GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

THIRD ANNUAL MEETING

PROGRAMS AND ABSTRACTS

November 7, 1985
Sheraton University Center
Durham, North Carolina
8:00 a.m. to 5:00 p.m.

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

Elected Officers for Terms Beginning October 1985

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Vice President	– Gene Elmore
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	– Michael Shelby (NIEHS)
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Genotoxicity and Environmental Mutagen Society

Dear GEMS member:

Thank you for attending the Third Annual Meeting of the Genotoxicity and Environmental Mutagen Society. Thanks to you, the meeting is once again a success. Each officer and board member hopes that this meeting will be beneficial to every participant; and we encourage your full participation. As a society, GEMS welcomes the participation of students, bench scientists, scientific managers, and others interested in these exciting areas of toxicology.

GEMS is pleased this year to have DR. MONTROSE MOSES as our keynote speaker. Dr. Moses is a professor in the Department of Anatomy, Duke University, Durham, North Carolina. We want to thank Dr. Moses for his contribution to this year's meeting.

This will be the second year that GEMS has been able to give a cash award to a junior scientist for the BEST ORAL PRESENTATION. We were very pleased with the quality of presentations last year, and we look forward to this year's presentations. GEMS is very fortunate to have so many students and junior scientists who make significant contributions to our disciplines and so many senior scientific investigators who encourage the training and advancement of their co-workers.

SUSTAINING MEMBERS provide a variety of services to GEMS and its members, and we encourage our members to visit the exhibits and thank their representatives. This meeting, and especially the meal, would not be financially possible without their aid.

As your outgoing president, I would like to thank all of you who have served the society—our officers, board members, committee helpers, seminar speakers, and all others who have contributed in any way. Also, congratulations to the new officers and board members, who I know are eager to serve the society and its members.

Again, WELCOME to the Third Annual Meeting of GEMS.

Sincerely,

Larry D. Claxton

Larry Claxton, President

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

CHARTER SUSTAINING CORPORATIONS AND MEMBERS

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PROGRAM
GEMS THIRD ANNUAL MEETING
November 7, 1985

Sheraton University Center, Durham, North Carolina
8:00 a.m.-5:00 p.m.

8:00 a.m. – 9:00 a.m.	Registration, exhibit and poster set-up
9:00 a.m. – 9:15 a.m.	Welcoming remarks, L. Claxton, President, GEMS
9:15 a.m. – 9:30 a.m.	**T1** TOWARDS AN UNDERSTANDING OF THE SALMONELLA MICROSUSPENSION ASSAY: HOW AND WHY IT WORKS, *J. P. Inmon* and D. M. DeMarini, US EPA, RTP, NC 27711
9:30 a.m. – 9:45 a.m.	**T2** MUTAGENIC ACTIVITY OF SIDESTREAM AND MAINSTREAM CIGARETTE SMOKE IN THE AMES/SALMONELLA ASSAY, *L. G. Monteith,*[1] D. M. Simmons,[1] L. D. Claxton,[2] K. H. Davis,[1] J. T. Keever,[1] and T. J. Hughes.[1] [1]RTI, RTP, NC 27709; [2]US EPA, RTP, NC 27711
9:45 a.m. – 10:00 a.m.	**T3** MUTATIONAL RESPONSE OF A PETROLEUM OIL EXTRACT IN THE AMES ASSAY AND MOUSE LYMPHOMA CELLS, *T. R. Barfknecht,* R. C. Nardone, L. J. Lane, and R. W. Naismith. Pharmakon Research International, Waverly, PA.
10:00 a.m. – 10:15 a.m.	**T4** SYNTHESIS AND BIOLOGICAL ACTIVITY OF CYCLOPENTA EPOXIDES OF PAH CONTAINING PERIPHERALLY FUSED CYCLOPENTA RINGS, *A. W. Bartczak,* R. Sangaiah, L. M. Ball, and A. Gold, UNC-CH, Chapel Hill, NC 27514
10:15 a.m. – 10:45 a.m.	Coffee Break
10:45 a.m. – 11:00 a.m.	**T5** MORPHOLOGICAL TRANSFORMATION OF MAMMALIAN CELLS BY CYCLOPENTA-FUSED ISOMERS OF BENZ(A)ANTHRACENE AND THEIR METABOLISM, *N. Mohapatra,*[1] S. Nesnow,[1] P. MacNair,[2] B. J. Bryant,[2] S. Ellis,[2] K. Rudo,[2] R. Sangaiah,[3] A. Gold.[3] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709; [3]UNC-CH, Chapel Hill, NC 27514
11:00 a.m. – 11:15 a.m.	**T6** GENOTOXIC ACTIVITIES OF ACEANTHRYLENE AND ACEPHENANTHRYLENE, *J. Ross,*[1] S. Nesnow,[1] N. Mohapatra,[1] A. Gold,[2] B. J. Bryant,[3] K. Rudo,[3] P. MacNair,[3] S. Ellis,[3] R. Gupta.[4] [1]US EPA, RTP, NC 27711; [2]UNC-CH, Chapel Hill, NC 27514; [3]EHRT, RTP, NC 27709; [4]Baylor College of Medicine, Houston, TX

11:15 a.m. – Noon	AIRBORNE MUTAGENS AND CARCINOGENS: WHAT DO WE KNOW? **Special Address** by Dr. L. Claxton, US EPA, RTP, NC
Noon – 1:30 p.m.	Lunch, Membership and Financial Reports, Installation of Elected Officers
1:30 p.m. – 1:45 p.m.	**T7** METABOLISM OF 1-NITRO[^{14}C]PYRENE BY PRIMARY TRACHEAL EPITHELIAL CELLS, *Leon C. King,*[1] Marcus Jackson,[2] Louise M. Ball,[3] and Joellen Lewtas.[1] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709; [3]UNC-CH, Chapel Hill, NC 27514
1:45 p.m. – 2:00 p.m.	**T8** EXPERIMENTAL METHODOLOGY TO DETERMINE THE VARIABILITY AND RESOLVING POWER OF AN IN VITRO TRANSFORMATION ASSAY, *J. D. Van Arnold,* W. A. Suk, J. E. Humphreys, M. A. Chapman, and P. H. Suk. Northrop, RTP, NC 27709
2:00 p.m. – 2:15 p.m.	**T9** EFFECTS OF THE TUMOR PROMOTER TPA AND DERIVATIVES ON COLONY FORMATION IN CULTURE OF HUMAN, HAMSTER, AND RAT RESPIRATORY EPITHELIAL CELLS, *D. K. Beeman,*[1] J. M. Siegfried,[2] M. J. Mass.[1] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709
2:15 p.m. – 2:30 p.m.	**T10** INDUCTION OF CYTOGENETIC DAMAGE IN RATS AFTER SHORT-TERM INHALATION OF BENZENE, *G. L. Erexson,*[1] J. L. Wilmer,[2] W. H. Steinhagen,[3] and A. D. Kligerman.[1] [1]EHRT, RTP, NC 27709; [2]Bristol Labs, Syracuse, NY 13221; [3]CIIT, RTP, NC 27709
2:30 p.m. – 2:45 p.m.	**T11** MOLECULAR ANALYSIS OF SPONTANEOUS AND INDUCED MUTATIONS, *Ronald R. Cobb,* Lois B. Barnett, and Susan E. Lewis, RTI, RTP, NC 27709
2:45 p.m. – 3:45 p.m.	THE SYNAPTONEMAL COMPLEX AS AN INDICATOR OF MUTAGENIC EFFECTS IN MAMMALIAN GERM CELLS, **Keynote Address** by Dr. Montrose J. Moses, Dept. of Anatomy, Duke University Medical Center, Durham, NC
3:45 p.m. – 4:45 p.m.	Poster Sessions, Vendor Exhibits, and Social
5:00 p.m.	Meeting Adjourns

