GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

FOURTH ANNUAL MEETING

PROGRAMS AND ABSTRACTS

November 20, 1986
Sheraton University Center
Durham, North Carolina
8:00 a.m. to 6:00 p.m.

Banquet and Scientific Evening 6:00 p.m. to 9:00 p.m.

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

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GEMS

Genotoxicity and Environmental Mutagen Society

Dear GEMS Member:

Thank you for attending the Fourth Annual Meeting of the Genotoxicity and Environmental Mutagen Society. Each officer and board member hopes that this meeting will be informative and intellectually stimulating. We hope not only that you benefit from the oral and poster presentations, but also that you use the time to interact with one another and meet colleagues and students from the Triangle area. We also encourage you to contact the officers and board members and give them suggestions for the Society and recommendations for future meetings and events.

GEMS is pleased to have **Dr. Marshall Anderson** as the keynote speaker at this year's meeting. He will discuss "The Role of Activated Oncogenes in Chemical Carcinogenesis." In a new experimental format for this year's meeting, a Scientific Evening will be presented in which **Drs. Don Clive and Richard Welch** will discuss "Short-Term Tests and the Whole Animal." In addition, scientists will be competing for the Second Annual GEMS Travel Award to attend the EMS meeting in San Francisco, and the award for best presentation by a junior scientist.

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

I would like to thank **Dr. Gene Elmore** for organizing this Fourth Annual Meeting. Having organized the meeting for the past two years, I know the amount of time and effort it takes to arrange this event. Also, I would like to congratulate the new board members and to thank the retiring members for their service to the Society.

Welcome to the Fourth Annual Meeting of GEMS.

Sincerely,

Andrew D. Kligerman
President

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

GEMS would like to gratefully acknowledge the following companies for providing additional financial support of the Fourth Annual Scientific Meeting:

FLOW LABORATORIES, INC., is sponsoring the coffee and danish at 10:10 a.m.

INTEGRATED LABORATORY SYSTEMS is co-sponsoring, with GEMS, the social hour at 4:45 p.m.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING is sponsoring the wine at the evening banquet at 6:00 p.m.

BOEHRINGER MANNHEIM BIOCHEMICALS is sponsoring the Best Talk by a Junior Scientist award.

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PROGRAM
GEMS FOURTH ANNUAL MEETING
November 20, 1986
8:00 a.m. to 6:00 p.m.

BANQUET and SCIENTIFIC EVENING
6:00 p.m. to 9:00 p.m.

Sheraton University Center, Durham, NC

8:00 a.m. - 9:00 a.m.	Registration
9:00 a.m. - 9:15 a.m.	Welcoming remarks, A. Kligerman, President, GEMS
9:15 a.m. - 9:55 a.m.	**T1** CHEMICAL CARCINOGENS: A LITERATURE ANALYSIS OF 506 CHEMICALS AND THE ESTABLISHMENT OF THE GENE-TOX CARCINOGEN DATA BASE. *S. Nesnow* and H. Bergman. US EPA, RTP, NC 27711.
9:55 a.m. - 10:10 a.m.	**T2** A RAPID *IN VITRO* BIOASSAY FOR THE DETECTION OF POTENTIAL CARCINOGENS AND FOR THE STUDY OF EARLY AND LATE TRANSFORMED PHENOTYPES IN RETROVIRUS-INFECTED RAT EMBRYO CELLS. *W. A. Suk,* J. E. Humphreys, and J. D. Van Arnold. Northrop, RTP, NC 27709.
10:10 a.m. - 10:30 a.m.	Coffee Break. Light refreshments.
10:30 a.m. - Noon	Posters, Exhibits, Coffee.
Noon - 1:30 p.m.	Lunch, Membership and Financial Reports, Recognition of Exhibitors, Installation of Newly-Elected Board members.
1:30 - 1:45 p.m.	**T3** ACTIVATION OF NITRO-SUBSTITUTED PAH BY CHEMICAL REDUCTION IN THE AMES PLATE INCORPORATION ASSAY. *J. Goldring,* L. M. Ball, A. Gold, and R. Sangaiah. UNC-CH, Chapel Hill, NC 27514.
1:45 p.m. - 2:00 p.m.	**T4** COMPARATIVE GENOTOXICITY OF SIDESTREAM AND MAINSTREAM CIGARETTE SMOKE. *L. G. Monteith,*[1] D. M. Simmons,[1] T. J. Hughes,[1] and L. D. Claxton.[2] [1]RTI, RTP, NC 27709: [2]US EPA, RTP, NC 27711.
2:00 p.m. - 2:15 p.m.	**T5** NON-RANDOM KILLING OF T-LYMPHOCYTES IN CRYOPRESERVATION. *W. L. Stanford,*[1] and G. H. S. Strauss.[2] [1]EHRT, RTP, NC 27709: [2]US EPA, RTP, NC 27711.

2:15 p.m. - 2:30 p.m. **T6** THE ESTABLISHMENT OF A MICROCOMPUTER SYSTEM DEVELOPED FROM THE "COMPUTERIZED LABORATORY NOTEBOOK" CONCEPT. *S. J. Berkowitz,*[1] W. L. Stanford,[1] and G. H. S. Strauss.[2] [1]EHRT, RTP, NC 27709: [2]US EPA, RTP, NC 27711.

2:30 p.m. - 2:45 p.m. **T7** CYCLOPHOSPHAMIDE-INDUCED DAMAGE TO SYNAPTONEMAL COMPLEXES AND METAPHASE CHROMOSOMES IN MOUSE MEIOTIC CELLS. *L. C. Backer,*[1] J. B. Gibson,[2] M. J. Moses,[2] D. A. Amtower,[3] and J. W. Allen.[1] [1]US EPA, RTP, NC 27711, [2]Duke Univ., Durham, NC 27710, [3]EHRT, RTP, NC 27709.

2:45 p.m. - 3:00 p.m. **T8** MEASUREMENT OF ONCOGENE AMPLIFICATION IN HUMAN LUNG TUMOR CELLS. *S. K. Hansen* and J. M. Siegfried. EHRT, RTP, NC 27709.

3:00 p.m. - 3:30 p.m. **T9** ACTIVATION OF C-HA-RAS IN DNA FROM CHEMICALLY INDUCED HEPATOMAS OF THE B6C3F$_1$ MOUSE. *R. W. Wiseman,*[1] S. J. Stowers,[1] E. C. Miller,[2] B. C. Stewart,[2], D. Grenier,[2] M. W. Anderson,[1] and J. A. Miller.[2] [1]NIEHS, RTP, NC 27709; [2]Univ. Wisc., Madison, WI 53706.

3:30 p.m. - 3:45 p.m. Break. Coffee and Tea, poster review, and exhibits.

