GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

EIGHTH ANNUAL MEETING
PROGRAM AND ABSTRACTS

OCTOBER 25, 1990
Mission Valley Inn & Conference Center
Raleigh, North Carolina

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

Officers for Terms Beginning October 1990

President	David J. Doolittle (RJR)
President-Elect	Bruce C. Casto (EHRI)
Secretary	Debra B. Walsh (EHRT)
Treasurer	Gregory L. Erexson (EHRT)

Board of Directors: Councilors

1990 - 1993	Janet R. Warner (RTI) Elisabeth Korytynski (NSI) Lisa Parker (EHRT)
1989 - 1992	Douglas A. Bell (EPA) Raymond W. Tennant (NIEHS) Ronald W. Williams (EHRT)
1989 - 1991	William Caspary (NIEHS)
1988 - 1991	Edilberto Bermudez (CIIT) Wendell H. McKenzie (NCSU)

David DeMarini

Genotoxicity and Environmental Mutagen Society

Dear GEMS Members:

As I am prone to say: "Time flies when you are having a good time!" This is especially true when I realize that this is the eighth annual meeting of GEMS. We have come a long way from the first annual meeting at the Governer's Inn in 1983. I welcome you to this GEMS meeting and I know that you will have both a scientifically fulfilling and socially entertaining day.

This past year as the GEMS president has been a great joy to me. I have been fortunate to have a very energetic Board of Directors, which has made the job of being President easy. David Doolittle, as President-elect, was responsible for the meetings this year. His committee consisted of Ron Williams, Ray Tennant, Doug Bell, Bruce Casto, and me. The exceptional success of the spring meeting in Greensboro and the PCR workshop this September were a credit to GEMS. In addition, the proceedings from the spring meeting, entitled "The Control of DNA Synthesis and Cell Proliferation," will be published in *Comments on Toxicology,* thanks to David's efforts. The success of the PCR workshop was due to the hard work of Doug Bell. Bruce Casto did an excellent job taking over from Louise Ball as Newsletter editor. The exceptional assistance of Debra Walsh in organizing the membership list in alphabetical order and in her secretarial duties for the Board meetings and all the Society's scientific meetings is greatly appreciated. I thank Greg Erexson for his efforts in getting GEMS a bulk mailing permit. Janet Warner and Kate Brock were responsible for the sustaining membership drive and did a wonderful job.

The Corporate members are a unique resource that GEMS has been very fortunate to have over the years. The idea of soliciting corporate support was the brainchild of Gene Elmore, who also worked at enlisting many of our current Corporate members. GEMS thanks the past and present Corporate members for their support. The listing of the Corporate members is contained in this booklet. Charter sustaining members supported GEMS the very first year and are recognized for doing so by the designation of *Charter* Sustaining Member. Please thank all the Corporate members as they made possible this eighth annual meeting.

Two junior scientists will win awards and plaques this year for the

best talk and the best poster. The best talk award winner will also be given support to attend the EMS meeting in Orlando, Florida from April 7–11, 1991. The best poster winner will receive a $100.00 cash prize. Please be present at 4:30 for the presentations. The judges for the posters and the talks are thanked for making difficult choices in determining the winners.

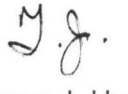
The theme of the eighth annual meeting is Environmental Toxicology. The invited speakers will discuss water (Dr. Carl Shy, UNC) and air pollution (yours truly), and the remediation of the Alaskan oil spill (Dr. Larry Claxton, EPA) and a toxic waste dump in North Carolina (Dr. Bruce Casto, EHRT). The environmental education program in North Carolina will be discussed by the Keynote Speaker, Dr. Linda Little of the Governor's Waste Management Board (GWMB). Linda is my former supervisor who has become a colleague and a great friend over the past thirteen years. Linda has done it all: tenured professor at the UNC School of Public Health, toxicologist at RTI, has owned her own environmental toxicology company, was on the Three Mile Island (TMI) Review Board, and has been on the GWMB for the last two administrations. I know that you will appreciate her insights into a very political and contoversial topic.

The new board members, elected to a three-year term by the membership are: Janet Warner, Elisabeth Korytynski, and Lisa Parker. Dr. Bruce Casto was named President-Elect, and Dr. David Doolittle takes over the position of the President for the next year. I have worked with all of these people and may I say that the membership elected a solid team. Paul Glover, Kate Brock, Bruce Casto, and I rotate off the board. Bruce did such a great job he was reelected. Thank you to all these scientists for their time and energy and intelligence and interest in GEMS. Thank you to all those who have served the Society over the past nine years. Many of the people who were instrumental in the formation of the society are still active today. For example, Dr. Larry Claxton, our first President, is giving a talk at today's annual meting.

Think about becoming involved in GEMS activities. It is great fun, a little bit of work, and a very rewarding experience. Thank you for allowing me to serve GEMS for the past eight years. It has truly been my pleasure.

Please enjoy the eighth annual meeting of GEMS. Thank you for your participation. May God bless you all. Remember what they say: "Old GEMS Presidents never die, they reappear as invited speakers!"

Sincerely,



Thomas J. Hughes,
President, GEMS

CHARTER SUSTAINING CORPORATIONS AND MEMBERS

ATLANTIC COAST CONSULTANTS, 2450 Honeysuckle Road, Chapel Hill, NC 27514. Representative: Allen L. Miller (919-929-6212). *Provides an extensive line of tissue culture equipment including incubators, freezers, centrifuges, plate readers, etc.*

BAXTER HEALTHCARE CORPORATION, 8350 Arrowridge Blvd., Charlotte, NC 28217. Representative: Andy Burnett (800-678-2298, Ext. 2322). *Provides general supplies to the scientific community.*

BURROUGHS WELLCOME COMPANY, 3030 Cornwallis Road, Research Triangle Park, NC 27709. Representatives: Donald Clive and Roberta Krehl (919- 248-3000). *Provides pharmaceuticals.*

COSTAR/NUCLEPORE, One Alewife Center, Cambridge, MA 02140. Representatives: Ed Krehl, 145 Clancy Circle, Cary, NC 27511 and Josie Wingfield, 2834 Clearbrook Drive, Marietta, GA 30067 (800-492-1110). *Provides labware lines in cell culturing, liquid handling, laboratory filtration, and immunology worldwide.*

FISHER SCIENTIFIC, 3315 Atlantic Avenue, Raleigh, NC 27604. Representatives: Vic Batts and Bob Sale (919-876-2352). *Provides supplies and equipment for biotechnology, environmental, electronic, pharmaceutical, clinical, chemical, and analytical labs: the dependable source for today's lab.*

GIBCO/BRL LIFE TECHNOLOGIES, INC., 8717 Grovemont Circle, P.O. Box 6009, Gaithersburg, MD 20877. Representative: Roger Thuotte, 1314 Poplar Lane, Hillsborough, NC 27278 (800-828-6686). *Provides research products for cell culture, cell biology, immunology, and molecular biology.*

MICROBIOLOGICAL ASSOCIATES, INC., 9900 Blackwell Road, Rockville, MD 20850. Representatives: Li Yang and David Jacobson-Kram (301-738-1000). *Provides a complete line of genetic toxicology testing services.*

NSI ENVIRONMENTAL SERVICES, P.O. Box 12313, Research Triangle Park, NC 27709. Representatives: Elisabeth Korytynski and Gail Wyatt (919-549-0611). *Provides high quality, innovative, dependable research support for cellular, molecular, and whole animal research/development studies.*

SUSTAINING CORPORATIONS AND MEMBERS

BARTLETT ENTERPRISES, 239 Flemington Road, Chapel Hill, NC 27514. Representatives: Steven Bartlett (303-371-9373) and Ann Williams (919-967-8111). *Independent sales agents representing several manufacturers with a concentration of products and services pertinent to the cell biology research laboratory, including Vanguard International labware, Skatron cell harvesters, and C.C. Laboratory's nutrients for cell culture.*