POSTERS

P1 QUALITY ASSESSMENTS: GLP COMPLIANCE VS. PEER REVIEW. *R. S. DeWoskin* and J. H. Haw. RTI, RTP, NC 27709

P2 PRACTICAL APPLICATION OF COMPUTERIZED SAR IN MUTAGENICITY TESTING. *D. Walsh,*[1] M. Miller,[1] E. Perry,[1] and L. Claxton.[2] [1]EHRT, RTP, NC 27711; [2]US EPA, RTP, NC 27711

P3 THE EFFECTS OF VARIOUS EXTRACTION METHODS AND SOLVENTS ON THE RECOVERY OF MUTAGENIC ACTIVITY FROM WOODSMOKE-IMPACTED AIR PARTICULATE. *Ron Williams,*[1] Linda Forehand,[1] Sarah Warren,[1] Andrew Stead,[2] Joyce Chappell,[2] Larry Claxton,[2] and Joellen Lewtas.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

P4 EFFECT OF PREINCUBATION TIME ON MUTAGENIC ACTIVITY IN THE AMES/SALMONELLA ASSAY. *D. M. Simmons,*[1] L. G. Monteith,[1] T. J. Hughes,[1] and L. D. Claxton.[2] [1]RTI, RTP, NC 27709; [2]US EPA, RTP, NC 27709

P5 COMPARISON OF $\sigma TK^{-/-}$ MUTANT FREQUENCY AND CYTOGENETIC DAMAGE IN L5178Y/$TK^{+/-}$ MOUSE LYMPHOMA CELLS. *P. A. Poorman,* R. Krehl, N. T. Turner, and D. Clive, Burroughs Wellcome, RTP, NC 27709.

P6 MUTAGENICITY OF NONCARCINOGENS, *Michael D. Shelby,* NIEHS, RTP, NC 27709

P7 EPIGENESIS BY 5-AZACYTIDINE: MISLABELLED GENOTOXICITY? *R. Krehl,* P. A. Poorman, and D. Clive, Burroughs Wellcome, RTP, NC 27709

P8 EFFECTS OF A 5-AZACYTIDINE ON THE PROGRESSION OF CELL TRANSFORMATION IN RAT EMBRYO CELLS. *M. A. Chapman,* E. P. Hays, and W. A. Suk, Northrop, RTP, NC 27709

P9 ANALYSIS OF SISTER CHROMATID EXCHANGE (SCE) IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES (PBLs) AND BONE MARROW FOLLOWING EXPOSURE TO CARCINOGEN/NONCARCINOGEN PAIRS. *A. D. Kligerman,*[1] G. L. Erexson,[1] B. Westbrook-Collins,[1] Y. Sharief,[2] J. A. Campbell,[2] and J. W. Allen.[3] [1]EHRT, RTP, NC 27709; [2]Integrated Laboratory Systems, RTP, NC 27709; [3]US EPA, RTP, NC 27711

P10 ISOLATION AND CULTURE OF PRIMARY LIVER, LUNG, AND KIDNEY CELLS OF MICE FOR SISTER CHROMATID EXCHANGE ANALYSIS FOLLOWING IN VIVO EXPOSURE TO VINYL CARBAMATE. *J. A. Campbell,*[1] A. D. Kligerman,[1] Y. Sharief,[2] A. B. Petro,[3] and J. W. Allen.[3] [1]EHRT, RTP, NC 27709; [2]Integrated Laboratory Systems, RTP, NC 27709; [3]US EPA RTP, NC 27711

P11 SCE AND CHROMOSOME ABERRATION ANALYSES IN MICE AFTER *IN VIVO* EXPOSURE TO ACRYLONITRILE, STYRENE, OR BUTADIENE MONOXIDE. *L. C. Backer,*[1] A. M. Brown,[2] J. W. Allen,[1] J. A. Campbell,[3] B. Westbrook-Collins,[3] A. G. Stead,[1] and Y. Sharief.[3] [1]US EPA, RTP, NC 27711; [2]US FDA, Rockville, MD; [3]EHRT, RTP, NC 27709

P12 COMPARATIVE TOXICOLOGY STUDIES IN CULTURED UPPER AIRWAY EPITHELIUM, *V. E. Steele* and J. T. Arnold, Northrop, RTP, NC 27709

ABSTRACTS
POSTERS

P1 QUALITY ASSESSMENTS: GLP COMPLIANCE VS. PEER REVIEW. *R. S. DeWoskin* and J. H. Haw. RTI, RTP, NC 27709

The quality of a scientific study is assessed by comparing its attributes to a set of reference standards. Conclusions based upon different standards may not agree. Two methods of assessing quality, the GLP Compliance Audit and the Peer Review, differ both in the attributes of interest and in the reference standards used. The GLP compliance audit generally assesses the integrity and quality of the data, whereas the peer review generally assesses the scientific merit of the study. Data integrity is possible in a study that is lacking in scientific merit, but scientific merit is unlikely in a study whose conclusions are based upon inaccurate or fraudulent data. To assure the overall quality of the study, the GLP audit results should be readily available to the peer review panel.

