with a standard S9 mix as well as with S9 mixes formulated with each of the three buffers.

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

P6 MUTAGENICITY OF FOUR ACRYLATE COMPOUNDS IN L5178Y MOUSE LYMPHOMA CELLS AND CHINESE HAMSTER OVARY CELLS.
C.S. Millis,[1] L. Parker,[1] K.H. Brock,[1] C.L. Doerr,[1] K.L. Dearfield,[2] and M.M. Moore.[3] [1]EHRT, RTP, NC 27709; [2]US EPA, Washington, DC 20460. [3]US EPA, RTP, NC 27711.

Mutagenicity of four acrylates (2-ethylhexylacrylate, discycopentenyloxyethyl methacrylate, tetraethylene glycol diacrylate, and trimethylolpropane triacrylate) was evaluated at the thymidine kinase (TK) locus in L5178Y/TK $^{+/-}$ -3.7.2C mouse lymphoma cells and at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovary (CHO) cells. L5178Y/TK $^{+/-}$ -3.7.2C cells were maintained in suspension culture and were treated for 4 h, allowed 2 days expression, and then cloned with trifluorothymidine (TFT) selection (1 μg/ml). CHO cells were maintained in suspension culture and were treated for 4 h, allowed 7 days expression, and cloned with 6-thioguanine selection (6.68μg/ml) in soft agar medium. The monoacrylate, 2-ethylhexyl, induced an equivocal response, and the methacrylate, dicyclopentenyloxethyl, induced a weakly positive response at the *tk* locus. The diacrylate, tetraethylene glycol, and the triacrylate, trimethylolpropane, induced

high mutant frequencies at the *tk* locus. However, all four compounds induced only a small number of *hgprt*-deficient mutants. This difference in results between the mouse lymphoma assay and the CHO assay may be due to the inability of the *hgprt* locus to fully quantitate the clastogenicity of test compounds. These compounds are being evaluated for their clastogenicity in both mouse lymphoma and CHO cells.

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

P7 EFFECTS OF α-NAPHTHOFLAVONE ON NUCLEOID SEDIMENTATION IN CHINESE HAMSTER OVARY CELLS AND IN LYMPHOCYTES FROM SMOKERS AND NONSMOKERS. *J.M. Goldring,*[1] G.W. Lucier,[2] and C.L. Thompson.[2] [1]UNC-CH, Chapel Hill, NC 27599; [2]NIEHS, RTP, NC 27709.

Recently, our laboratory reported that α-naphthoflavone (ANF) induces sister chromatid exchanges (SCEs) when cultured with Chinese hamster ovary (CHO) cells only in the presence of TCDD-induced rat liver microsomes. Moreover, ANF induces greater SCE frequencies in the lymphocytes of smokers than in those of nonsmokers. To investigate the nature of ANF-induced damge in human lymphocytes and CHO cells, the "nucleoid sedimentation assay" was performed on lymphocytes and CHO cells cultured with ANF and TCDD-induced rat liver microsomes. "Nucleoids" are the DNA/RNA/protein aggregates formed when cells are exposed to nonionic detergents and high salt concentrations. The assay measures the sedimentation distance of nucleoids on a sucrose density gradient; damaged DNA sediments more slowly than undamaged DNA. Our results demonstrate that the nucleoid sedimentation assay is capable of detecting ANF-induced damage in both human lymphocytes and CHO cells. The extent of ANF-induced damage in human lymphocytes after 2 h of in vitro exposure is highly variable among individuals. Moreover, repair of such damage is essentially complete after an additional 2 h. Nucleoids from untreated lymphocytes sediment about 32 mm on a 15-30% sucrose density gradient, while nucleoids from ANF-treated cells sediment at about 25 mm; the difference in nucleoid sedimentation between treated and untreated cells consistently ranged from 2 to 10 mm. Mechanistic studies will be performed in the future to determine the nature of differential susceptibility between individuals to ANF-induced damage. Attempts will also be made to examine the utility of the assay in large-scale screening for environmental exposures.