BECTON DICKINSON LABWARE, 3625 Top of the Pines Court, Raleigh, NC 27604. Representative: Chris Kennedy (919-467-7074). *Provides tissue culture products for the lab, pipettes, flasks, filters, conical tubes, round bottom tubes, Petri dishes, and multiwell plates.*

DYNA TECH LABS, 14340 Sullyfield Circle, Chantilly, VA 22721. Representative: Ken Anderson (703-631-7800). *Provides electronic colony counters.*

E.I. DUPONT COMPANY, 4280 North East Expressway, Atlanta, GA 30340 (404-452-6411). Representatives: Nancy McGranahan, 18 Kingsmont Court, Durham, NC, 27713 (919-493-0358); William Smith, 5805 Timber Ridge Drive, Raleigh, NC 27609 (919–878–0780). *Provides radiolabeled chemicals.*

EM SCIENCE, 480 Democrat Road, P.O. Box 70, Gibbstown, NJ 08027 (800-222-0342). Representative: Melanie Moore (919-383-8603). *Provides Omnisolve high-purity solvents for HPLC and HR-GC solvents for capillary GC analysis. Also provides sorbents, TLC/HPTLC plates, HPLC columns, new polymeric columns, and environmental and safety products.*

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC., P.O. Box 12199, Research Triangle Park, NC 27709. Representatives: Bruce Casto and Lorrie Backer (919-544-1792). *Provides testing for environmental toxicology, hazardous waste site remediation, drug abuse, analytical chemistry, mutagens and carcinogens, cytogenetics, organic synthesis, and SAR.*

GLAXO, INC., 5 Moore Drive, Research Triangle Park, NC 27709. Representative: Jane Allen (919-248-2100). *Provides pharmaceuticals.*

HAZELTON LABORATORIES AMERICA, INC., 5516 Nicholson Lane, Kensington, MD 20895. Representative: Steve Haworth (301-230-0001). *Provides contract research services in toxicology, molecular toxicology, biotechnology services, and research products.*

HYDRO SERVICE AND SUPPLIES, P.O. Box 12197, Research Triangle Park, NC 27709. Representatives: Christine Doss and Jeff McCormick (919-544-5580 and 717-766-7209). *Provides ultrapure water systems for research laboratories; technologies include deionization, reverse osmosis, ultraviolet sterilization, and ultrafiltration.*

INTEGRATED LABORATORY SYSTEMS, P.O. Box 13501, Research Triangle Park, NC 27709. Representatives: T.K. Rao and Ray Tice (919-544-4589). *Provides quality assurance and testing services.*

KRAECKELER SCIENTIFIC, INC., P.O.Box 11326, Durham, NC 27703. Representatives: Claude Wrenn and L.A. Walker (919-596-7373 and 919-471-1908). *Provides general laboratory supplies.*

RJR-NABISCO INC., Toxicology Research Division, Building 630/2, Winston-Salem, NC 27102. Representatives: David J. Doolittle and Wally Hayes (919-741-5762). *Provides consumer products.*

TRIANGLE SCIENTIFIC, 7474 Creedmoor Road, Suite 312, Raleigh, NC 27613-9975. Representatives: Bill Fairman and Kim Smith (919-847-3333 and 919-846-6721). *Provides representation for several chemical/biomedical research equipment companies; major products include biological safety cabinets, chemical fume hoods, CO_2 incubators, ultra-low temperature freezers and environmental walk-in rooms.*

USA/SCIENTIFIC PLASTICS, INC., P.O. Box 3565, Ocala, FL 32678. Representatives: Rob Blackman and Gene Platter (800-522-8477). *Provides disposable plastics such as pipet tips, microcentrifuge tubes, sample vials, electrophoresis equipment, Brinkmann/Eppendorf liquid handling products, radiation safety shielding products, racking and inventory systems.*

ZYMARK, Zymark Center, Hopkinton, MA 01748. Representatives: Russell Salisbury, 309 Cornwall Road, Cary, NC (919-467-0168), and Eric Herr, 4808G Trapers Drive, Raleigh, NC. *Provides laboratory robotics and automation products*

ACKNOWLEDGMENTS

GEMS gratefully acknowledges the financial support provided by the Sustaining Corporations. GEMS would especially like to thank the following companies for additional financial support for the eighth annual meeting: **RJR** (afternoon social); **EHRT** (continental breakfast for Sustaining Member Corporations); and **ILS** (afternoon break).

As members of GEMS, **please visit the booths during the morning** and thank our Corporate Members for their support.

Program
GEMS EIGHTH ANNUAL MEETING
October 25, 1990

Mission Valley Inn and Conference Center
Raleigh, North Carolina

7:30–9:00 **Registration**, exhibits, poster setup

8:55–9:00 Welcoming remarks, T.J. Hughes, President, GEMS

9:00–9:15 **T1** CYTOTOXICITY AND MUTAGENESIS IN HUMAN CELLS WITH DIFFERING DNA REPAIR CAPACITIES, *S.M. Bronstein*[a], J.E. Cochrane, T.R. Craft, J.A. Swenberg, and T.R. Skopek. [a]Duke University and University of North Carolina at Chapel Hill

9:15–9:30 **T2** THE MUTAGENICITY OF SULFAPYRIDINE, A PRIMARY METABOLITE OF THE ANTI-INFLAMMITORY DRUG SALICYLAZOSULFAPYRIDINE (SASP), *K.L. Witt*[a] and J.B. Bishop[b]. [a]ORAU, Oak Ridge, TN 37831, and [b]NIEHS, RTP, NC 27709

9:30–9:45 **T3** COMPARISON OF DNA ADDUCTS IN MICE FOLLOWING DERMAL APPLICATION OF SMOKE CONDENSATES FROM CIGARETTES WHICH BURN OR HEAT TOBACCO. C.K. Lee, *B.G. Brown,* E.A. Reed, A.T. Mosberg, D.J. Doolittle, and A.W. Hayes. Cellular and Molecular Biology Division, R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102

9:45–10:00 **T4** URETHANE INDUCTION OF MORPHOLOGICAL AND ANCHORAGE INDEPENDENT TRANSFORMATION IN BALB/3T3 CELLS WITH EXOGENOUS ACTIVATION. *C. Marr*[1], V. Ney[1], M. Roesch[1], J. Lasley[1], B. Casto[1], L. Schechtman[2], and V. Dunkel[2]. [1]Environmental Health Research and Testing, Inc., RTP, NC and [2]U.S.F.D.A., Washington, D.C.

10:00–11:30 **Exhibits,** posters, coffee and danish

11:30–1:30 **Lunch; business meeting; Speaker:** Dr. Carl Shy, UNC School of Public Health: "Epidemiological Viewpoint on Water Pollution."

1:30–2:00 **Invited Talk:** Dr. Larry Claxton, EPA: "Bioremediation of the Alaskan Oil Spill."

2:00–2:30 **Invited Talk:** Thomas J. Hughes, President, GEMS, EHRT: "The Development of Methodology for the Direct Bioassay of Atmospheric Volatile Organic Chemicals."

2:30–3:00 **Invited Talk:** Dr. Bruce Casto, EHRT: "Toxic Waste Site Biomonitoring in North Carolina."

3:00–3:30 Iced tea and soda break

3:30–4:30 **Keynote Speaker:** Dr. Linda Little, Governor's Waste Management Board: "Environmental Education in North Carolina: Replacing Fear with Reason in Tackling Environmental Problems."