P2 PRACTICAL APPLICATION OF COMPUTERIZED SAR IN MUTAGENICITY TESTING. *D. Walsh,*[1] M. Miller,[1] E. Perry,[1] and L. Claxton.[2] [1]EHRT, RTP, NC 27711; [2]US EPA, RTP, NC 27711

Mutagenicity laboratories are frequently improved by modifying testing methods and computerizing data collection. A recent practical application of computerized technology is the capability of predicting mutagenicity of an untested chemical by using computerized structure activity relationships (SAR) analysis. The ADAPT system was used to study the relationship between molecular structure and mutagenicity of 70 halogenated aromatic compounds. A compound was considered a nonmutagen only if there was a negative response in Ames tester strains TA98, TA100, TA1535, TA1537, and TA1538 when bioassayed with and without metabolic activation. A positive response in any of the strains, either with or without activation, classified the compound a mutagen. The data set of 25 mutagens and 45 nonmutagens was correctly classified using nine molecular descriptors. The average correct predictability of these descriptors applied to structurally similar compounds was evaluated by a holdout method in the system, which measures the internal consistency of the data. Analyzing and averaging of 80 training/prediction subsets of the data base, as determined by the holdout method, gave an average correct predictability of 92%. Mutagenicity of 13 halogenated pyridines not included in the data base was predicted using this set of structural descriptors. The ADAPT predicted all 13 compounds as negative. When these compounds were tested in our laboratory in the Ames tester strains mentioned above and TA102 both with and without activation all 13 compounds were negative. The ADAPT's correct prediction provides a definite linkage between SAR studies and the testing laboratory to help establish priorities for testing new compounds.

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

P3 THE EFFECTS OF VARIOUS EXTRACTION METHODS AND SOLVENTS ON THE RECOVERY OF MUTAGENIC ACTIVITY FROM WOODSMOKE-IMPACTED AIR PARTICULATE. *Ron Williams,*[1] Linda Forehand,[1] Sarah Warren,[1] Andrew Stead,[2] Joyce Chappell,[2] Larry Claxton,[2] and Joellen Lewtas.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

A systematic study of the effects of extraction methodology on mutagenic recovery from urban air particulate was conducted using the Ames *Salmonella typhimurium* plate incorporation assay. Conducted as part of the U.S. EPA Integrated Air Cancer Project, the objective of the research was to optimize sample preparation procedures for the recovery of mutagens. Particulate extraction solvents methanol, acetone, dichloromethane and 3:1 v/v toluene/ethanol were compared via sonication or Soxhlet techniques. Protocols established from historical literature were utilized on replicate particulate samples and mutagenic dose response curves obtained from the extracts. Activity data was then used to indicate a preferred extraction method and complementary solvent (dichloromethane and sonication). This technique was optimized for parameters involving necessary solvent volumes, extraction periods, and extraction frequency. A finalized protocol was then used to extract individual urban air particulate filters from regional sites where health concerns have been raised due to woodsmoke impaction (from residential heating).

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

P4 EFFECT OF PREINCUBATION TIME ON MUTAGENIC ACTIVITY IN THE AMES/SALMONELLA ASSAY. *D. M. Simmons,*[1] L. G. Monteith,[1] T. J. Hughes,[1] and L. D. Claxton.[2] [1]RTI, RTP, NC 27709; [2]US EPA, RTP, NC 27709

The effect of preincubation time on the mutagenic activity of 11 volatile organic liquids was investigated in the Ames/Salmonella test system. These compounds were tested utilizing Salmonella strains TA100 and TA102, with and without Aroclor-induced hamster liver S9. The chemicals tested were: trichloroethylene (TRC), ethylene dichloride (EDC), formalin (FM), epichlorohydrin (EPC), 2-nitropropane (NP), ethylene dibromide (EDB), 1-bromo-2-chloroethane (BCE), ethyl bromide (EBR), ethyl iodide (EID), propylene oxide (PO) and methylene chloride (MC). Each chemical was initially tested at the minimum effective dose that produced a positive mutagenic response, as determined in previous studies. The chemical, bacteria, and S9 mix were incubated at 37° in glass vials, which were rotated at 60 RPM for eight preincubation times (0, 5, 10, 15, 20, 25, 30 and 60 min), with zero min preincubation being the standard plate incorporation technique. Results suggested that the preincubation technique was a more sensitive test for mutagenic evaluation of volatile chemicals than the standard plate incorporation method. The mutagenic response can be maximized with the correct preincubation time. For example, a preincubation time of 5 to 10 min allowed for greater bacterial survival and an increased mutagenic response when toxic chemicals were tested (e.g., FM, MC). A mid-range preincubation time (15 to 30 min) was suitable to detect the highest mutagenic response for the majority of the chemicals. A longer preincubation time (60 min) produced the highest mutagenic response for weak mutagens (e.g., NP, EBR). A study of mutagens at five dose levels suggested an inversely proportional relationship between dose and preincubation time; the higher the dose or the more potent the mutagen, the lower the preincubation time necessary to achieve maximum mutagenic response.

Research supported by EPA Contract No. 68-02-3992-17.

P5 COMPARISON OF $\sigma TK^{-/-}$ MUTANT FREQUENCY AND CYTOGENETIC DAMAGE IN L5178Y/$TK^{+/-}$ MOUSE LYMPHOMA CELLS. *P. A. Poorman*, R. Krehl, N. T. Turner, and D. Clive, Burroughs Wellcome, RTP, NC 27709.

In an earlier study with the mouse lymphoma assay, a nearly constant ratio was found between the frequency of small colony thymidine kinase-deficient ($\sigma TK^{-/-}$) mutants and the incidence of chromosomally aberrant cells for three compounds (caffeine, methotrexate, and methapyrilene), while a fourth compound (colchicine) produced neither $TK^{-/-}$ mutants nor chromosome aberrations [Clive *et al, Environ. Mutagen.* **7** (1985) 33]. In the present study, six additional compounds, including two candidate antiviral drugs, were tested at 3-7 concentrations each. These showed a wider variation in the ratio of $\sigma TK^{-/-}$ mutants to chromosome aberrations, as tabulated:

Compound	Max.$\sigma TK^{-/-}$ mut. freq. ($\times 10^{-6}$)	Max. % cells with aberrs	Ratio (σ/CA) ($\times 10^{-4}$)
Hycanthone	1072	68	16
2-AAF	240	42	5.7
HMPA	101*	19*	5.7
Antiviral B	325	76	4.3
5-Azacytidine	263	75	3.5
Antiviral A	52*	31	1.7

*These responses are not considered to be significantly above their respective concurrent backgrounds.