3:45 p.m. - 4:45 p.m. **Keynote Address:** THE ROLE OF ACTIVATED ONCOGENES IN CHEMICAL CARCINOGENESIS. *Marshall Anderson,* NIEHS, RTP, NC 27709.

4:45 p.m. - 6:00 p.m. Social. Light refreshments, drinks.

Scientific Evening

6:00 p.m. - 7:30 p.m. Banquet

7:30 p.m. - 9:00 p.m. SHORT-TERM TESTS AND THE WHOLE ANIMAL. *Don Clive* and *Richard Welch.* Burroughs Wellcome, RTP, NC 27709.

POSTERS

P1 OBSERVATIONS ON THE EFFECT OF *IN VIVO* ADMINISTRATION OF CYCLOPHOSPHAMIDE (CP) AND PHOSPHORAMIDE MUSTARD (PAM) ON SISTER CHROMATID EXCHANGES (SCEs) IN MULTIPLE TISSUES OF THE C57Bl/6 MOUSE. *J. A. Campbell,*[1] G. L. Erexson,[1] M. F. Bryant,[1] J. W. Allen,[2] and A. D. Kligerman.[1] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

P2 A COMPARISON OF THE FREQUENCIES OF CHROMOSOMAL ABERRATIONS AND MICRONUCLEI IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES EXPOSED TO GAMMA IRRADIATION *IN VITRO*. *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]DUMC, Durham, NC 27710.

P3 THE INDUCTION OF SISTER CHROMATID EXCHANGES IN MOUSE HEPATOCYTES AND PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING *IN VITRO* EXPOSURE TO PHOSPHORAMIDE MUSTARD. *M. F. Bryant,*[1] J. A. Campbell,[1] G. L. Erexson,[1] A. D. Kligerman,[1] and J. W. Allen.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

P4 A COMPARISON OF THE FREQUENCIES OF CHROMOSOME BREAKAGE IN MOUSE AND HUMAN PERIPHERAL BLOOD LYMPHOCYTES (PBLs) FOLLOWING *IN VITRO* GAMMA IRRADIATION. *B. Westbrook-Collins,*[1], A. D. Kligerman,[2] G. L. Erexson,[2] J. W. Allen,[1] E. C. Halperin,[3] and G. Honoré.[3] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709; [3]DUMC, Durham, NC 27710.

P5 MUTAGENICITY OF TOPOISOMERASE-ACTIVE AGENTS IN PHAGE T4. *B. K. Lawrence* and D. M. DeMarini, US EPA, RTP, NC 27711.

P6 INDUCTION OF PHAGE LAMBDA IN *E. COLI* BY CHLORINATED PESTICIDES. *V. S. Houk* and D. M. DeMarini, US EPA, RTP, NC 27711.

P7 GENOTOXIC EFFECTS OF HAZARDOUS WASTES IN A SET OF SHORT-TERM TESTS. D. M. DeMarini,[1] *J. P. Inmon,*[1] V. S. Houk,[1] S. Warren,[2] S. Sandhu,[1] G. Acedo,[2] K. Brock,[2] M. M. Moore,[1] T. Pasley,[2] and R. W. Williams.[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

P8 SOLID PHASE ADSORPTION AND RECOVERY OF URINARY MUTAGENS FROM ENVIRONMENTAL TOBACCO SMOKE. *R. Williams,*[1] T. Pasley,[1] R. Watts,[2] J. P. Inmon,[2] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27709.

P9 MUTAGENICITY OF INDOOR AIR IN A RESIDENTIAL PILOT FIELD STUDY. *K. Williams,*[1] J. Chappell,[1] S. Goto,[2] M. E. Miller,[3] and J. Lewtas.[1] [1]US EPA, RTP, NC 27711; [2]Inst. of Public Health, Tokyo, Japan; [3]EHRT, RTP, NC 27709.

P10 MUTAGENICITY OF WOOD COMBUSTION SOURCE AND AMBIENT SAMPLES IN THE SALMONELLA PLATE INCORPORATION ASSAY. *S. Warren,*[1] R. Zweidinger,[2] V. S. Houk,[2] R. Highsmith,[2] J. Lewtas,[2] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

P11 RELATIONSHIP OF EARLY, CARCINOGEN-INDUCED, ATYPICAL CELL COLONIES TO *IN VIVO* MALIGNANT TRANSFORMATION IN THE RAT TRACHEAL EPITHELIAL FOCUS ASSAY. *V. E. Steele,*[1] J. T. Arnold,[1] and M. J. Mass.[2] [1]Northrop, RTP, NC 27709; [2]US EPA, RTP, NC 27709.

P12 DEVELOPING AN SAR BATTERY APPROACH FOR PREDICTING GENOTOXICITY. *D. Walsh,*[1] M. Miller,[1] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

P13 A NULL MUTATION AT *CAR-2* IN THE MOUSE INDUCED BY ETHYLNITROSOUREA. RESULTS IN A NEW ANIMAL MODEL OF A HUMAN DISEASE. *S. E. Lewis,*[1] R. P. Erickson,[2] L. B. Barnett,[1] P. Venta,[2] and R. Tashian.[2] [1]RTI, RTP, NC 27709; [2]Univ. Michigan, Ann Arbor, MI 48109.

ABSTRACTS
TALKS

T1 CHEMICAL CARCINOGENS: A LITERATURE REVIEW AND ANALYSIS OF 506 CHEMICALS AND THE ESTABLISHMENT OF THE GENE-TOX CARCINOGEN DATA BASE. *S. Nesnow* and H. Bergman, US EPA, RTP, NC 27711

The literature on 506 selected chemicals has been evaluated for evidence that these chemicals induce cancer in experimental animals. This evaluation comprises the Gene-Tox Carcinogen Data Base. Three major sources of information were used to create this evaluated data base: assessments from the Gene-Tox Carcinogenesis Panel based on a critical review of the literature of 293 selected chemicals, assessments from the International Agency for Research on Cancer (IARC) Monographs, and National Toxicology Program/National Cancer Institute Bioassay Technical Reports. The 293 chemicals examined by the Gene-Tox Carcinogenesis Panel were selected for analysis based mainly on their previous Gene-Tox evaluation in genetic toxicology bioassays. The literature data on these chemicals were evaluated in an organized, rational, and consistent manner. Criteria were established to assess individual studies employing single chemicals, and four categories of response were developed: Positive, Negative, Inconclusive (Equivocal), and Inconclusive. After evaluating each of the individual studies on a chemical, the Panel placed each chemical in an overall classification category based on the strength of the evidence indicating the presence or absence of carcinogenic effects. An eight-category decision scheme was established using a modified version of the IARC approach, and included two categories of Positive and two categories of Negative, a category of Equivocal, and three categories of Inadequate. Of the 506 chemicals in the Gene-Tox Carcinogen Data Base, 252 were evaluated as Sufficient Positive, 99 as Limited Positive, 40 as Sufficient Negative, 21 as Limited Negative, 1 as Equivocal, 13 as Inadequate with the data suggesting a positive indication, 32 as Inadequate with the data suggesting a negative indication, and 48 as Inadequate. The Gene-Tox Carcinogen Data Base provides a basis for future in-depth analyses of genetic toxicology bioassay systems with regard to their ability to predict the carcinogenic effects of chemicals.