4:30–5:00 **Awards**

5:00–6:00 **Social**

6:00 **Adjourn**

ABSTRACTS
TALKS

T1 CYTOTOXICITY AND MUTAGENESIS IN HUMAN CELLS WITH DIFFERING DNA REPAIR CAPACITIES
S.M.Bronstein[a], J.E.Cochrane, T.R.Craft, J.A.Swenberg, and T.R.Skopek. [a]Duke University and University of North Carolina at Chapel Hill)

Three human B-lymphoblastoid cell lines with different DNA repair capacities were exposed to the alkylating agent ethylnitrosourea (ENU). The first cell line is competent in both O^6alkylguanine-DNA-alkyltransferase (ATase) and nucleotide excision repair (NER); the second cell line, which is derived from a patient with xeroderma pigmentosum (complementation group A), is deficient in NER, and the third cell line does not express ATase. The cell lines lacking ATase and NER both showed increased cytotoxicity from ENU treatment relative to the repair-competent cell line, with the ATase-negative cells showing the highest toxicity. Mutation frequency at the hypoxanthine-guanine phosphoribosyl transferase locus (HPRT) was measured by plating cells in the presence of the purine analog 6-thioguanine. Measured mutant fractions suggest that the importance of ATase and NER in preventing mutations are similar to their roles in preventing cell killing by ENU: both the ATase-negative and the NER-negative cell lines had induced mutant fractions several times greater than did the repair-competent cells. Taken together, these data suggest that both ATase and NER are important for the repair of DNA damage induced by ENU. To further characterize the roles of ATase and NER in ENU-treated cells, individual HPRT mutant clones were isolated and the mutations were identified by cDNA production followed by PCR amplification and sequencing. Preliminary results reveal the presence of transition and transversion mutations at both AT and GC base pairs in all three cell lines. The similarity in spectra obtained thus far have not permitted any definitive conclusions about the contribution of either repair mechanism in determining the types of mutations produced by ENU. However, the lack of striking differences in spectra suggests that the profile of promutagenic DNA adducts in the DNA as mutations occur is similar in all three cell lines.

T2 THE MUTAGENICITY OF SULFAPYRIDINE, A PRIMARY METABOLITE OF THE ANTI-INFLAMMATORY DRUG SALICYLAZOSULFAPYRIDINE (SASP),
K. L. Witt[a] and J. B. Bishop[b], [a]ORAU, Oak Ridge, TN, 37831, and [b]NIEHS, RTP, NC 27709

Salicylazosulfapyridine, commonly known as sulfasalazine or SASP, is a drug that is widely used in the treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn's Disease. Bacteria in the large intestine cleave SASP into sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA) by reducing the diazo bond linking these two moieties. The bulk of the 5-ASA stays in the intestine where it exercises its therapeutic, anti-inflammatory effect, while the SP is readily absorbed into the body, where it is ultimately hydroxylated and N-acetylated in the liver, and then glucuronidated and excreted in the urine (Das et al., 1974). We recently reported the induction of micronuclei in mice treated with SASP (Bishop, et al., 1990). The results of micronucleus (MN) tests on the metabolites of SASP demonstrate that the SP rather than the 5-ASA portion of the molecule is responsible for the mutagenicity of SASP. Using a fluorescent antikinetichore stain, we have further demonstrated that the mutagenic damage induced by SASP results primarily in the formation of MN which contain kinetochores (KC), while the mutagenic damage induced by SP results in MN both with and without KC. The increases in MN induced by SASP and SP were well-correlated with dose. These data on SP and SASP will be discussed in relation to our understanding of the pharmacokinetics of SASP metabolism and the increased risk of colon cancer for IBD patients.

T3 COMPARISON OF DNA ADDUCTS IN MICE FOLLOWING DERMAL APPLICATION OF SMOKE CONDENSATES FROM CIGARETTES WHICH BURN OR HEAT TOBACCO

C. K. Lee, B. G. Brown, E. A. Reed, A. T. Mosberg, D. J. Doolittle, and A.W. Hayes. Cellular and Molecular Biology Division, R.J. Reynolds Tobacco Co. Winston-Salem, NC. 27102

A new cigarette developed by R.J. Reynolds Tobacco Company which only heats tobacco has yielded consistently negative results in a standard battery of *in vivo* and *in vitro* genetic toxicology tests. The 32P DNA adduct assay has been reported to be an extremely sensitive assay for detection of chemically induced alterations to DNA. The objective of the present study was to evaluate the potential of the new cigarette to alter DNA in mouse skin and lung. CD-1 mice were skin-painted with cigarette smoke condensate (CSC) from reference and new (TEST) cigarettes for 4 weeks. The greatest concentrations of CSC applied were 180 mg CSC per week per animal for both reference and TEST cigarette. DNA adducts in skin and lung tissues were analyzed using the 32P-postlabeling method with P1 nuclease modification. Distinct diagonal radioactive zones (DRZ), charateristic of smoke-induced DNA adducts, were observed in the DNA from both skin and lung tissues of animals exposed to reference CSC, while no corresponding DRZ was observed from the DNA of animals exposed to either TEST or vehicle control (acetone). The relative adduct labeling (RAL) values of skin DNA from animals exposed to reference were 38.5×10^{-9} after one week and 22.5×10^{-9} after 4 weeks. The RAL values of lung DNA from the same animals were 22.9×10^{-9} after 1 week and 80.7×10^{-9} after 4 weeks. The RAL values of skin and lung DNA from TEST were no greater than those of vehicle control and were less than 3×10^{-9}. The present results on TEST cigarettes are consistent with all the previous results of *in vivo* as well as *in vitro* genetic toxicology testing and provide compelling additional evidence on the nongenotoxicity of smoke condensate from the new cigarette.

T4 URETHANE INDUCTION OF MORPHOLOGICAL AND ANCHORAGE INDEPENDENT TRANSFORMATION IN BALB/3T3 CELLS WITH EXOGENOUS ACTIVATION. C. Marr, V. Ney, M. Roesch, J. Lasley, B. Casto, L. Schechtman*, and V. Dunkel*. Environmental Health Research and Testing, Inc., RTP, NC. and *U.S.F.D.A., Washington, D.C.

Urethane, a naturally occurring component of many fermented foods and beverages, has been classified as a carcinogen in experimental animals. Since urethane may comprise a larger portion of the American diet than previously estimated, the FDA was interested in expanding the earlier urethane bioassay studies to include *in vitro* transformation. Subconfluent Balb/3T3 cells ($2x10^5$ cells) were incubated with urethane-induced hamster S9, the necessary cofactors, and urethane (5-1500 ug/ ml). Controls were benzo(a)pyrene [B(a)P] (5-10 ug/ml) or diethylnitrosamine (DEN) (250-1000 ug/ml) and DMSO [0.5% as B(a)P control]. Two hour incubations were conducted at 37°C. Cells were washed 1x in Eagle's medium (E-MEM) containing 10% fetal bovine serum (FBS) and plated at 200 cells per dish for cytotoxic analyses (n=5) and at $1x10^4$ cells per dish for assessment of morphological transformation (n= 20). Seven days later, clones on cytotoxicity dishes were fixed, stained, and scored. Transformation dishes were maintained in culture for six weeks, then overlayed with agar and viewed under a phase-contrast microscope. Foci displaying morphology typical of a type II or III focus were isolated and expanded in culture as cell lines. Transformation dishes were then fixed, stained and scored for the presence of type I, II or III foci. Urethane was an active transforming agent inducing an average of 3.4 Type II or III foci per dish in 100% of dishes treated with 62.5 ug/ml and 5% S9. Cell lines arising from individual foci were evaluated for their ability to grow in soft agar at passages 5 to 25. Cells were plated at 100, 1,000, and 10,000 cells per dish in medium containing 20% FBS and 0.33% BBL agar. Dishes were maintained for 3 weeks, then stained and scored under a dissecting microscope. Cloning efficiencies in agar increased with increasing passage level and ranged from 0.1 to 40%.