This work was supported in part by US PHS Traineeship No. 5 T32 ES 07126.

P8 SCREENING OF CODED TERATOGENS IN TWO IN VITRO ASSAYS. *B.P. Wilkinson,*[1] E.L. Elmore,[1] R.E. Morrissey,[2] J.C. Lamb, IV,[2] D.G. Rocha,[1] D.D. Murphy,[1] and V.E. Steele.[1] [1]Northrop, RTP, NC 27709; [2]NIEHS, RTP, NC 27709.

Two in vitro test systems, the human embryonic palatal mesenchymal (HEPM) cell growth inhibition assay and the mouse ovarian tumor (MOT) cell attachment inhibition assay, were evaluated for their ability to detect 44 coded teratogens and nonteratogens using standard protocols. Following completion of testing, the chemicals were decoded and ranked according to the minimum IC50 value (the mM concentration of compound that inhibits growth or attachment by 50% relative to the solvent control). The in vitro test concordance with established in vivo results was examined over a range of concentration levels above which the in vitro results were called positive and below which they were considered negative. At the mM level the concordance of data from the combined in vitro assay with in vivo data was 65.9%. The maximum agreement between the combined in vitro and in vivo data was reached at the 20 mM level, where there was a 72.7% concordance of results. At that level there was a 16.1% incidence of false negative results. Several of these false negative compounds may require metabolic activation. The use of either assay alone did not reach this high level of concordance. Agreement of the in vitro data at the 10 mM level with available human data was 70.8%.

This work was supported by NIEHS Contract No. NO1-ES-38046.

P9 GENETIC STUDIES OF A BEHAVIORAL MUTATION INDUCED BY ETHYLENE OXIDE IN THE MOUSE. *L.B. Barnett,*[1] S.E. Lewis,[1] and M. Davisson.[2] [1]RTI, RTP, NC 27709; [2]Jackson Lab, Bar Harbor, ME 04609.

A male mouse with neurological abnormalities was found among the progeny of male DBA/2J mice treated with ethylene oxide. Although this (C57BL/6J x DBA/2J)F_1 male had reduced fertility, he did transmit the variant trait to offspring from a backcross to a C57BL/6J mouse. Affected animals display a progressively worsening circling defect beginning at about 3 weeks of age. In addition to the neurological defect, corneal clouding is observed and worsens as the affected animals age. The study of mitotic chromosomes in peripheral lymphocytes in animals studied to date shows that carriers of the mutation have a 17/4 translocation. Further cytogenetic studies are in progress to determine if the translocation in these mice is also associated with the mutation causing the neurological defect.

This work was supported by NIEHS Contract NO1-ES-55078.

P10 ON DNA ADDUCTS USING A ^{32}P-POSTLABELING TECHNIQUE. S.L. Huang. EHRT, RTP, NC 27709.

To quantitatively evaluate the degree of exposure to environmental agents, the level of DNA adduct formation can be accurately measured using the ^{32}P-postlabeling technique. In the ^{32}P-postlabeling test for DNA adducts, DNA was enzymatically digested to 3'-phosphorylated normal and adducted mononucleotides, which were then labeled by T4 polynucleotide kinase catalyzation. ^{32}P-labeled nucleotides were resolved by t.l.c. Separation of modified from nonmodified nucleotides has been a difficult step in this ^{32}P-postlabeling technique. Different carcinogen-nucleotide adducts exhibit varying degrees of binding affinity to PEI-cellulose; therefore, solvents of different ionic strengths were required to improve recovery and resolution of modified nucleotides. Several protocols for detecting DNA adducts were used to demonstrate the existence of benzo(a)pyrene, diaziquone,3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, and cyclophosphamide binding to DNA.