This abstract does not necessary reflect EPA policy.

T2 A RAPID *IN VITRO* BIOASSAY FOR THE DETECTION OF POTENTIAL CARCINOGENS AND FOR THE STUDY OF EARLY AND LATE TRANSFORMED PHENOTYPES IN RETROVIRUS-INFECTED RAT EMBRYO CELLS *W. A. Suk*, J. E. Humphreys, and J. D. Van Arnold. Northrup, RTP, NC 27711.

A mammalian cell culture system using Rauscher leukemia virus-infected Fischer rat embryo (2FR$_4$50) cells was used as a model to study the ability of chemical and physical carcinogens to induce early and late phenotypes of neoplastic transforma-

tion. When suspended in liquid media above an agar base, anchorage-dependent control cells showed a rapid decline in cell survival, whereas cells that had previously been treated with carcinogen survived in suspension as multicellular aggregates. The bioassay takes 11 days, and the early transformation end point, survival in cellular aggregates (SAg^+), is measured by counting viable cells dissociated from aggregates that were suspended for four days. Chemical carcinogens (7,12-dimethylbenzanthracene, benzo(a)pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitroquinoline-N-oxide, and diethylstilbestrol), complex environmental mixtures, and the physical carcinogen, ultraviolet irradiation, induced SAg^+ dose-dependently. The bioassay discriminated between carcinogens and noncarcinogens without the addition of a metabolic activation system. The induction of the end point was correlated with progression to neoplastic phenotypes in the same cells, as evidenced by their morphological transformation, growth in semisolid medium, and tumorigenicity in nude mice. The selection process of suspension of cells in liquid medium resulted in the rapid expression of transformed phenotypes. The SAg^+ end point appeared to require the presence of the exogenous retrovirus, or the induction of the endogenous viral genome, since uninfected cells did not show a differential survival response when carcinogen-treated, noncarcinogen-treated, or control cells were compared. The results indicate that agents can be assayed for carcinogenic potential rapidly by *in vitro* transformation of target cells selected by the integration and expression of retrovirus, and that the formation in suspension of aggregated cells following carcinogen treatment selects for the expression of subsequent neoplastic phenotypes.

T3 ACTIVATION OF NITRO-SUBSTITUTED PAH BY CHEMICAL REDUCTION IN THE AMES PLATE INCORPORATION ASSAY. *J. M. Goldring,* L. M. Ball, A. Gold, and R. Sangaiah, UNC-CH, Chapel Hill, NC 27514.

Cyclopenta-fused isomers of pyrene and benz(a)anthracene, nitrated on the etheno bridge, were synthesized and tested in the Ames assay. Since enzymatic reduction would form arylhydroxylamines, which in turn would form highly stabilized arylnitrenium ions, we hoped to test the hypothesis that the direct-acting mutagenic activity of nitro-PAH correlates with the degree of stabilization of the electrophilic intermediate. We found that, while these compounds are mutagenic without S9, they are much less active than other nitro PAH of similar molecular weight.

We reasoned that these compounds may be poor substrates for the nitroreductase enzymes. If this were the case, chemical reduction should produce stable active intermediates. We therefore reduced one of these compounds, 2-nitrobenz(1)acenanthrylene (2-nitroB(1)A), with zinc dust and ammonium chloride and immediately incubated the reaction mixture with Salmonella strain TA98. As a control, we performed the identical procedure using 1-nitropyrene (1-NP) in TA98NR, which lacks the "classical" nitroreductases and is therefore insensitive to 1-NP. 1-NP was also tested in TA98 and 2-nitroB(1)A in TA98NR.

1-NP was extemely active in TA98NR (2800 rev/plate at 1 μg/plate with reduction compared to 60 rev/plate without zinc), indicating that chemical reduction does occur in this system and is capable of producing a reactive intermediate. However, activity of 2-nitroB(1)A is only marginally increased by chemical reduction (80 rev/plate at 5 μg/plate with reduction, compared to 60 rev/plate without zinc). We conclude that, whether or not nitroreduction of B(1)A occurs in Salmonella, any intermediates formed during the reductive process may not be reactive enough to generate genotoxic damage.

Supported by EPA Grant CR#811817 and a UNC-CH Graduate School Assistantship to J.M.G.

T4 COMPARATIVE GENOTOXICITY OF SIDESTREAM AND MAINSTREAM CIGARETTE SMOKE, *L. G. Monteith,*[1] D. M. Simmons[1], T. J. Hughes,[1] and L. D. Claxton.[2] [1]RTI, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

Methods were developed to collect and fractionate particles, semivolatiles and volatile organic compounds from mainstream and sidestream cigarette smoke (GEMS 1985). A trapping train composed of a bubbler/sand/cold trap collected: 1) respirable particles 2) semivolatiles, and 3) volatiles with bp $<25°C$. Results suggested that sidestream cigarette smoke accounted for 60% of the total mutagenicity per cigarette in the Ames/Salmonella assay. To further investigate the potential genotoxicity of sidestream and mainstream cigarette smoke, samples were recently collected with two solvents — acetone and ethanol. Data are being evaluated for mutagenicity in the Ames/Salmonella assay. Comparison of the efficiency of these two solvents to collect organic compounds from cigarette smoke will be presented. Dose-responsive mutagenicity of these sidestream and mainstream cigarette smoke samples were also detected in the mouse lymphoma assay. In addition, these cigarette smoke fractions are being evaluated in the *in vitro* rat hepatocyte unscheduled DNA synthesis/repair assay. Genotoxicity results of sidestream and mainstream cigarette smoke from these three *in vitro* bioassays will be presented.

Research sponsored by EPA 68-02-3992-42 and 60 and RTI Project No. 311-5311-4.