ABSTRACTS
POSTERS

P1 Development of an Anchorage-Independence Assay with A427 Human Lung Tumor Cells for the Screening of Chemopreventive Agents, E.A. Koryznski*, G.J. Kelloff*, W.A. Suk*, and E. Elmore*, *NSI Technology Services Corporation, P.O. Box 12313, RTP, NC 27709, *National Cancer Institute, NIH, 9000 Rockville Pike, Bethesda, MD 20892, and *National Institute of Environmental Health Sciences, P.O. 12233, RTP, NC 27709.

The human lung tumor cell line, A427, was used to develop an anchorage-independence assay (AIA) for the screening of chemopreventive agents. The culture medium was optimized and the A427 cells grew as colonies in soft agarose at frequencies of 0.37 to 2.2%. The cells were seeded in 0.3% soft agarose in the presence of test agent and then were fed weekly with medium. After approximately four weeks, the colonies were stained and enumerated. 13-*cis*-retinoic acid (RA) was evaluated in the A427 AIA and was found to inhibit colony growth. To ensure the measurement of a reduction in colony frequency was due to the agents' mechanism and not toxicity, and anchorage-dependent cytotoxicity assay was performed concurrently. In 25 experiments, 10μg/mL 13-*cis*-RA consistently inhibited colony growth at 61.15% ± 16.29. Data from the evaluation of several potential chemopreventive agents will also be presented. The A427 AIA has demonstrated the capability for screening potential chemopreventive agents.
(Supported by NCI Contract NO1-CN-55503-02).

P2 **ESTIMATION OF INDUCED MUTATION IN MOUSE LYMPHOMA CELLS,**
Kimberly C. Hines, Diane S. Daston, and William J. Caspary, NIEHS/NIH, RTP, NC 27709.

Mammalian cell mutagenicity assays may not accurately measure the number of mutations induced by chemical treatment. During expression, mutant and wild-type cells are mixed; thus, if a population of mutants grows more slowly than wild-type cells, the ratio of mutants to wild-type cells will decrease during the expression period. Because the *in situ* procedure segregates and immobilizes cells during expression, mutations are captured as individual colonies so the measured mutation frequency accurately reflects the mutational events that occur. In addition, segregating colonies during expression insures the independence of mutant colonies. Our laboratory has previously described the *in situ* assay protocol and evaluated the expression of spontaneous mutation at the thymidine kinase (tk) locus in the L5178Y mouse lymphoma cell line (*Genetics* 126:435-442). We determined that the mutation rate measured in the *in situ* assay is 50-fold greater than when the cells expressed the phenotype in suspension. In the present work, we evaluated mutation frequencies induced by chemical treatment by generating expression curves for a number of compounds. In these experiments, mouse lymphoma cells heterozygous at the tk locus were plated in semisolid medium immediately after treatment and allowed to form microcolonies. The selective agent, trifluorothymidine (TFT) was added as an overlay at specified times permitting only $tk^{-/-}$ cells to survive. The induced mutation was determined by subtracting the spontaneous background from the total number of colonies on the induced plates. The numbers of TFT^r colonies at various times after treatment indicated that chemically treated cultures had higher mutation frequencies than the solvent controls. For most chemicals tested, the numbers of chemically induced mutant colonies began to increase after an expression time of 20-30 hours and began to plateau at about 50 hours. Based on our results, a 40 to 50 hour expression time would be recommended for use in determining chemical dose response.

P3 A COMPARISON OF MICRONUCLEUS FREQUENCIES IN MOUSE BONE MARROW AND PERIPHERAL BLOOD ERYTHROCYTES USING MULTIPLE SAMPLING TIMES AFTER EXPOSURE TO MITOMYCIN C IN VIVO.

R.M. McGee, J.L. Huston, G.L. Erexson, and B.C. Casto. Environmental Health Research and Testing, Inc. P.O. Box 12199, RTP, NC 27709.

An experiment was designed to investigate the persistence of micronuclei (MN) in bone marrow and peripheral blood polychromatic erythrocytes (PCEs) after multiple intraperitoneal (i.p.) injections of mitomycin C (MMC). MMC was dissolved in phosphate buffered saline (pH=7.2). Three consecutive daily i.p. injections of either 0, 0.2, or 0.4 mg MMC/kg were administered to male B6C3F1 mice. Bone marrow and blood PCEs were harvested at either 6, 12, 24, 36, 48, or 72h post-injection. Bone marrow slides were prepared from one femur of each mouse using the direct method and peripheral blood smears were made from tail vein blood. All slides were fixed for 5 minutes in methanol and stained in acridine orange (125 ug/ml). Two thousand PCEs and 200 PCEs and normochromatic erythrocytes were scored for MN and %PCEs, respectively, Dose-dependent increases in MN-PCEs were observed in both tissues. Analyses revealed the peak incidence of MN-PCEs in bone marrow occurred at 24h post-injection. The peak in MN-PCEs in peripheral blood was seen at 48h post-injection. Allowing for the differences in bone marrow and blood PCE cycling times, no significant difference in MN frequency was observed between the two tissues. The %PCEs in BM was significantly suppressed at the 72h sampling time. In addition, a significant decrease in %PCEs was observed at the 12h post-injection sampling time in peripheral blood. This study reveals that peripheral blood PCEs can be analyzed for MN with equal efficacy as the bone marrow. Scoring of mouse peripheral blood smears for MN is less labor intensive and more adaptable to automated analysis than bone marrow.

P4 DNA DAMAGE EVALUATION USING THE RODENT IN VITRO HEPATOCYTE CULTURE SYSTEM AND THE SINGLE CELL GEL (SCG) ELECTROPHORETIC ASSAY.

[1]Hirai, O., [2]Andrews, P. W., and [2]Tice, R. R. [1]Fujisawa Pharmaceutical Company, Osaka, Japan, [2]Integrated Laboratory Systems, P.O. Box 13501, Research Triangle Park, NC.

The ability of the SCG technique to detect chemically-induced DNA damage in rodent hepatocytes treated in vitro was investigated. Several chemicals were examined using cells isolated from the B6C3F1 mouse and Fischer 344 rat by in situ EDTA and collagenase perfusion of the liver followed by incubation with the test chemical for 2-8 hours (kinetic studies) and 6 hours (dose response studies. DNA damage in individual cells was detected as electrophoretic DNA migration under alkaline conditions using the SCG technique. Qualitative and quantitative differences in DNA migration were observed for every chemical. Hepatocytes isolated from the rat and mouse treated with cyclophosphamide (CP), dimethylnitrosamine (DMN) and ethylmethanesulfonate (EMS) all yielded dose related increases in DNA migration. Rat hepatocytes yielded a strong positive response following exposure to 2-acetylaminofluorene and a weak positive response following exposure to 4-acetylaminofluorene, whereas mouse hepatocytes yielded no DNA migration. Treatment with benzo(a)pyrene produced a positive response in mouse hepatocytes. In some studies, parenchymal and nonparenchymal cells were distinguished on the basis of nuclear size after DNA unwinding with DNA damage evaluated in both cell types. DMN, which requires metabolic activation, produced DNA damage primarily in parenchymal cells and some damage in nonparenchymal cells when tested at higher doses. EMS, a direct acting chemical, produced equal levels of DNA damage in both cell types. These results indicate that the SCG assay can easily be applied to the in vitro rodent hepatocyte culture system and suggests that this approach may be a useful technique for identifying and differentiating between genotoxic chemicals. Supported by EPA Contract 68-C8-0069.

P5 EVALUATION OF DNA DAMAGE IN GOLDEN MICE (OCHROTOMYS NUTTALLI) INHABITING A HAZARDOUS WASTE SITE USING THE SINGLE CELL GEL (SCG) ASSAY. [1]Phillips, M.D., [1]Croom, D.K., [1]Andrews, P.W., [1]Tice, R.R., and [2]Nauman, C.H. [1]Integrated Laboratory Systems, Research Triangle Park, NC, [2]EPA-EMSL, Las Vegas, NV.