We are currently exploring possible explanations for these differences in ratios of $\sigma TK^{-/-}$ mutants/chromosomally aberrant cells, including site specificity of chromosome breaks for certain compounds, and cell sampling time after dosing for analysis of cytogenetic damage.

P6 MUTAGENICITY OF NONCARCINOGENS, *Michael D. Shelby*, NIEHS, RTP, NC 27709

Prior to 1980, there were few chemicals known that produced no evidence of carcinogenicity following long-term, high-dose studies in rodents. Because of this, studies to determine the performance of short-term tests in predicting chemical carcinogenicity have been frequently characterized by a poor representation of adequately defined noncarcinogens among the test chemicals. As a result, we have a limited understanding of both the ability of short-term tests to discriminate between carcinogens and noncarcinogens, and the frequency with which noncarcinogens exhibit genetic toxicity. The recent publication of a large number of reports on the results of NCI or NTP long-term studies in rodents have provided a source of noncarcinogens for addressing the two problems. If it is accepted that a mutational change can contribute to the neoplastic process, then a chemical with intrinsic mutagenicity, as demonstrated by in vitro testing, should be considered potentially carcinogenic. If negative results are obtained in thorough, short-term in vivo assays, concern for the chemical's potential carcinogenicity may be diminished. The National Toxicology Program/NIEHS has accumulated in vitro test data on many of the chemicals that produced no evidence of carcinogenicity when tested for two years in male and female rats and mice. Of 70 such chemicals, at least 50% exhibit a positive response in one or more in vitro tests. Furthermore, the four in vitro tests (Salmonella, L5178Y, CHO aberrations, CHO SCE) all gave positive results for some noncarcinogens. These findings emphasize the importance of more thoroughly assessing the performance of individual assays for carcinogens and, in general, of more clearly defining the role of in vitro tests in identifying carcinogens.

P7 EPIGENESIS BY 5-AZACYTIDINE: MISLABELLED GENOTOXICITY? *R. Krehl*, P. A. Poorman, and D. Clive, Burroughs Wellcome, RTP, NC 27709

5-Azacytidine (AZA) is purported to alter the phenotype of certain eukaryotic cells via an epigenetic mechanism. Most of these claims rest on the relatively high specificity and frequency of induction of these phenotypic alterations, the incomplete stability of the altered phenotype, and a claimed absence of genotoxicity for this compound [*e.g.*, Frost *et al.*, J. Exp. Med **159** (1984) 1491-1501]. The present study shows that low concentrations (⟨1.0 μg/ml) of AZA are both mutagenic and clastogenic in the L5178Y/TK$^{+/-}$ mouse lymphoma assay, producing primarily small colony TK$^{-/-}$ mutants. These results are consistent with recent reports that AZA is clastogenic [Lavia *et al.*, Mutat. Res. **149** (1985) 463–467] and induces fragile sites [Sutherland *et al.*, Hum. Genet. **69** (1985) 233–237]. In light of the demonstrated mutagenicity and confirmed clastogenicity of AZA (and other compounds — *e.g.*, methapyrilene, DES, asbestos — purported to act via epigenetic mechanisms), we propose that the term epigenesis be restricted to (i) high frequency events (*e.g.*, affecting >50% of cells at risk), which (ii) occur in the demonstrated absence of mutagenesis and chromosomal aberrations. The hypothesis that the unusual effects of AZA result from the properties of fragile site induction (high frequencies, cell line specificity and incomplete stability) represents a specific and testable alternative to "epigenesis."

P8 EFFECTS OF A 5-AZACYTIDINE ON THE PROGRESSION OF CELL TRANSFORMATION IN RAT EMBRYO CELLS. *M. A. Chapman*, E. P. Hays, and W. A. Suk, Northrop, RTP, NC 27709

Our studies have shown that carcinogens induce enhancement of anchorage-independent survival (AIS$^+$), a preneoplastic event in retrovirus-infected Fischer rat embryo cells, and subsequent neoplastic transformation, and that these phenotypic changes require the presence of a nontransforming retrovirus. In correlative studies, uninfected Fischer rat embryo (FRE) cells did not show this progression toward neoplasia. Since the expression of endogenous virus genomes and of cellular phenotypes is regulated by DNA modifications and can be activated by 5-azacytidine (AZA), this cytidine analog was used to study AIS$^+$, neoplastic progression, and endogenous retrovirus expression in the FRE cells. Exposure of FRE cells to AZA (0.3–8μg/ml) induced AIS$^+$ dose dependently. AIS$^+$ cells were then isolated in aggregate form, plated into flasks, and subcultured. Progression to neoplastic phenotypes was observed by the appearance of morphological transformation and growth in semisolid agar. During this progression, AZA-treated cultures expressed phenotypes of mesenchymal origin: myoblasts, adipocytes, and chondrocytes. Moreover, AZA induced the expression of endogenous retrovirus proteins, observed by immunoflourescence, at concentrations that were effective at causing preneoplastic, differentiated, and neoplastic phenotypes. These studies provide a relative system to study the regulation of specific gene expression.

Supported by NIEHS/NTP Contract No. NO1-ES-15798.

P9 ANALYSIS OF SISTER CHROMATID EXCHANGE (SCE) IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES (PBLs) AND BONE MARROW FOLLOWING EXPOSURE TO CARCINOGEN/NONCARCINOGEN PAIRS. *A. D. Kligerman,*[1] G. L. Erexson,[1] B. Westbrook-Collins,[1] Y. Sharief,[2] J. A. Campbell,[2] and J. W. Allen.[3] [1]EHRT, RTP, NC 27709; [2]Integrated Laboratory Systems, RTP, NC 27709; [3]US EPA, RTP, NC 27711

As part of the World Health Organization IPCS collaborative study on in vivo tests, SCE analyses were performed on the PBLs and bone marrow of male C57B1/6 mice to determine possible differences in SCE induction potencies between carcinogen/noncarcinogen pairs. Mice were treated by gavage with either 2-acetylaminofluorene (2AAF) or 4-acetylaminofluorene (4AAF), or benzo(a)pyrene (BP) or pyrene (PYR). Twenty-four hours after exposure, some animals were bled by cardiac puncture for analysis of SCEs in PBLs and other animals were killed by cervical dislocation for femoral bone marrow SCE analysis according to standard published protocols. Both 2AAF and BP caused dose-related increases in SCE frequencies in bone marrow and PBLs, with 2AAF clearly being the less potent of the two chemicals in both systems. In the mouse PBL system, both 4AAF and PYR produced no statistically significant increase in SCE frequency at any dose examined. In bone marrow cells, 4AAF produced a small but statistically significant increase in SCE frequency while PYR was negative. It is concluded that SCE analysis in either bone marrow or PBLs can aid in discriminating carcinogens from noncarcinogens on the basis of their relative abilities to induce this form of cytogenetic damage.