T5 NON-RANDOM KILLING OF T-LYMPHOCYTES IN CRYOPRESERVATION. *W. L. Stanford,*[1] and G. H. S. Strauss.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

To eliminate between-test error in longitudinal studies it is necessary to freeze freshly separated lymphocytes as well as continuous T-lymphocyte (CTL) lines. To test the efficacy of a programmable freezer (in which the temperature falls at an optimal rate), freshly separated lymphocytes and CTLs were aliquoted into three sets of vials. Two sets each were frozen in a 1:1 mixture of 15% DMSO in Mixed Medium (MM), and in 20% FBS in MM using the standard styrofoam freezer insert for liquid N_2 refrigerators and a programmed freezer, respectively. The remaining set was left in an incubator. The cells were thawed and/or washed and assayed in both a fluorochrome-mediated viability test and fluorescent antibody and ELISA T-helper (T_H) / T-suppressor (T_S) tests. Representative results from 2 studies each, presented as (% Viable), T_H/T_S for cells unfrozen, program frozen, or styrofoam frozen, respectively, were:

for fresh cells from 1 individual:

(100%), ± .03; (95 ± 1%), 1.15 ± .05 & (91), 0.88 ± .01

and for 2 lines of Con A-activated CTLs:

(100%), 1.20 ± .01; (98%), 1.24 ± .03 & (92 ± 2%), 0.74 ± .01.

We show that inadequate freezing non-randomly kills cells of T-cell subpopulations, T_H being more sensitive than T_S, and assert that a preprogrammed freezer is indicated. The Battery of Lymphocyte Tests (BLT), under development in our laboratory, is designed to detect toxic, immunotoxic, and genotoxic effects of *in vivo* mutagen exposure on human blood. We discuss how minimization of non-random cell losses (as quantitated on the basis of morphology) and preservation of related regulatory cell function is essential if we would assess the *in vivo* or *in vitro* state of heterogeneous cells.

T6 THE ESTABLISHMENT OF A MICROCOMPUTER SYSTEM DEVELOPED FROM THE "COMPUTERIZED LABORATORY NOTEBOOK" CONCEPT. *S. J. Berkowitz,*[1] W. L. Stanford,[2] and G. H. S. Strauss.[1] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

We describe a microcomputer system developed in our laboratory for automating the Battery of Lymphocyte Tests (BLT). The BLT was designed to evaluate blood specimens for toxic, immunotoxic, and genotoxic effects after *in vivo* exposure to putative mutagens. A system was developed with the advantages of low cost, limited spatial requirements, ease of use for personnel inexperienced with computers, and applicability to specific testing yet flexibility for experimentation. This system eliminates cumbersome record keeping and repetitive analysis inherent in genetic toxicology bioassays. Statistical analysis of the vast quantity of data produced by the BLT would not be feasible without a central database. Our central database is maintained by an integrated package, which we have adapted to develop a computerized "laboratory notebook." The clonal assay of lymphocyte mutagenesis (CALM) section of the "notebook" is demonstrated. PC-Slaves expand the microcomputer to multiple workstations so that our computerized notebook can be used next to a hood while other work is done in an office and instrument room simultaneously. Communication with peripheral instruments is now an indispensable part of many laboratory operations, and we include a program, written to acquire and analyze CALM data, for communicating with both a liquid scintillation counter and an ELISA plate reader. In conclusion we discuss how our computer system could easily be adapted to the needs of other laboratories.

T7 CYCLOPHOSPHAMIDE-INDUCED DAMAGE TO SYNAPTONEMAL COMPLEXES AND METAPHASE CHROMOSOMES IN MOUSE MEIOTIC CELLS. *L. C. Backer,*[1] J. B. Gibson,[2] M. J. Moses,[2] D. A. Amtower,[3] and J. W. Allen.[1] [1]US EPA, RTP, NC 27711; [2]Duke Univ., Durham, NC; [3]EHRT, RTP, NC 27709.

Synaptonemal complex (SC) analysis represents a new approach for evaluating chemically-induced damage to mammalian germ cells (Moses et al., 1985, In: Dellarco et al. (eds.) Aneuploidy: Etiology and Mechanisms, Plenum Publishing Corp., N.Y., pp. 337-352.) Cyclophosphamide (CP) administered ip to male mice causes SC breakage and abnormalities of synapsis. The nature of any mechanistic or predictive relationships between prophase SC alterations and subsequent meiotic metaphase I and II chromosome aberrations remains uncertain. In the present study, male mice were injected ip with CP. Unilateral orchidectomies were performed on day 3 and SC damage was analyzed by both light and electron microscopy. The remaining testis was removed on day 13 and metaphase I and II chromosomes were analyzed for structural aberrations and hyperploidy. Increases in SC breakage and metaphase structural aberrations were found. Although synaptic abnormalities were observed, no metaphase hyperploidy was apparent. No statistically significant correlations between SC and chromosome damage were found. Further efforts to evaluate potential correlations involve the analysis of exposed cell populations identified at prophase and metaphase by tritiated thymidine label administered at the time of drug treatment. These studies are intended to provide a more precise analysis of the significance and fate of unrepaired SC damage as germ cells progress through meiosis.

T8 MEASUREMENT OF ONCOGENE AMPLIFICATION IN HUMAN LUNG TUMOR CELLS. *S. K. Hansen* and J. M. Siegfried, EHRT, RTP, NC 27709.

In recent studies, amplification of several oncogenes, c-*myc*, L-*myc*, N-*ras*, and c-Ki-*ras*2, have been correlated with the transformed state of certain lung cancers. In particular, the c-*myc* and L-*myc* genes have been shown to be amplified in the variant forms of small cell lung carcinomas. We were interested in determining if other oncogenes are amplified in human lung tumor cells and whether a pattern emerges with respect to oncogene and cell type. The approach used to address these questions was to isolate DNA from lung tumor cell lines and solid lung tumors, then to probe these DNAs with lung-relevant oncogenes to determine gene copy number relative to normal cell copy number. DNA from cell lines or solid tumors of 6 adenocarcinomas of the lung, 4 small cell lung carcinomas, 3 squamous cell lung carcinomas, 1 bronchiolo-alveolar carcinoma and 1 large cell lung carcinoma were tested for amplification of the c-*myc*, L-*myc*, N-*ras*, c-Ki-*ras*2, and B*lym* oncogenes by DNA:DNA hybridization experiments. Two of the adenocarcinoma cell lines, H-23 and SKLU-1, showed amplified c-*myc* and a solid adenocarcinoma tumor had an altered c-*myc* gene. None of the other genes were amplified in any of the cell lines or tumors tested. These observations led us to conclude that amplification of oncogenes is not a generalized phenomenon and may not be useful in determining patterns of transformation events in human lung tissue. However, alterations in the c-*myc* gene may be important in the development of adenocarcinoma of the lung.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy. Work performed in support of US EPA contract 68-02-4301.