Wild animal populations inhabiting hazardous waste sites provide an opportunity to assess the extent of exposure to genotoxic pollutants as measured by the presence or absence of increased levels of DNA damage. Golden mice (Ochrotomys nuttalli) were live-trapped along the perimeter of the NCSU Hazardous Waste (HW) Facility, Raleigh, NC and at three nearby control sites. The HW site has been classified as a Superfund site with the predominant pollutants including trichloroethylene, chloroform, carbon tetrachloride, various pesticides, laboratory solvents, and other chemicals. O. nuttalli were sacrificed via CO_2 asphyxiation. Peripheral blood, liver, brain, and bone marrow samples were obtained from each animal and evaluated for DNA damage using the SCG technique. The level of DNA damage, as measured by mean migration length, was increased in all four tissues, but only significantly in the brain ($P <= 0.05$). Dispersion analyses showed that the bone marrow cells from the HW mice exhibited a significantly increased dispersion coefficient over that calculated for the control mice ($P <= 0.05$). Analyses conducted to evaluate the correlation among tissues within animals in the extent of DNA migration or dispersion revealed only a significant correlation for the dispersion in brain and liver. Bone marrow and peripheral blood smears were also prepared for each animal to evaluate micronuclei (MN) frequency in polychromatic erythrocytes (PCEs) and the percentage of PCE among total erythrocytes. No difference between the HW and control groups in either the MN-PCE frequency or %PCE was observed. The end results demonstrate the utility of the SCG technique for evaluating DNA damage induced by environmental pollutants. This project was supported by the U.S. EPA through contract number 68-C8-0069 to ILS.

P6 EVALUATION OF CHEMICALLY-INDUCED DNA DAMAGE IN GERM CELLS OF MALE MICE USING THE SINGLE CELL GEL (SCG) ASSAY. Croom, D.K., Andrews, P.W., Nascimbeni, B., and Tice, R.R., Integrated Laboratory Systems, P.O. Box 13501, Research Triangle Park, NC 27709.

The SCG electrophoretic technique has been developed for directly evaluating, in individual cells, the frequency of single strand breaks and/or alkali labile sites. This technique, which requires only a few thousand cells, can be used to evaluate the level and intercellular distributioin of DNA damage induced by genotoxic agents in virtually any eukaryotic cell population. The focus of this study was to detect chemically induced DNA damage in the gonadal cells of male mice. The effects of three agents, acrylamide (ACR), ethylmethane-sulfonate (EMS) and dimethylnitrosamine (DMN) were assayed at three dose levels. Male B6C3F1 mice were treated by gavage and sampled at 3, 6, 12, 24 and 48 hours after treatment. One testis was removed from each animal, minced and the cells mixed with agarose and layered onto microscope slides according to the SCG technique. Diploid and haploid cells were distinguished on the basis of nuclear size after DNA unwinding. DNA migration lengths were measured to determine the kinetics of the induction and repair of the induced damage. In addition, whole blood was also sampled and the leukocytes analyzed for comparision. The results indicate that this technique can easily provide useful data on the chemical induction of DNA damage in germ-line cells. Supported by NIEHS SBIR # IR43-ES-05383-01.

P7 DIRECT SEQUENCE ANALYSIS OF ras ONCOGENES USING PCR AMPLIFIED DNA
J. Ginsler, C. Walker, S. Lane, and L. Recio CIIT, RTP, NC

Direct sequence analysis of DNA amplified using the polymerase chain reaction (PCR) is a rapid and efficient method for detection of DNA mutations. Utilizing this technique, sequence information can be obtained from a small amount of genomic DNA using the dideoxynucleotide sequencing method. We have applied this methodology to determine the sequence of ras oncogenes in a panel of rat kidney epithelial cell lines derived from 6 independent spontaneous rat renal cell carcinomas. DNA was isolated from these cell lines and the DNA sequence of H-, K-, and N-ras for each line was determined. The first 80 amino acids of H-, K-, and N-ras are identical and conserved among mammalian species, although the DNA sequence coding for these amino acids differs between members of the ras gene family and between species. Using PCR primers for the rat ras genomic regions encompassing codons 12, 13, and 61 (sites of mutational activation for these oncogenes), DNA was amplified and sequenced. H-ras and K-ras sequences were normal in each of the 6 lines. However, the DNA sequence of codons 13 and 14 of N-ras in all of the cell lines was the same as the "activated" form of this gene reported by McMahon et al (PNAS, 87:1104, 1990). At codon 13, N-ras in all the cell lines had a gly → val alteration (GGT → GTT), and in codon 14, a val → ile alteration (GTT → ATT) was observed. In addition, the same N-ras alterations were present in normal rat kidney DNA. Thus, the rats in which the tumors arose carried this gene alteration in their germ line as a "silent" oncogene. These results indicate that N-ras DNA sequence alterations alone are insufficient for tumor formation and that other events are required to express a fully transformed phenotype.

P8 **2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD)-INDUCED CELLULAR RESPONSES IN A HUMAN SQUAMOUS CELL CARCINOMA CELL LINE (SCC-12F),** S.C. MANESS AND K.W. GAIDO, CIIT, RTP, NC 27709

TCDD has a strong modulating effect on growth and differentiation of epithelial cells. This response is highly dependent on cell type and culture conditions. The work presented here characterizes the effect of TCDD on growth and differentiation of a human keratinocyte cell line (SCC-12F), cultured under preconfluent, proliferative conditions. The advantage of using the immortalized SCC-12F cell line is that it has been shown to express a normal cell phenotype. Cells were plated at high density (2.5×10^4 cells/cm^2) and treated, while still preconfluent, with TCDD at a final concentration of 10 nM. Cell growth, DNA synthesis, cross-linked envelope formation (CLE), and colony forming efficiency (CFE) were measured at specific times after treatment with TCDD. Although cell number continued to increase following TCDD treatment, overall proliferative capacity was significantly reduced as compared to control values. DNA synthesis, as measured by ^{3}H-thymidine incorporation, showed a significant reduction compared to control values 48 hrs after treatment with TCDD. However, by 72 hrs, DNA synthesis in TCDD-treated cultures was comparable to control values. Treatment with TCDD resulted in a significant increase in terminal differentiation as measured by CLE. Basal cell number, as assayed by CFE, was reduced by TCDD. These results indicate that under preconfluent conditions, TCDD reduces proliferation in a human keratinocyte cell line while enhancing differentiation. This finding is similar to what has been shown in normal human epithelial cells and demonstrates the usefulness of the SCC-12F cell line for studying the mechanism of TCDD action in human epithelial cells.

P9 Optimization of the Chick Embryo Retina Cell Assay to Screen Teratogens. M.P. Fitzgerald*, R.D. Sorrell*, G.P. Daston^, and E.L. Elmore*, *NSI Technology Services Corporation, Research Triangle Park, North Carolina and ^The Procter & Gamble Company, Cincinnati, Ohio.

In response to a growing need for reliable short term in vitro assays to test the potential teratogenicity of compounds, the Chick Embryo Retina Cell Assay is under investigation by our laboratories. This in vitro screening system assesses the effect of potential teratogens on three endpoints: inhibition of cell aggregation, growth inhibition of cell aggregation, growth inhibition and inhibition of cortisol induced differentiation. Test chemicals may interfere with any one of these endpoints and the endpoint mechanisms are not necessarily related. This method also allows for the assessment of all three endpoints from the same treated cell population. All tests are performed using neural retina cells from 7.0 ± 0.5 day White Leghorn chick embryos. The effects of serum lot, cell density, aggregate size, pH and shaker speed on assay variability were evaluated. Differences in the optimal shaker speeds for aggregation and differentiation were observed. Cell densities were best at $7x10^4$/35mm dish. Vinblastine, 13-cis retinoic acid, bromodeoxyuridine, 6-aminonicotinamide and TPA, which are known teratogens with differing mechanisms, were used in assay optimization. All of these chemicals affect at least one endpoint at concentrations less than 1mM. An evaluation of the optimized Chick Embryo Retina Cell Assay to determine its accuracy in predicting the teratogenicity of coded agents is currently in progress.