P10 ISOLATION AND CULTURE OF PRIMARY LIVER, LUNG, AND KIDNEY CELLS OF MICE FOR SISTER CHROMATID EXCHANGE ANALYSIS FOLLOWING IN VIVO EXPOSURE TO VINYL CARBAMATE. *J. A. Campbell,*[1] A. D. Kligerman,[1] Y. Sharief,[2] A. B. Petro,[3] and J. W. Allen.[3] [1]EHRT, RTP, NC 27709; [2]Integrated Laboratory Systems, RTP, NC 27709; [3]US EPA RTP, NC 27711

Techniques are presented for the short-term culture (48 to 56 h) of liver, lung, and kidney cells from C57B1/6 mice. With this methodology mice can be exposed in vivo to test compounds and the cells grown on cover glasses in the presence of 5-bromo-2′-deoxyuridine (5 μM) for analysis of sister chromatid exchange (SCE) and cell cycle kinetics. Mice exposed to a single ip injection of vinyl carbamate (VC) (10 to 60 mg/kg) were used in the initial examination of this system. Cultured lung and kidney cells from animals exposed to 60 mg/kg VC exhibited significant increases in SCE frequencies (approximately 3 to 5× baseline). Liver cells, however, were much less responsive and showed less than a twofold increase over baseline SCE levels. Lung cultures initiated as long as 320 h after VC exposure (60 mg/kg) revealed a persistence of lesions leading to an SCE frequency of more than 2× baseline. These protocols yield substantial numbers of metaphases for the analysis of cytogenetic damage in organs with very low in vivo mitotic activity.

P11 SCE AND CHROMOSOME ABERRATION ANALYSES IN MICE AFTER IN VIVO EXPOSURE TO ACRYLONITRILE, STYRENE, OR BUTADIENE MONOXIDE. *L. C. Backer,*[1] A. M. Brown,[2] J. W. Allen,[1] J. A. Campbell,[3] B. Westbrook-Collins,[3] A. G. Stead,[1] and Y. Sharief.[3] [1]US EPA, RTP, NC 27711; [2]US FDA, Rockville, MD; [3]EHRT, RTP, NC 27709

The use of polymers in plastic and rubber products has generated concern that monomers potentially active in biological systems may be eluted from these substances. We have evalu-

ated two such monomers, acrylonitrile and styrene, for the induction of chromosome damage in mice. Butadiene monoxide, a presumed metabolite of a third important monomer, 1,3-butadiene, was also tested. These chemicals were administered as a single ip injection; SCEs and chromosome aberrations were analyzed in bone marrow cells. Acrylonitrile and styrene were largely negative for these endpoints when tested at doses ranging to 60 mg/kg and 1,000 mg/kg, respectively. Butadiene monoxide, which previously had not been tested in a mammalian system, was determined to be a very effective inducer of SCEs and chromosome aberrations. Both endpoints showed a clear dose response and a greater than tenfold increase over control levels at high doses. These studies represent an initial step in our efforts to evaluate genetic risk associated with exposure to common polymers.

P12 COMPARATIVE TOXICOLOGY STUDIES IN CULTURED UPPER AIRWAY EPITHELIUM, *V. E. Steele* and J. T. Arnold, Northrop, RTP, NC 27709

To compare the cytotoxic responses of nasal epithelial cells from experimental animals and humans, cell cultures were exposed to carcinogens and a tumor promoter. Two techniques were developed to remove nasal epithelial cells from experimental animals. A surgical technique was used to remove specific regions of the upper airway for study. This tissue was then incubated in an enzyme solution and the cells were harvested the next day. The second method was an *in situ* enzyme incubation technique in which the enzyme was placed directly into the nasal cavity and the cells harvested after two 30 min incubation periods. Human cells were isolated from surgical specimens by incubating the tissue overnight in an enzyme solution. Response to toxicants in primary cell cultures was measured by counting viable cell numbers at 5 to 7 days after exposure. A decrease of up to 10 fold in growth rate was seen when F-344 rat and human nasal epithelial cells were exposed for 24 h to sodium chromate between 0.1 and 3 μg/ml. The human cells were more sensitive than the rat cells to the cytotoxic effects of chromium. However, human cells were more resistant than rat cells when cultures were exposed to cadmium sulfate. In addition, human cells grew at a much slower rate than rat cells when exposed to the tumor promoter, TPA. These studies demonstrate that rat and human epithelial cells from the upper airways may differ in their response to carcinogens and tumor promoters.

Supported by US EPA Contract No. 68-02-4032.

ABSTRACTS
TALKS

T1 TOWARDS AN UNDERSTANDING OF THE SALMONELLA MICROSUSPENSION ASSAY: HOW AND WHY IT WORKS. *J. P. Inmon* and D. M. DeMarini, US EPA, RTP, NC 27711

Because of our need to determine the mutagenicity of environmental samples, such as air and water, we have been interested in developing assays that are highly sensitive and require only small amounts of sample. In pursuit of this goal, we have investigated various aspects of a Salmonella microsuspension assay that was developed by Kado *et al.* (Mutat. Res. 121:25; 1983). The assay consists of adding 100 μl of a 10-X concentrate of an overnight culture of cells; 10 μl of S9 mix or buffer; and only 5 μl of mutagen or solvent to a small test tube. This suspension is incubated for 90 min at 37°C, top agar is added, and the contents of the tube are poured onto minimal medium. By altering the volume of the reaction mixture, the number of cells, and incubation time; and by comparing the results to those obtained from the plate-incorporation assay, the preincubation assay, and from modifications of both of these assays, we have determined that: (1) essentially all of the detectable mutagenesis occurs in the reaction tube and (2) the mutagenic response is a function of the amount of mutagen per ml of the reaction mixture and not of the amount per plate. Thus, we suggest that the dose of mutagen for this assay be expressed as μg/ml, not μg/plate. The 10-fold greater number of cells provides more target cells than the standard preincubation assay, and the reduced volume and increased incubation time enhance the sensitivity of the assay by at least 10-fold over the plate-incorporation or preincubation assay. We have used the following compounds: 2-nitrofluorene, 2-anthramine, benzo(a)pyrene, sodium azide, and 2-acetylaminofluorene—as well as three complex mixtures (air and water samples and rodent urine)—in these comparative studies. In all cases, the microsuspension assay was more sensitive, i.e., detected mutagenicity at a lower dose, than the standard assays. In addition, nine noncarcinogens that are not mutagenic in the standard Salmonella assays were not mutagenic in the microsuspension assay.