T9 ACTIVATION OF C-HA-*RAS* IN DNA FROM CHEMICALLY INDUCED HEPATOMAS OF THE B6C3F$_1$ MOUSE. *R. W. Wiseman*[1], S. J. Stowers[1], E. C. Miller,[2] B. C. Stewart,[2], D. Grenier,[2] M. W. Anderson[1], and J. A. Miller.[2] [1]NIEHS, RTP, NC 27709; [2]Univ. Wisc., Madison, WI 53706.

Activation of the C-Ha-*ras* proto-oncogene has been examined in DNA from well-differentiated hepatomas initiated by a single ip dose of a carcinogen to male B6C3F$_1$ mice at 12 days of age (R. W. Wiseman, et al., (1986) *PNAS 83*, 5825-5829). DNA from 32 of 39 hepatomas (7/7 induced by vinyl carbamate, 7/7 from N-hydroxy-2-acetylaminofluorene, 11/11 from 1'-hydroxy-2', 3'-dehydroestragole, and 7/14 from diethylnitrosamine) demonstrated transforming activity in the NIH 3T3 transfection assay. Southern analysis of 3T3 focus DNA from 31 of 32 of the positive hepatomas revealed amplified and/or rearranged restriction fragments homologous to a Ha-*ras* probe; the other tumor contained an activated c-Ki-*ras* proto-oncogene. Immunoprecipitation of 3T3 foci demonstrated expression of a p21 protein that had increased mobility on SDS-PAGE analysis. The activating mutations were characterized at the DNA sequence level by selective oligonucleotide hybridization or restriction fragment length polymorphism analyses; each of

these tumors contained one of three base substitutions at the 1st or 2nd position of codon 61 in the c-Ha-*ras* gene. These results have been extended to the direct analysis of base changes in codon 61 for tumor DNA from 65 additional $B6C3F_1$ hepatomas induced by the following carcinogens: 7,12-dimethylbenz(a)anthracene, benzo(a)pyrene, aflatoxin B_1, ethyl carbamate, vinyl carbamate, safrole, estragole, N-hydroxy-4-aminoazobenzene, and N-hydroxy-2-acetylaminofluorene. At least 80% of these hepatomas also contained point mutations at the first or second position of the 61st codon. The distinct patterns of c-Ha-*ras* mutations observed in these hepatomas for each chemical imply that these alterations can be a direct result of the interaction of electrophilic ultimate carcinogens with this gene *in vivo*. These observations are also consistent with the hypothesis that c-Ha-*ras* activation is frequently an early event of hepatocarcinogenesis in the $B6C3F_1$ mouse.

ABSTRACTS
POSTERS

P1 OBSERVATIONS ON THE EFFECT OF *IN VIVO* ADMINISTRATION OF CYCLOPHOSPHAMIDE (CP) AND PHOSPHORAMIDE MUSTARD (PAM) ON SISTER CHROMATID EXCHANGES (SCEs) IN MULTIPLE TISSUES OF THE C57Bl/6 MOUSE. *J. A. Campbell,*[1] G. L. Erexson,[1] M. F. Bryant,[1] J. W. Allen,[2] and A. D. Kligerman.[1] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

Cytogenetic studies were undertaken to investigate the relative SCE-inducing potency of CP and its presumed active metabolite, PAM, in several tissues from male C57Bl/6 mice following *in vivo* exposure. After ip administration of either CP or PAM, hepatocytes were grown on coverglasses, and peripheral blood lymphocytes (PBLs) were grown in 1 ml cultures (Campbell et al. 1986; Kligerman et al. 1986) for comparison with the SCE response in bone marrow. At equimolar concentrations (50 μM) bone marrow exhibited a significantly elevated SCE frequency after exposure to PAM or CP (Control: 5 SCEs; PAM: 44 SCEs; CP: 32 SCEs). Contrary to expectations, hepatocytes and PBLs were much less responsive than bone marrow to the two compounds. At the same dose, hepatocytes from mice treated with PAM had an increase of 12 SCEs per cell over baseline, but only a 4 SCE per cell increase was observed in mice treated with CP. The same 50 μM dose of CP or PAM induced an increase of 16 SCEs/metaphase or 10 SCEs/metaphase, respectively, in PBLs from treated mice. We are investigating reasons for the divergence in SCE responses seen among these tissues.

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

P2 A COMPARISON OF THE FREQUENCIES OF CHROMOSOMAL ABERRATIONS AND MICRONUCLEI IN MOUSE PERIPHERAL BLOOD LYMPHOCYTES EXPOSED TO GAMMA IRRADIATION *IN VITRO*. *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]DUMC, Durham, NC 27710.

Three replicate experiments were designed to investigate both the induction of chromosomal aberrations (CAs) and micronuclei (MN) in mouse peripheral blood lymphocytes (PBLs). Fifty male C57Bl/6 mice were bled by cardiac puncture, and mononuclear leucocytes (MNLs) were isolated using Lymphocyte Separation Medium.[®] The cells were then placed in 6 ml polystyrene culture tubes containing 6.5×10^6 MNLs in 4 ml of RPMI 1640. The tubes were exposed to cobalt-60 at a rate of 83 cGy/min to yield total doses of 0, 100, 200, 300, and 400 cGy. PBLs were

cultured in RPMI 1640 medium supplemented with 8 μg phytohemagglutinin (PHA)/ml. At 21 h, the medium was removed and replaced with medium lacking PHA but containing either 5 μM bromodeoxyuridine for CA analysis in first division cells or 3 μg cytochalasin B/ml for MN analysis in binucleated PBLs. CA cultures were harvested at 42 h after a 3 h colcemid treatment (0.5 μg/ml). MN cultures were harvested at 47 h using a cytocentrifuge. In each replicate experiment, CAs were scored in 200 metaphases/dose and MN were quantitated in 1000 cells/dose. A dose-related increase in both CAs and MN was observed in the PBLs. At a dose of 400 cGy, CA analysis in first division metaphases revealed 1.6-fold more damage than MN analysis in binucleated PBLs. MN frequencies closely parallel the percentages of dicentrics at all doses studied. Metaphase analysis for CAs in mouse PBLs reveals more damage than does scoring for MN after irradiation. However, the analysis of both CAs and MN in mouse PBLs correlate well with the latter being a rapid and therefore more efficient assay.