EVIDENCE THAT METHANOL INHALATION DOES NOT INDUCE CHROMOSOME DAMAGE IN MICE, J.A. Campbell[a], D.R. Howard[a]. L.C. Backer[a], and J.W. Allen[b] , [a]EHRT, Inc, P.O. Box 12199, RTP, NC 27709, and [b]EPA, MD-68, RTP, NC, 27711

P10 Methanol is a solvent widely used in industry; e.g., in the production of formaldehyde, methyl chloride, acetic acid, and in a host of consumer products which include anti-freeze, enamel, and paint removers. It has been proposed as an alternative fuel for automobiles, which would lead to greatly increased human exposure to this substance. Consequently, interest in public health risks associated with exposure to methanol has intensified. There is relatively little information available concerning the potential genotoxicity of methanol following exposure by inhalation. In the present study, multiple mouse tissues were examined for chromosome damage following inhalation exposure to methanol. Male C57BL/6J mice were exposed to 0, 800 or 4,000 ppm methanol 6 hours per day for 5 days. Several cytogenetic endpoints were then analyzed. Micronuclei (MN) were evaluated in peripheral blood erythrocytes; structural chromosome aberrations (CAs), MN, sister chromatid exchange (SCE) frequencies and cell replication indices were assessed in primary lung cell cultures. Synaptonemal complex (SC) damage was analyzed in spermatocytes. The results were uniformly negative; no increased frequencies of micronuclei in blood cells, of SCEs, CAs, or MN in lung cells, or of SC damage in spermatocytes were found. From the standpoint of risk assessment, these experimental studies do not reveal any evidence of a cytogenetic hazard associated with inhalation of methanol at these concentrations.

This is an abstract of a proposed poster and does not necessarily reflect U.S. EPA policy.

P11 Differential Growth Response to Exogenous Calcium in Normal and Carcinogen-Exposed Primary Human Keratinocyte Cell Cultures, G.P. Wyatt*, E.L. Elmore*, and V.E. Steele*, *NSI Technology Services Corporation, Research Triangle Park, N C 27709 and *National Cancer Institute, NIH, 9000 Rockville Pike, Bethesda, MD 20892.

The purpose of these studies was to examine an early carcinogen-induced change in primary human epithelial cell cultures and to attempt to reverse this change with a chemopreventive agent. Methods for the routine isolation of epithelial cells were developed. Primary cultures of human foreskin keratinocytes were prepared and exposed to the carcinogen, propane sultone. In a series of experiments it became evident that carcinogen exposed cells continued to grow in the presence of added calcium. Solvent control cell growth was decreased under such conditions. This new phenotype became pronounced after the fourth subculture. The addition of retinoic acid to the culture medium at each medium change reduced this effect and the keratinocytes grew more slowly in the presence of added calcium. The results suggest that carcinogen-exposed human keratinocytes acquire a resistance to calcium-induced differentiation or growth cessation and that retinoic acid can ameliorate this process. (Supported by NCI contract NO1-CN-55503-01).

P12 APPLICABILITY of CYANOPROPYL BONDED SILICA HPLC for BIOASSAY DIRECTED-FRACTIONATION of COMPLEX COMBUSTION EXTRACTS, R. Williams[1], L. Brooks[1], M. Taylor[1] and D. DeMarini[2], [1]EHRT,Inc. RTP,NC 27709, and [2]EPA, HERL, RTP, NC 27711.

Potential human health effects have been reported to be associated with some products of incomplete combustion (PICs). These products from sources such as gas and diesel engines, woodstoves, and municipal waste incinerators have been found to be extremely complex in nature. Municipal waste incinerators have proven to be extremely challenging in trying to determine both the chemical composition of the particle emissions from these sources as well as their potential for health effects. The US EPA is currently involved in development of methods to overcome some of these difficulties. Captured samples are extracted and then fractionated using HPLC. One HPLC column, cyanopropyl-bonded silica (CN), has shown promise in being able to efficiently and reproducibly fractionate incinerator extracts. Extract fractions are then analyzed for mutagenic effects using a microsuspension mutagenicity assay. The CN column has been found to recover low microgram quantities of mass in the range of 80-112% with excellent resolution of selected polyaromatic hydrocarbon (PAH) standards. Fractionation of combustion extracts has revealed that several classes of compounds are responsible for incineration mutagenicity. [This is a proposed abstract and does not necessarily reflect the views of the US EPA.]

P13 A COMPARISON OF DNA ADDUCTS INDUCED BY ADMINISTRATION OF BENZO(a)PYRENE AND BENZO(a)PYRENE METABOLITES IN RAT LUNG, LIVER AND PERIPHERAL BLOOD LYMPHOCYTES. G. Nelson[a], J. Ross[b], G. Erexson[a], A. Kligerman[b], M. Bryant[a], S. Nesnow[b], K. Earley[c], R. Gupta[c]. [a]EHRT, Inc., RTP, NC 27709, [b]U.S. E.P.A., RTP, NC 27711, and [c]University of Kentucky, Lexington, KY 40536.

The i.p. administration of benzo(a)pyrene (B(a)P) in the rat produced DNA adducts specific to tissue type. Lung and peripheral blood lymphocytes (PBLs) formed one major DNA adduct and two minor adducts. Liver exhibited two major DNA adducts of similar intensity and two minor adducts. The following synthetic B(a)P metabolites and ring-substituted derivatives were i.p. administered to rats: 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, and 12-hydroxy-B(a)P, B(a)P-trans-4,5-dihydrodiol, B(a)P-trans-7,8-dihydrodiol, B(a)P-trans-9,10-dihydrodiol, and B(a)P-7,8-dione. DNA adducts were produced by the i.p. administration of several B(a)P metabolites and derivatives. B(a)P-7,8-dihydrodiol leads to the formation of the major DNA adducts observed in all three tissues upon i.p. administration of B(a)P. 9-hydroxy-B(a)P leads to the formation of a DNA adduct observed in lung and PBLs dosed with B(a)P. 2-hydroxy-B(a)P produces three major DNA adducts that are observed in all three tissues. However, the adducts produced by 2-hydroxy-B(a)P are not observed with the administration of B(a)P. The administration of 12-hydroxy-B(a)P leads to the formation of two major adducts in lung, which are present at low levels in liver and PBLs. B(a)P administration does not lead to the formation of the adducts observed with 12-hydroxy-B(a)P.

This is an abstract of a proposed poster and does not reflect U.S. EPA policy.