T2 MUTAGENIC ACTIVITY OF SIDESTREAM AND MAINSTREAM CIGARETTE SMOKE IN THE AMES/SALMONELLA ASSAY, *L. G. Monteith*,[1] D. M. Simmons,[1] L. D. Claxton,[2] K. H. Davis,[1] J. T. Keever,[1] and T. J. Hughes.[1] [1]RTI, RTP, NC 27709; [2]US EPA, RTP, NC 27711

The objective of this research was to develop methods to collect and fractionate organic compounds from mainstream and sidestream cigarette smoke and to test these fractions in the Ames/Salmonella mutagenicity assay. Trapping trains were attached to a 30-port cigarette-smoking machine. A large Tedlar® plastic tent was constructed around the cigarette-smoking machine to trap the sidestream smoke. Constant draft, puff mode and constant burn were the smoking conditions. Thirty reference cigarettes (IR3) from the University of

Kentucky Tobacco Health Department were burned per smoking condition. Two trapping trains were used: 1) ethanol bubbler/sand/cold trap and 2) XAD resin/Pallflex® filters/cold trap. Both sidestream and mainstream smoke were collected from the cigarette smoking machine. Three fractions were collected from each train: 1) respirable particulates and some semivolatiles; 2) semivolatiles; 3) volatiles with boiling points $<60°C$. The semivolatiles and particulate fractions were tested with strains TA98 and TA100, both with and without 5% hamster liver S9 in the preincubation modification with rotation at 60 RPM for 5 or 15 min at 37°C. The volatile fractions were tested in Tedlar® bags with the same strains and activation conditions. Results suggested semivolatile and particulate fractions tested with TA98 and S9 were mutagenic; those tested with TA100 and S9 were not mutagenic; and those tested with TA100 and TA98 without S9 were toxic. Cold trap volatiles were not mutagenic under any test condition. Slope analysis of the data is in progress. Mutagenic activity of the mainstream and sidestream fractions under the varying test conditions and efficiency of the different sampling trains to collect mutagenic compounds will be discussed.

Research sponsored by EPA Contract No. 68-02-3992-17.

T3 MUTATIONAL RESPONSE OF A PETROLEUM OIL EXTRACT IN THE AMES ASSAY AND MOUSE LYMPHOMA CELLS. *T. R. Barfknecht*, R. C. Nardone, L. J. Lane, and R. W. Naismith, Pharmakon Research International, Waverly, PA.

Two methods of extraction were evaluated with a petroleum crude oil from the National Bureau of Standards. One method was a 1:5 extraction (oil/DMSO). The second method was a 1:1:4 extraction (oil/DMSO/methylene chloride) with subsequent removal of the methylene chloride with a stream of N_2 gas to yield a concentrated extract. The extracted samples were initially evaluated in Ames/Salmonella strains TA1538 and TA98 with induced hamster liver S9 at a concentration of 400 μl/plate with a pre-incubation protocol. Mean mutational slope values for the unconcentrated extract in strains TA1538 and TA98 were 0.83 and 0.73 respectively. The corresponding slope values for the concentrated extract were 2.9 and 10.6 respectively. Employing the concentrated extract and strain TA98 the optimal level of S9 was determined for 2 lots of induced hamster S9 and 1 lot of induced guinea pig S9. The optimal concentration of S9 was 100 μl/plate for all three S9 preparations. Utilizing the optimal level of S9 fraction for the three S9 preparations, dose response studies were performed with strain TA98. Mutational slope values observed over a dose range of 1 – 10 μl/plate with induced hamster liver S9 lots A & B and guinea pig S9 were 25.5, 32.2, and 30.9 respectively. Of the four tester strains, TA1538, TA97, TA98, and TA100, only TA98 exhibited a positive dose-response with the concentrated extract. In the Mouse Lymphoma Cell Mutation Assay with an induced rat liver S9 preparation the concentrated extract induced a positive mutational dose response with a slope value of 0.34. However, no mutational response was detected with induced hamster liver S9. The data suggest that extract concentration is superior to simple DMSO extraction for the detection of genotoxicity of a petroleum oil.

T4 SYNTHESIS AND BIOLOGICAL ACTIVITY OF CYCLOPENTA EPOXIDES OF PAH CONTAINING PERIPHERALLY FUSED CYCLOPENTA RINGS, *A. W. Bartczak*, R. Sangaiah, L. M. Ball, and A. Gold, UNC-CH, Chapel Hill, NC 27514

Initial work demonstrated the mutagenicity of all four benzanthracene isomers and aceanthrylene and has supported involvement of the cyclopenta ring in each case on the basis of the S9 concentration-dependence curves of mutagenicity in the Ames test. We have

synthesized all four benzanthracene cyclopenta epoxides by reacting each fused cyclopenta ring benzanthracene isomer with N-bromosuccinimide in wet DMSO and subsequent reaction of the bromohydrin with sodium methoxide in THF. Preliminary Ames testing done on the oxides has shown them to be active without further metabolism and considerably more active than the parent hydrocarbon, suggesting that, consistent with previously published S9 curves, the cyclopenta epoxides are responsible for the biological activity shown by the parent hydrocarbon.

T5 MORPHOLOGICAL TRANSFORMATION OF MAMMALIAN CELLS BY CYCLOPENTA-FUSED ISOMERS OF BENZ(A)ANTHRACENE AND THEIR METABOLISM, *N. Mohapatra,*[1] S. Nesnow,[1] P. MacNair,[2] B. J. Bryant,[2] S. Ellis,[2] K. Rudo,[2] R. Sangaiah,[3] A. Gold.[3] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709; [3]UNC-CH, Chapel Hill, NC 27514