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

P3 THE INDUCTION OF SISTER CHROMATID EXCHANGES IN MOUSE HEPATOCYTES AND PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING *IN VITRO* EXPOSURE TO PHOSPHORAMIDE MUSTARD. *M. F. Bryant,*[1] J. A. Campbell,[1] G. L. Erexson,[1] A. D. Kligerman,[1] and J. W. Allen.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

In vitro studies were conducted to examine sister chromatid exchange (SCE) induction by phosphoramide mustard (PAM), the presumed active metabolite of cyclophosphamide (CP), in mouse hepatocytes and peripheral blood lymphocytes (PBLs). Hepatocytes were isolated from mice by enzyme perfusion and cultured in medium containing 5 μM BrdUrd according to published methods (Campbell et al. 1986). Cultures were treated for the first 1 h, first 2 h, first 21 h, or the last 21 h of a 52 h culture with 1 or 5 μM PAM. PBLs were removed from mice by cardiac puncture, and mononuclear leucocytes were isolated using Lymphocyte Separation Medium® and cultured according to published methods (Kligerman et al. 1986) using RPMI 1640 medium supplemented with phytohemagglutinin (8 μg/ml). Cells were treated with 1 or 5 μM PAM for either 1 h or 24 h before mitogenic stimulation, or during the first 21 h or the last 21 h of a 50 h culture period. *In vitro* exposure of mouse hepatocytes and PBLs to PAM generated dose-related increases in SCE frequencies. The results also indicate that SCE induction by PAM is not cell cycle specific but depends upon the amount of time PAM is in contact with the target cells.

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

P4 A COMPARISON OF THE FREQUENCIES OF CHROMOSOME BREAKAGE IN MOUSE AND HUMAN PERIPHERAL BLOOD LYMPHOCYTES (PBLs) FOLLOWING *IN VITRO* GAMMA IRRADIATION. *B. Westbrook-Collins,*[1] A. D. Kligerman,[2] G. L. Erexson,[2] J. W. Allen,[1] E. C. Halperin,[3] and G. Honoré.[3] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709; [3]DUMC, Durham, NC 27710.

As part of a series of studies designed to develop risk assessment models for chromosome damage in humans exposed to genotoxicants, *in vitro* experiments were designed to investigate the relative radiosensitivity of mouse and human

PBLs. Whole blood was obtained from a human male subject by venipuncture and from 50 male C57Bl/6 mice by cardiac puncture. The mononuclear leucocytes were isolated by centrifugation on a density gradient and suspended in 4 ml of RPMI 1640. They were exposed to cobalt-60 gamma radiation at a rate of 83 cGy/min to yield total doses of 0, 100, 200, 300, and 400 cGy. Standard PBL cultures were established with BrdUrd; the mouse cultures were harvested at 42 h and the human cultures at 48 h. Chromosome aberrations were scored from first division cells only. Human PBLs were more sensitive to the induction of dicentrics than were mouse PBLs. The preliminary dose-response curve obtained for dicentrics in mouse PBLs was: $Y = 0.00524x + 0.000140x^2$. The respective curve for human PBLs was: $Y = 0.0327x + 0.000333x^2$. A comparison of the number of undamaged cells after irradiation also demonstrates that the human PBLs are approximately 1.75 times more sensitive to gamma radiation than the mouse PBLs. Since the DNA content of the PBLs is approximately equal, this suggests that more than target size alone is responsible for the sensitivity differences.

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

P5 MUTAGENICITY OF TOPOISOMERASE-ACTIVE AGENTS IN PHAGE T4. *B. K. Lawrence* and D. M. DeMarini, US EPA, RTP, NC 27711.

Biochemical studies have identified a series of agents that stimulate DNA scission by mammalian topoisomerase II. This group of agents includes: adriamycin, actinomycin D, *m*-AMSA, ellipticine, and teniposide (VM-26). Of these, only *m*-AMSA has been studied thus far for its ability to stimulate DNA scission by prokaryotic (phage T4) topoisomerase II, and *m*-AMSA was found to stimulate DNA cutting by the T4 enzyme (Rowe et al. 1984). Because *m*-AMSA is a potent mutagen in T4 (DeMarini, Lawrence, and Ripley, unpublished results), we have investigated the mutagenicity in T4 of additional agents that have been shown to stimulate DNA scission by mammalian topoisomerase II or that may inhibit either the prokaryotic or eukaryotic enzyme. Log-phase *E. coli* BB was infected with T4 FC11 (an *rIIB* frameshift mutant) at an m.o.i. of 5 in the presence of the drug for 1 h at 37°C. Cells were diluted and lysed, and the phage were plated onto *E. coli* K38 (which selects for wild-type revertants) and onto *E. coli* BB (which permits both mutants and wild type to grow, thus giving a measure of survival). Plaques were counted the next day, and mutant frequencies were calculated. In addition to *m*-AMSA, only ellipticine, mitomycin C, nalidixic acid, and oxolinic acid were clearly mutagenic; 9-aminoellipticine and 9-methoxyellipticine were weakly mutagenic (2-4 fold over background at 10% survival). Interaction studies indicated that novobiocin reduced the mutagenicity of *m*-AMSA; similar studies with naladixic acid are in progress. Many of these agents are potent clastogens/mutagens in mammalian cells. Thus, these agents appear to exhibit a prokaryotic/eukaryotic specificity that may reflect differences in topoisomerase II from the two types of organisms. Mutants induced by the mutagenic agents have been isolated and will be sequenced to determine the mutagenic specificity of these agents. Preliminary sequence data suggest that *m*-AMSA and ellipticine induce mutations at a hot spot that may coincide with the phage T4 topoisomerase II primary cut site. Thus, topoisomerase II may mediate the mutagenicity of these and related compounds.

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

P6 INDUCTION OF PHAGE LAMBDA IN *E. COLI* BY CHLORINATED PESTICIDES. *V. S. Houk* and D. M. DeMarini, US EPA, RTP, NC 27711.

No single assay can detect all classes of genotoxic compounds. Thus, several assays are often used complementarily so that the strengths of one can compensate for the limitations of another. The Salmonella mutagenesis assay is used frequently because it is relatively quick, inexpensive, and has been validated extensively. However, one important class of carcinogens that is not detected reliably by Salmonella is chlorinated organics. As an adjunct to the Salmonella assay, we have investigated the Microscreen assay developed by Rossman et al. (1984) to evaluate its potential usefulness for detecting this chemical class. The assay measures the induction of λ prophage in *E. coli* WP2s(λ) and has been shown to detect some carcinogenic compounds that are not detected by Salmonella. After making some modifications in the protocol and in the method of data analysis, we tested a group of chlorinated pesticides (most of which are carcinogenic) for their ability to induced phage λ. Included were the pesticides monuron, malathion, mirex, p,p'-DDT, lindane, nitrofen, chlordane, captan, toxaphene, and dichlorvos. All but the first four induced phage. The phage-induction assay detected chlordane and lindane, which are not mutagenic in Salmonella but which are limited positives for carcinogenicity.

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

P7 GENOTOXIC EFFECTS OF HAZARDOUS WASTES IN A SET OF SHORT-TERM TESTS. D. M. DeMarini,[1] *J. P. Inmon,*[1] V. S. Houk,[1] S. Warren,[2] S. Sandhu,[1] G. Acedo,[2] K. Brock,[2] M. M. Moore,[1] T. Pasley,[2] and R. W. Williams.[2] [1]US EPA, RTP, NC 27711; [2]EHRT, RTP, NC 27709.