P14 INDUCTION OF DNA DAMAGE IN CULTURED HUMAN LUNG CELLS AND BINDING TO DNA BY SUBSTITUTED AROMATIC COMPOUNDS REACTED WITH HYPOCHLOROUS ACID (HOCL). W.J. Kozumbo[a], S. Agarwal[a] and H.S. Koren[b], [a]University of North Carolina, Chapel Hill, NC 27599 and [b]U.S. EPA, Research Triangle Park, NC 27711

Lung inflammation is typically characterized by an influx of polymorphonuclear leukocytes (PMNs), and can be induced by various air-polluting oxidants and particles. During phagocytosis of particles by PMNs, H_2O_2 and myeloperoxidase (MPO) are released into extracellular spaces and intracellular phagosomes. In the presence of H_2O_2 and MPO, there is opportunity not only for HOCl formation but also for its reaction with compounds associated with pollutant particles. Using 1-naphthylamine and 1-naphthol as noncarcinogenic model compounds associated with particulate matter in tobacco smoke and diesel exhaust, we examined whether HOCl (⁻OCl) could react with and transform them into products that can damage DNA in cultured human lung fibroblasts (CCD18Lu). Compounds (15 uM) dissolved in phosphate-buffered saline (pH 7.2) were first incubated with HOCl (12.5 to 100 uM) for 20 min at 37°C. Cells were then treated for 1h with the reaction mixture at 37 or 4°C and analyzed for DNA single-strand breaks by alkaline elution. HOCl-reacted compounds induced up to a maximum of 400 to 500 rad-equivalents of DNA damage. Damage was greater in cells treated at 37 than at 4°C. Neither HOCl nor compounds alone damaged DNA. DNA breakage was proportional to the HOCl concentration used in the transformation reaction, with maximal breakage produced at about a 6 to 1 molar ratio of HOCl to compound. DNA breakage also correlated with changes that occurred in the UV spectra of compounds following their reaction with HOCl. In other studies, HOCl-reacted [^{14}C] 1-naphthol was found to bind to purified DNA. Taurine, an HOCl trapping agent, blocked HOCl-mediated formation of the DNA-reactive products. When stimulated *in vitro* for 1h, human PMNs ($4x10^6$/ml) generated 277 +/- 23 uM of HOCl. Taken together, these results suggest that HOCl produced during an inflammatory response may transform various pollution-associated substituted aryl compounds into products that can react with and break cellular DNA. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

P15 HPRT SEQUENCE ALTERATIONS IN MALE PATIENTS RECEIVING CISPLATIN-BASED TREATMENT. T.R. Skopek, N.F. Cariello, T.R. Craft, J. Cochrane, J. Nicklas[a], J.P. O'Neill[a], and R. Albertini[a]. University of North Carolina, Chapel Hill, NC and [a]University of Vermont, Burlington, VT.

We are determining the DNA sequence specificity of mutagenesis occurring in vivo in the peripheral T-lymphocytes of male patients receiving cisplatin-based therapy. For comparison, the mutational specificity of cisplatin is also being determined in vitro under controlled culture conditions. The genetic target in both the in vivo and cell culture studies is the X-linked hypoxanthine-guanine phosphoribosyl transferse (hprt) gene. In vivo mutational specificity is determined primarily by automated DNA sequence analysis of PCR-amplified hprt cDNA from 6-thioguanine-resistant mutant colonies. Mutants displaying aberrant mRNA splicing (primarily exon loss) are further characterized by sequence analysis of the appropriate exon/intron regions of hprt following PCR amplification from genomic DNA. Complex mutant populations generated in vitro with cisplatin are being analyzed using denaturing gradient gel electrophoresis (DGGE). The in vivo spectrum collected to date is a complex mixture of base substitutions, frameshifts, and deletions in the hprt coding region at both AT and GC base pairs, as well as mutations leading to aberrant hprt mRNA splicing. Many of the mutations observed were associated with Pu-Pu or Pu-X-Pu sequences, regions known to be adducted by cisplatin. Only 6/32 of the mutant sites observed in the coding region of cisplatin-treated patients have been seen previously in vivo in unexposed individuals. Preliminary work in vitro has revealed four mutant sites in exon 3, one of which has been observed in vivo in a cisplatin patient.

P16 EFFICIENT DNA ISOLATION FROM SMALL TISSUE SAMPLES, K. Holden[1], G. Nelson[1], and J. Ross[2]. [1]EHRT, RTP, NC 27709; [2]U.S. EPA, RTP, NC 27711.

Phenol-chloroform and spermine DNA isolation techniques currently used with large amounts of tissue (>300mg) are not efficient with small amounts (<300mg) of tissue. Therefore, we developed a procedure using saturated salt and spermine isolation to increase DNA recovery. Tissues are minced and lysed, and the DNA is precipitated by a saturated salt solution and absolute ethanol. The DNA is then reprecipitated with spermine. We compared the saturated salt/spermine technique to the phenol-chloroform isolation in tissues under 300mg in Strain A mice lung. In the phenol-chloroform the average recovery was 0.195 ± 0.42 μg/mg. The average recovery with the salt-spermine techniques was 3.058 ± 1.34 μg/mg. We also compared recovery from rat peripherial blood lymphocytes (PBLs) using spermine isolation alone to the salt-spermine technique. For the salt-spermine extraction recovery was 3.6 ± 0.8 μg DNA/10^6 PBLs and recovery from the spermine alone was 3.3 ± 1.5 μg DNA/10^6 PBLs showing that this technique works equally well on PBLs. While using this technique we have seen a dramatic increase in recovery levels with a good DNA/RNA ratio. Although the saturated salt-spermine technique was developed because of our work with small tissue samples, it is a generally useful technique for isolating DNA from tissues.

This is an abstract of a proposed poster and does not necessarily reflect U.S. EPA policy.

P17 EVIDENCE FOR SOS-INDEPENDENT INDUCTION OF MUTATIONS BY N-ACETOXY-N-2 ACETYLAMINOFLUORENE IN TWO E. COLI STRAINS, S.A. Leavitt and J. Ross, U. S Environmental Protection Agency, MD-68, Research Triangle Park, NC 27711

Plasmid pT7T3α-18 DNA was modified in vitro to various extents with the live carcinogen N-acetoxy-N-2-acetylaminofluorene (N-AcO-AAF) and then transforme into E. coli strains with different repair capabilities. The survival of th plasmid, measured as the ratio of ampicillin resistant colonies per inpu plasmid molecule, decreased with increasing levels of adduction, and showe no differences between the recombination deficient strain DH5α and the wild type strain JM83. The mutagenicity of the adducts formed was assayed b measuring loss of B-galactosidase activity coded for by a portion of the Lac gene present on the plasmid. There was a 6.3-fold increase in mutatio frequency at the 29 adducts/molecule of pT7T3α-18 in JM83, and a 27-fol increase for DH5α. There did not appear to be a difference between mutatio frequency at the 0 adducts/ molecule of pT7T3α-18 and the 14 adduct/molecul of pT7T3α-18 for JM83. For DH5α, at the 14 adducts/ molecule of pT7T3α-1 level, there was a 20-fold increase over spontaneous mutation frequency. Th mutation spectra, as determined by DNA sequencing, were dominated by a 2 bas pair deletion for both strains. (This abstract does not reflect the views an policies of the US EPA)

P18 Cytogenetic Evaluation of Three Structurally-related Phenylenediamines in th Mouse Micronucleus Assay. L. Soler-Niedziela[1], J. Nath[2], and T. Ong[2,3] [1]Research Triangle Institute, Research Triangle Park, NC, [2]West Virgini University and Division of Respiratory Disease Studies, and [3]NIOSH Morgantown, WV.

Three structurally-related compounds, 4-chloro-o-phenylenediamine (COP), 4-nitro-o-phenylenediamine (NOP), and p-phenylenediamine dihydrochloride (PPD are used in fur dyes, inks, and hair-coloring formulations. COP has beer reported to be carcinogenic in both rats and mice. NOP and PPD are noncarcinogens but have consistently tested positive in short-term, in vitro, genotoxicity assays. This study was undertaken to evaluate their activity in an in vivo assay, the mouse bone marrow micronucleus assay. Five CD-1 male mice per dose were injected i.p. with the compounds and sacrificed at intervals of 24, 48, and 72 h to obtain bone marrow cells. Two thousand cells per animal were scored to determine the number of micronucleated polychromatic erythrocytes (MN-PCE). In addition, polychromatic to normochromatic erythrocyte (PCE/NCE) ratios were determined to give an indication of compound toxicity. COP induced significant dose-related increases in MN-PCE over the three doses tested at each of the sampling intervals. The peak response occurred at 24 h. Doses of 100 mg/kg and 400 mg/kg induced 4.8 and 7.4 MN-PCE, respectively, per 1,000 PCE scored. The solvent control value was 1.9. No increase in MN-PCE was observed in animals treated with PPD. However, PCE/NCE ratios exhibited significant reductions related to time and dose. NOP neither induced MN-PCE nor changed PCE/NCE ratios. The positive results for COP and negative results for NOP and PPD correlate with carcinogenicity results rather than in vitro genotoxicity data. This illustrates the usefulness of micronucleus evaluation.