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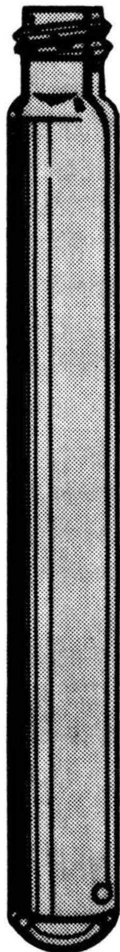

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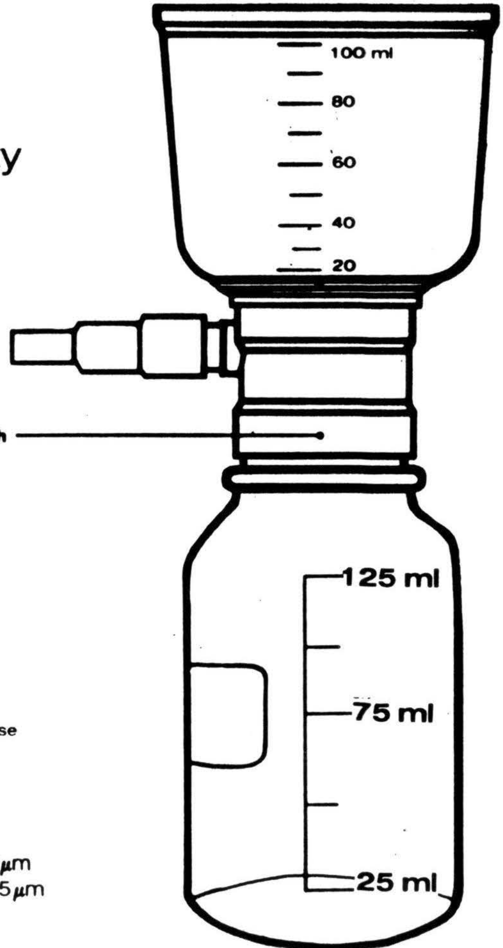

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Characteristics of HL-1 Supplement

pH	7.2±0.1
Protein content	(less than) 30 μg/ml when diluted in basal medium
Mycoplasma	None
Sterility	Tested as per USP Vol. 20, 71
Stability	Minimum 90 days from date of manufacture (stabilities ongoing)
Sterilization	Positive membrane filtration, 0.22 μm
Pyrogen level	0.25 EU/ml LAL Pyrotell®
Customer shelf life	Minimum 60 days

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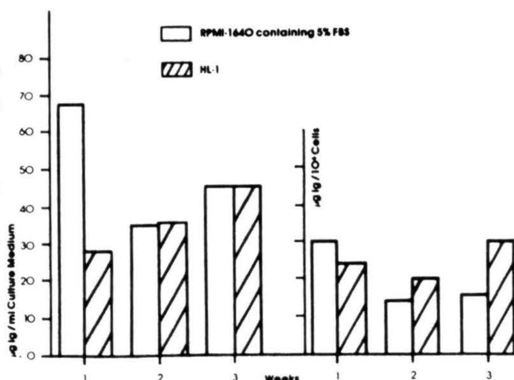
HL-1 Supplement is a 100X concentrate. When diluted in basal medium, the final total protein concentration is less than 30 μg/ml. Monoclonal antibody purification is facilitated and greater product yields are often realized. Additional benefits resulting from low protein media include lower non-specific binding in screening assays and increased cell sensitivity to regulatory factors and drugs.

Comparison Between Monoclonal Antibody Production by Hybridoma Cells grown in RPMI-1640 containing either Fetal Bovine Serum or HL-1 Supplement

Figure 1. Medium was collected from cultures that were allowed to overgrow. These were the seventh day cultures for a hybridoma cell line (P3X63Ag8.653 derivative) that was cultured in RPMI-1640. Cells were maintained in medium containing either HI-1 Supplement or 5% FBS for three consecutive weeks and were passaged three times per week. The medium was evaluated by ELISA. The open bars represent the Ig concentration in the cultures which were supplemented with 5% FBS and the striped bars represent the cultures which contained 1% HL-1 Supplement. The left panel illustrates the μg Ig/ml of culture medium and the right panel illustrates the μg Ig/10⁶ hybridoma cells per ml medium.



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