Sixteen harzardous wastes of various types were evaluated for genotoxicity in a set of short-term tests. Dichloromethane (DCM) extracts of the wastes as well as the unextracted wastes were tested for mutagenicity in the Salmonella plate-incorporation assay using strains TA98 and TA100 with and without Aroclor 1254-induced rat liver S9. Ten of the wastes were fed by gavage to male F-344 rats, and the unextracted urine as well as C-18/methanol extracts of the urine were tested in the Salmonella assay. All of the wastes were evaluated for their ability to induce phage lambda in *E. coli* by use of the Microscreen assay. Six of the wastes were tested for mutagenicity in a plant (Arabidopsis), and two were evaluated in L5178Y/TK$^{\pm}$ -3.7.2C cells. Seven of the unextracted wastes were mutagenic in Salmonella, but three of the DCM extracts were not. Only three of the mutagenic unextracted wastes produced mutagenic urine when fed to rats. In general, the C-18/methanol extraction procedure eliminated the toxicity associated with the mutagenic urine samples and resulted in a urine that was more potent than unextracted urine. The wastes that were mutagenic in Salmonella were also mutagenic in Arabidopsis, and one waste that was not mutagenic in Salmonella was mutagenic in Arabidopsis. One of the mutagenic wastes in Salmonella was tested in L5178Y/TK$^{\pm}$ cells and was mutagenic in this mammalian cell assay. The genotoxicity of the wastes in the phage-induction assay will also be presented.

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

P8 SOLID PHASE ADSORPTION AND RECOVERY OF URINARY MUTAGENS FROM ENVIRONMENTAL TOBACCO SMOKE. *R. W. Williams,*[1] T. Pasley,[1] R. Watts,[2] J. P. Inmon,[2] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

Solid phase adsorbents (bonded silicas) were evaluated for their ability to recover urinary mutagens from cigarette smokers. Cyano, octadecyl, and cyclohexyl phases were individually loaded with a pooled composite of smokers' urine followed by subsequent elution of the retained mutagens using methanol. The mutagens were solvent exchanged into dimethylsulfoxide then bioassayed using the Kado *Salmonella* micro assay with metabolic activation. Mutagenic recovery from the various solid phases was found to be dependent upon the volume of urine loaded and the phase being evaluated. Cyano bonded silica columns were found to consistently recover greater quantities of mutagenic activity as compared with the other phases. Overall results indicate that the use of prepacked solid phase adsorbents when combined with micro volume bioassays (ex. Kado) can offer a fast, efficient means to determine the mutagenic activity found in cigarette smokers' urine.

P9 MUTAGENICITY OF INDOOR AIR IN A RESIDENTIAL PILOT FIELD STUDY. *K. Williams,*[1] J. Chappell,[1] S. Goto,[2] M. E. Miller,[3] and J. Lewtas.[1] [1]US EPA, RTP, NC 27711; [2]Institute of Public Health, Tokyo, Japan; [3]EHRT, RTP, NC 27709.

The mutagenicity of indoor air was measured in a pilot field study of homes in Columbus, Ohio during the winter of 1984. The study was conducted in two all-electric homes and eight homes with natural gas heat and appliances. The combustion source variables included woodburning in fireplaces and cigarette smoking. Samples were collected in three indoor areas (kitchen, living room, and bedroom) and outdoors adjacent to each home. Particulate matter and semivolatile organic compounds were collected using modified EPA medium-volume samplers with a semivolatile (PUF) trap. A micro-forward mutation bioassay employing *Salmonella typhimurium* strain TM677 was used to quantify the mutagenicity in solvent extracts of microgram quantities of the organics. Both the particulate matter and the semivolatile organics collected indoors and outdoors were mutagenic in this assay. The mutagenicity of the particulate matter, expressed as mutant frequency/m^3, was highly correlated with the number of cigarettes smoked in the home. This was particularly evident for the kitchen samples. The mutagenicity (MF/m^3) of the semivolatile organics did not appear to significantly increase as a function of the number of cigarettes smoked.

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

P10 MUTAGENICITY OF WOOD COMBUSTION SOURCE AND AMBIENT SAMPLES IN THE SALMONELLA PLATE INCORPORATION ASSAY. *S. Warren,*[1] R. Zweidinger,[2] V. Houk,[2] R. Highsmith,[2] J. Lewtas,[2] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

The Integrated Air Cancer Project Study (IACP) is an interdisciplinary research program designed to develop methods and a data base to determine the major sources and the identities of airborne carcinogens to which humans are exposed. As part of this project, particulate samples from both ambient air and woodstove sources were collected in Raleigh, NC and Albuquerque, NM. These samples were extracted and tested for mutagenicity using the Salmonella plate incorporation assay with strain TA98. Woodstove source samples showed increased mutagenic

activity with the addition of S9 in comparison with roadway and background samples which were more mutagenic in the absence of S9. The mutagenicity of laboratory source samples was comparable to that of home source samples. Ambient samples showed more mutagenicity per cubic meter of air when sampled in the afternoon than in the morning.

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

P11 RELATIONSHIP OF EARLY, CARCINOGEN-INDUCED, ATYPICAL CELL COLONIES TO *IN VIVO* MALIGNANT TRANSFORMATION IN THE RAT TRACHEAL EPITHELIAL FOCUS ASSAY. *V. E. Steele,*[1] J. T. Arnold,[1] and M. J. Mass.[2] [1]Northrop, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

To define and study the initial stages of neoplastic transformation in respiratory epithelial cells, a series of morphologically transformed cell colonies from carcinogen-exposed rat tracheal epithelial (RTE) cell cultures was characterized. Tracheal epithelial cells were isolated from F-344 rats, plated on collagen-coated dishes, and exposed to 7,12-dimethylbenz(a)anthracene on day 1 for 24 h. Between days 26 and 30, colonies of morphologically altered cells were isolated and classified into three major groups based on cell density: class I—less than 1300 cells/mm^2; class II—1300-2500 cells/mm^2; and class III—more than 2500 cells/mm^2. Following plating the cell populations were assayed for their ability to grow in various media and on various substrata. In general, classes II and III had a higher colony forming efficiency when replated in various media and grew better on all substrates tested. The population doubling times generally decreased faster in class II and III cells than in class I cells. The cells were placed into denuded tracheal grafts which were transplated subdermally into nude mice. Untreated cells produced a mucociliary epithelium, while the progression from class I to class III in culture was reflected by a progression in the animal towards a more transformed phenotype. These studies show that early carcinogen-induced premalignant RTE cells differ in a variety of characteristics and that at least three classes initially exist which become homogeneous with time.