Cyclopenta-fused PAH, identified in coal combustion and wood smoke effluents, are a novel class of environmental pollutants. In previous studies, the four isomeric cyclopenta-fused benz(a)anthracenes — benz(j)aceanthrylene [B(j)A], benz(e)aceanthrylene [B(e)A], benz(k)acephenanthrylene [B(k)A] and benz(l)aceanthrylene [B(l)A] — were found to be active in mutating *S. typhimurium* and V79 cells (at the HGPRT locus). For B(l)A it was determined that up to 50% of its mutagenic activity could be ascribed to metabolic activation at the cyclopenta ring. In this study, we present the ability of these four cyclopenta-fused benz(a)anthracenes to morphologically transform C3H10T1/2CL8 (10T1/2) mouse embryo fibroblasts. Two of the four isomers [B(e)A and B(l)A] transformed 10T1/2 cells at concentrations of 0.5–10.0 μg/ml. B(j)A was active at concentrations of 0.01–2.5 μg/ml. When compared to B(a)P, B(e)A and B(l)A were 10-fold less active while B(j)A was equivalent in activity. Both type II and type III foci were produced at each concentration and a concentration-related response was observed for each active isomer. B(k)A was inactive even after the exposure time was increased to 96 h. None of these PAH were cytotoxic at these concentrations. Metabolism of [^{14}C]B(l)A and [^{3}H]B(j)A to unconjugated, sulfate conjugated, and glucuronide conjugated metabolites has been studied in 10T1/2 cells. Unconjugated metabolites were the predominant form isolated. Metabolism at the cyclopenta-fused ring of these PAH was a minor pathway in 10T1/2 cells, in contrast to the results found in the Aroclor-1254 induced rat liver microsome system. The major metabolites formed were B(j)A-9,10-dihydrodiol and B(l)A-7,8-dihydrodiol. Additional investigations in the area of metabolism and DNA adduct analyses are needed to resolve the exact mechanisms of activation of these unique PAH in 10T1/2 cells.

T6 GENOTOXIC ACTIVITIES OF ACEANTHRYLENE AND ACEPHENANTHRYLENE S. Nesnow,[1] N. Mohapatra,[1] *J. Ross,*[1] A. Gold,[2] B. J. Bryant,[3] K. Rudo,[3] P. MacNair,[3] S. Ellis,[3] R. Gupta.[4] [1]US EPA, RTP, NC 27711; [2]UNC-CH, Chapel Hill, NC 27514; [3]EHRT, RTP, NC 27709; [4]Baylor College of Medicine, Houston, TX

Cyclopenta-fused PAH are a novel class of environmental PAH of which a well known example is cyclopenta(cd)pyrene. The fusion of an ethylene fragment on a PAH to form a cyclopenta-ring generally enhances its activity as a mutagen in bacteria and mammalian cells, as a rodent cell transforming agent, and as a mouse skin tumor initiator. The fusion of a cyclopenta-ring to anthracene and phenanthrene produces aceanthrylene (ACA) and acephenanthrylene (ACP) whose major Aroclor-1254 rat liver microsomal metabolites are the dihydrodiols arising from oxidation at the cyclopenta-ring. ACA and ACP are active as gene mutagens in the Aroclor-1254 rat liver S9 mediated *S. typhimurium* bioassay (TA98)

unlike the parent PAH, anthracene and phenanthrene. Neither ACA or ACP was active in transforming C3H10T1/2CL8 mouse embryo fibroblasts to morphologically altered foci at concentrations up to 16 μg/ml, the limits of solubility, similar to reported results with the parent PAH. In contrast, cyclopenta(cd)pyrene and benzo(a)pyrene [B(a)P] transform these cells at 1-10 μg/ml. The major metabolites of both ACA and ACP formed by C3H10T1/2CL8 cells were the cyclopenta-ring dihydrodiols, suggesting that lack of metabolism at that site was not responsible for the lack of foci formation. PAH–DNA adducts were studied using the ^{32}P post-labeling technique. Both ACA and ACP formed adducts to the DNA of C3H10T1/2CL8 cells under conditions similar to those employed in the transformation assay. Repair of ACA–DNA adducts and B(a)P-DNA adducts in C3H10T1/2CL8 cells using a hydroxyurea block of DNA replication could not be demonstrated within 48 h. Post-labeling studies using polydeoxynucleotides to identify the sites of adduct formation are in progress. We conclude that the inability of ACA and ACP to transform C3H10T1/2CL8 cells is not a result of deficient metabolic activation or of efficient DNA repair. We postulate that the DNA lesions formed are inefficient at producing a transformed phenotype.

T7 METABOLISM OF 1-NITRO[^{14}C]PYRENE BY PRIMARY TRACHEAL EPITHELIAL CELLS, *Leon C. King,*[1] Marcus Jackson,[2] Louise M. Ball,[3] and Joellen Lewtas.[1] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709; [3]UNC-CH, Chapel Hill, NC 27514

The metabolism of 1-nitro[^{14}C]pyrene (8.1 μM) and the binding of its reactive intermediates to DNA and protein of rabbit tracheal epithelial cells have been examined. Primary cultures of rabbit tracheal cells preserved normal cellular morphology as indicated by light and electron microscopy. No gross degeneration of the cells (cellular viability, ATP, DNA, and protein) occurred throughout a 4-h in vitro incubation period. Metabolites from the incubation medium and cell lysates were extracted, analyzed and quantitated by high pressure liquid chromatography. Metabolism of 1-nitro[^{14}C]pyrene by tracheal cells occurred by both ring oxidation (1-NP-Diol and hydroxynitrophenols) and reduction of the nitro moiety (NAAP and 1-AMP). The cells were isolated from the trachea following either a 1 or 20 protease digestion. They metabolized 1-nitro[^{14}C]pyrene to reactive intermediates that were bound to DNA and protein. The highest rates of metabolism and binding were observed in cultures of tracheal cells treated immediately after isolation by 1-h protease digestion of the trachea at 37°C as compared to a 20-h protease digestion at 4°C.

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

T8 EXPERIMENTAL METHODOLOGY TO DETERMINE THE VARIABILITY AND RESOLVING POWER OF AN IN VITRO TRANSFORMATION ASSAY *J. D. Van Arnold,* W. A. Suk, J. E. Humphreys, M. A. Chapman, and P. H. Suk. Northrop, RTP, NC 27709

A common problem with current in vitro transformation assays is the inability to discern weak positive responses. Even in tests with statistically significant results, questions arise as to the biological interpretation of the events that fall within the range of historical controls. To address this issue, our laboratory has instituted the use of multiple controls in a short-term assay using retrovirus-infected Fischer rat embryo cells. Our assay attempts to detect carcinogens by measuring a preneoplastic event, anchorage-independent survival. Any biological response displays a range of values; hence, independent, duplicate negative controls are used to define the variability of the untreated cell population. The potent carcino-

gen 7,12-dimethylbenzanthracene is used as a positive control at two doses; the high dose defines the variability of the treated cell population, and the low dose is used to define the resolving power of the assay. Determining the variability and resolving power of the assay system, along with developing a data base from a large number of experiments, provides a statistical basis for detecting weak carcinogenic substances.