Supported by US EPA Contract #68-02-4103.

P12 DEVELOPING AN SAR BATTERY APPROACH FOR PREDICTING GENOTOXICITY. *D. Walsh,*[1] M. Miller,[1] and L. Claxton.[2] [1]EHRT, RTP, NC 27709; [2]US EPA, RTP, NC 27711.

A computerized SAR system (ADAPT) was used to classify a Salmonella data base of 114 nitrogenous cyclic compounds with 19 molecular descriptors. The average correct predictability for this data set was 89% and was determined by evaluating 100 training/prediction subsets. Actual predictive ability of the discriminants was demonstrated by predicting the Salmonella mutagenicity of 40 aromatic amines. The aromatic amines prediction set was from the data base analyzed by the computer program CASE (computer automated structure evaluator). ADAPT correctly predicted the mutagenicity of 35 of the 40 aromatic amines resulting in 88% correct predictability, which correlated closely with the 89% established by the training/prediction subsets. This predictive ability was slightly better than the 84% correct prediction stated in the CASE publication. The ADAPT correctly identified some compounds that CASE missed and CASE correctly identified some compounds that ADAPT missed. Analysis of compounds in a battery of computerized SAR systems would greatly increase the ability to determine biological activity much the same way as using different strains of bacteria and different assays are used to determine biological activity.

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

P13 A NULL MUTATION AT *CAR-2* IN THE MOUSE INDUCED BY ETHYLNITROSOUREA RESULTS IN A NEW ANIMAL MODEL OF A HUMAN DISEASE. *S. E. Lewis,*[1] R. P. Erickson,[2] L. B. Barnett,[1] P. Venta,[2] and R. Tashian.[2] [1]RTI, RTP, NC 27709; [2]Univ. of Michigan, Ann Arbor, MI 48109.

Screening of (C57Bl/6J x DBA/22J)F_1 progeny of male mice treated with ethylnitrosourea (ENU) revealed a mouse that lacked the paternal carbonic anhydrase II (CAII). Breeding tests showed that this trait was heritable and due to a null mutation at the *Car-2* locus on Chromosome 3. Animals homozygous for the new null allele are runted and have impaired acid/base balance. These abnormalities are very similar to those of humans with the same biochemical defect. Studies to determine the molecular defect caused by the mutation are now in progress. The mutant gene is apparently intact, and is transcribed.

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Wiseman, R. W. (T9)
Zweidinger, R. (P10)

New Brunswick Scientific Co., Inc.

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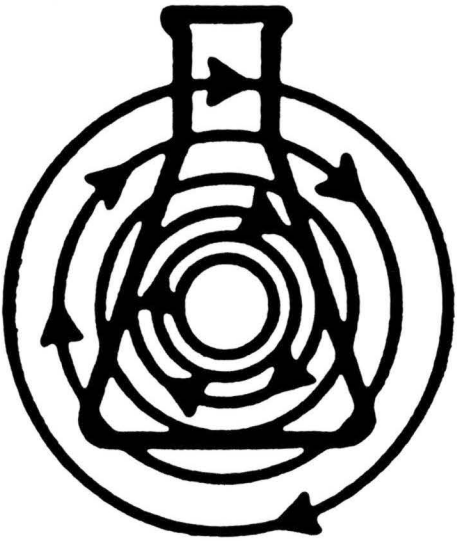

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NJ 1-201-287-1200
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The Spin-X filters multiple samples of 25 µl to 500 µl.

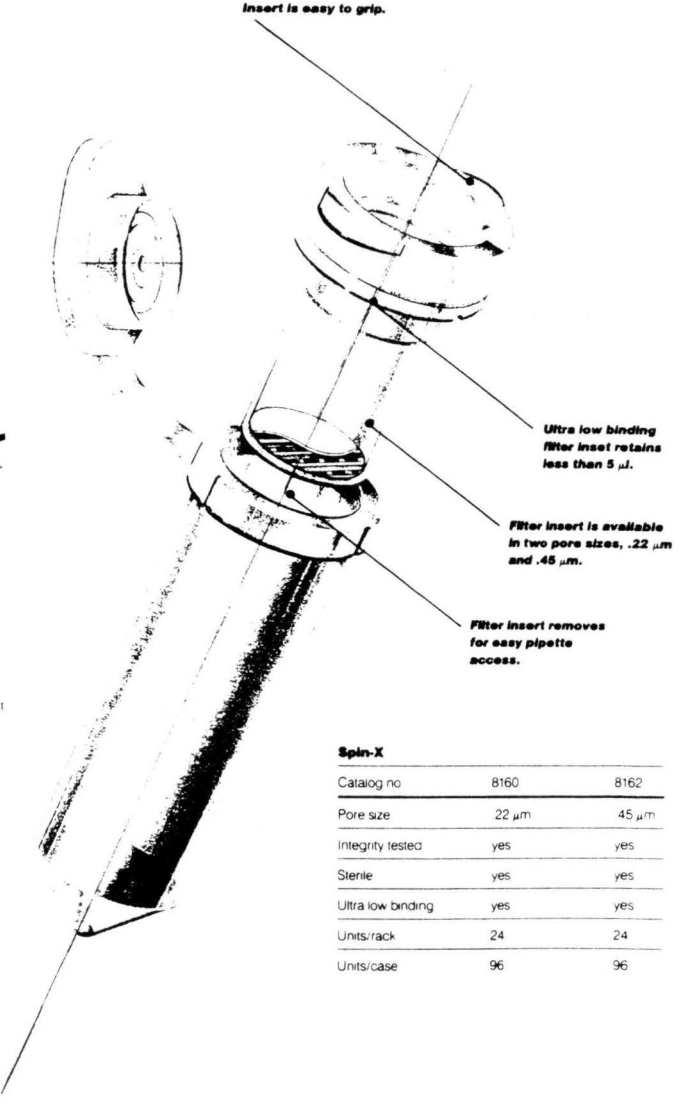
The Spin-X system allows rapid filtration of up to 24 samples. The Spin-X filter insert features an ultra low binding membrane. And, after centrification, the hold up volume is less than 5 µl.

The Spin-X s package tray holds 24 units to aid sample loading. Which means samples can be transferred quickly using standard pipetting techniques.

costar
Performance builds reputation

For additional information, technical assistance, or samples phone **800-492-1110**

Ed Krehl
Technical Application Specialist



Spin-X

Catalog no	8160	8162
Pore size	22 µm	45 µm
Integrity tested	yes	yes
Sterile	yes	yes
Ultra low binding	yes	yes
Units/rack	24	24
Units/case	96	96

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