Supported by NIEHS/NTP Contract NO1-ES-15798.

T9 EFFECTS OF THE TUMOR PROMOTER TPA AND DERIVATIVES ON COLONY FORMATION IN CULTURE OF HUMAN, HAMSTER, AND RAT RESPIRATORY EPITHELIAL CELLS, *D. K. Beeman,*[1] J. M. Siegfried,[2] M. J. Mass.[1] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709

Our laboratory is interested in the responses of various species to known tumor promoters and in the detection of environmental agents that are active as tumor promoters. The response of colony-forming efficiency (CFE) in culture to 12-0-tetradecanoylphorbol-13-acetate (TPA) has been measured for rat tracheal, human bronchial, and hamster tracheal epithelial cells. The normal colony-forming efficiency for rat tracheal epithelial cells averages between 2% and 5%; that for human bronchial epithelial cells varies from 3% to 22%; and that for hamster tracheal epithelial cells averages around 2%. In rat cells exposure to TPA at time of plating primary cells results in a concentration-dependent stimulation of colony-forming efficiency, while in explanted human cells it results in a concentration-dependent inhibition of colony-forming efficiency. TPA also inhibits colony formation in hamster cells. In rat cells the maximal colony formation observed (at 30 nM TPA or greater) was 10-fold higher than controls. Although a great variability was seen in the response of cells from individuals tested (130-fold difference for 24 individual specimens tested at 1.6 nM TPA) the colony-forming efficiency of human cells was reduced to virtually zero by 100 nM TPA. Other phorbol esters elicited a similar response generally in proportion to their activity as tumor promoters. In rat tracheal epithelial cells a time dependency of stimulation was observed and, in contrast, response of human bronchial cells to TPA was little affected by time. This study demonstrates the variable response of different species to phorbol esters and implies that the mechanism of action of tumor promoters may vary from species to species. Finally, the study emphasizes the importance of comparing more than one species when attempting to extrapolate to develop a model of human tissue.

T10 INDUCTION OF CYTOGENETIC DAMAGE IN RATS AFTER SHORT-TERM INHALATION OF BENZENE. *G. L. Erexson,*[1] J. L. Wilmer,[2] W. H. Steinhagen,[3] and A. D. Kligerman.[1] [1]EHRT, RTP, NC 27709; [2]Bristol Labs, Syracuse, NY 13221; [3]CIIT, RTP, NC 27709

Experiments were designed to investigate both the induction of sister chromatid exchanges (SCEs) in peripheral blood lymphocytes (PBLs) and micronuclei (MN) in bone marrow polychromatic erythrocytes (PCEs) of rats after inhalation of benzene (BZ). Male Sprague-Dawley rats (11 – 14 weeks old) were exposed to target concentrations of either 0, 0.1, 0.3, 1, 3, 10, or 30 ppm BZ for 6 h. Blood was obtained by cardiac puncture 18 h after exposure, and PBLs were cultured in the presence of concanavalin A (30 μg/ml) to stimulate blastogenesis of T-cells for SCE analysis. Five-bromo-2′-deoxyuridine (2 μM) was added 24 h after culture initiation for SCE analysis, and the cultures were harvested at 52 h following a final 4 h demecolcine (0.5 μg/ml) treatment. Rat PBLs showed a significant increase in the SCE

frequency after exposure to either 1, 3, 10, or 30 ppm BZ. Rat PCEs revealed a significant concentration-related increase in MN after inhalation of either 1, 3, 10, or 30 ppm BZ. Rat PBLs showed significant decreases in mitotic activity only after exposure to 3 and 30 ppm BZ, whereas cell-cycle kinetics and leucocyte counts were unaffected. These results show that BZ induces significant cytogenetic effects in PBLs and PCEs of rats after a 6 h inhalation of BZ to a concentration as low as 1 ppm.

T11 MOLECULAR ANALYSIS OF SPONTANEOUS AND INDUCED MUTATIONS

Ronald R. Cobb, Lois B. Barnett, and Susan E. Lewis, RTI, RTP, NC 27709

The understanding of the molecular structure of genes has been significantly increased by current developments in recombinant DNA technology. These techniques have recently been utilized to study the molecular aspects of mutagenesis. Mechanisms and specific patterns of mutagenic responses can be discovered by studying the structural alteration in mutant genes. Short-term tests for mutagenesis provide a basis for choosing which compounds industry will develop and which compounds will be chosen for priority testing by regulatory agencies. Long-term in vivo tests, using laboratory animals (mice, rats, etc.), play a crucial role in determining which compounds are mutagenic to mammalian systems. A combination of short-term testing, in vivo studies, and molecular techniques should allow for a more thorough understanding of the mutation induction mechanism.

The molecular mechanism of induced and spontaneous mutations was investigated using the cytosolic malic enzyme locus (*Mod-1*). Several mutations have been discovered by starch gel electrophoresis and enzyme activity. Each mutation has been identified by our laboratory and classified as either: 1) electrophoretic mobility variants; 2) altered enzyme activity or protein concentrations; or 3) null mutations. Genomic DNA was isolated from homozygous mutant mice, as well as C57BL/6J, DBA/2J and F_1(C57BL/6J $\times$ DBA/2J), and blotted onto nitrocellulose membranes. The restriction fragment banding patterns of each mutation were compared to the normal banding patterns. For a more comprehensive analysis of each mutation, the size and concentration of poly(A) + RNA were compared to normal poly(A) + RNA. The focus of this work is on the analysis of these mutant DNA fragments.

This research was supported by NIEHS Contract No. NO1-ES-55078.

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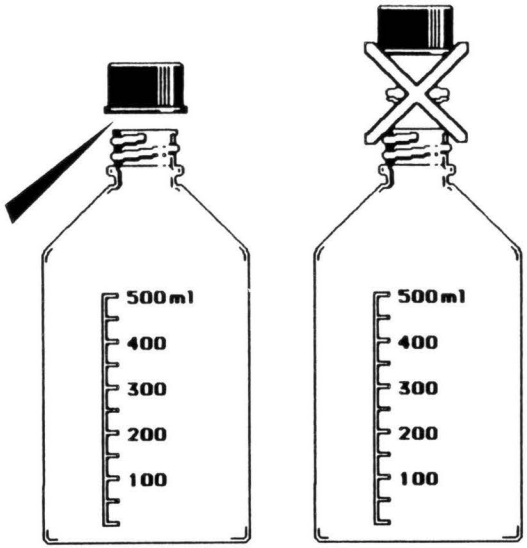

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