INDEX OF AUTHORS

(T=Talk, P=Poster)

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC.

P.O. BOX 12199 RESEARCH TRIANGLE PARK, NC 27709 (919) 544-1792

INTRODUCTION

EHRT is a small business with over 180 employees that specializes in short-term toxicity testing, both *in vitro* and *in vivo*. Key locations are in Research Triangle Park, North Carolina; Lexington, Kentucky; Birmingham, Alabama; and Cincinnati, Ohio.

NEW FACILITIES IN RTP

EHRT is presently building an office and laboratory complex which will eventually consist of five buildings. The location for this complex is across the street from the EPA on Alexander Drive and Highway 54, in Research Triangle Park, North Carolina. The first building is scheduled for completion in the Spring of 1991. It will be a three story building of black granite and glass and will contain 50,000 sq. ft. of floor space.

INFORMATION

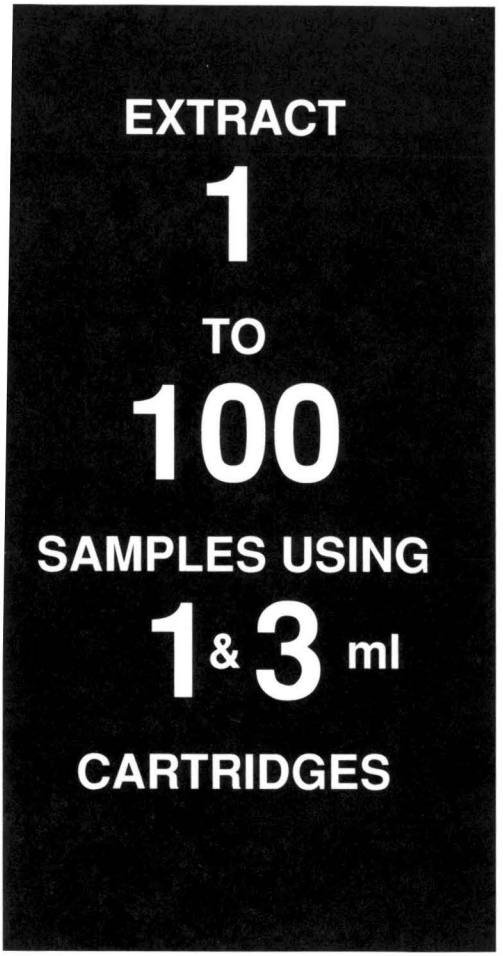
EHRT invites you to visit our booth at the GEMS meeting on October 25, 1990 for more information on our new RTP facilities. If you wish additional information on the capabilities of EHRT and how EHRT can assist your company, either see us at GEMS or contact Dr. Bruce Casto or Tom Hughes at:

Phone: (919) 544-1792
Fax: (919) 544-7986

Thank you.

OTHER LABORATORIES: 3235 Omni Drive, Cincinnati, Ohio 45245, Corporate Headquarters; 2514 Regency Rd., Lexington, KY 40503; 1075 South 13th St., Birmingham, AL 35205

OTHER OFFICES: Alexandria, VA.; Denver, CO; Philadelphia, PA



Zymark®

Attention HPLC Users!

Get better value from your HPLC.

The BenchMate™ Workstation can automatically extract up to 100 samples using standard 1 ml and 3 ml Solid Phase Extraction cartridges. The purified fractions can even be injected into an on-line HPLC.

For more information on automating your Solid Phase Extractions, call

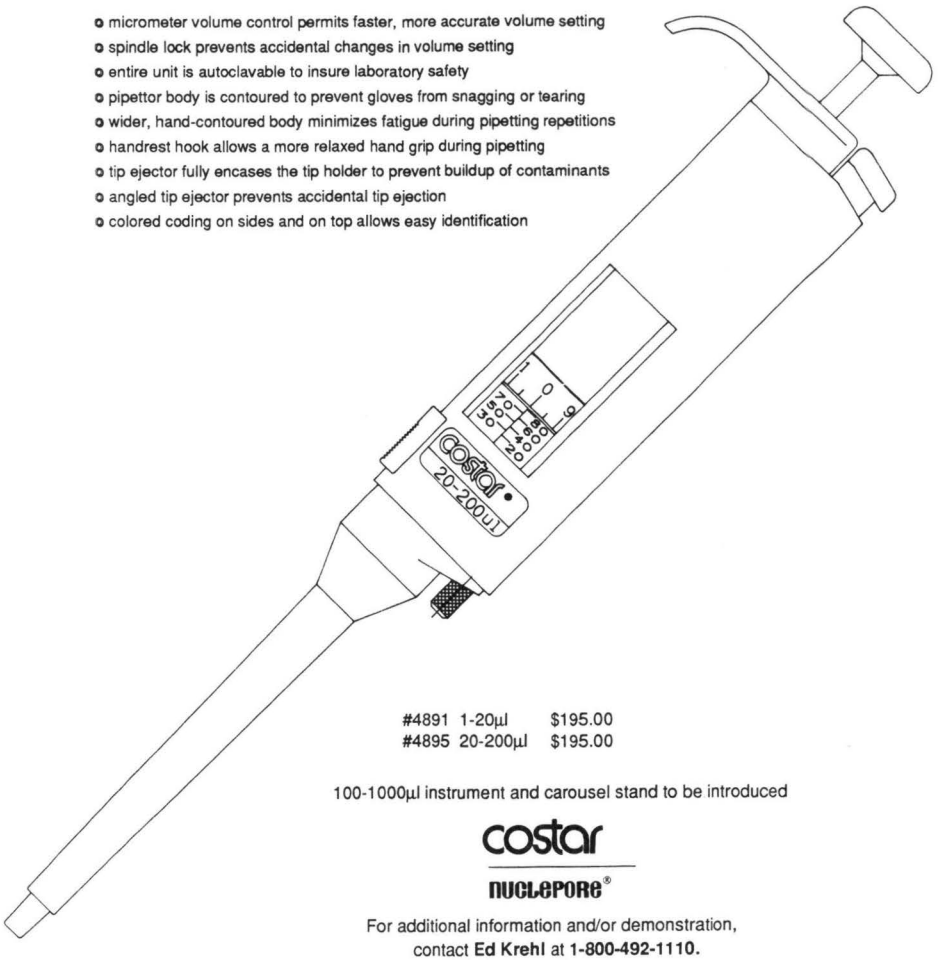
Russell Salisbury
Account Manager
(919) 467-0168
or

Zymark Corporation
Zymark Center
Hopkinton, MA 01748
(508) 435-9500
Fax: (508) 435-3439

Costar introduces the OnePette.
The pipettor your hand was designed for.

The Costar OnePette provides the accuracy and reproducibility your work requires and the comfort and convenience your hand deserves. It is the new standard to measure by!

- micrometer volume control permits faster, more accurate volume setting
- spindle lock prevents accidental changes in volume setting
- entire unit is autoclavable to insure laboratory safety
- pipettor body is contoured to prevent gloves from snagging or tearing
- wider, hand-contoured body minimizes fatigue during pipetting repetitions
- handrest hook allows a more relaxed hand grip during pipetting
- tip ejector fully encases the tip holder to prevent buildup of contaminants
- angled tip ejector prevents accidental tip ejection
- colored coding on sides and on top allows easy identification

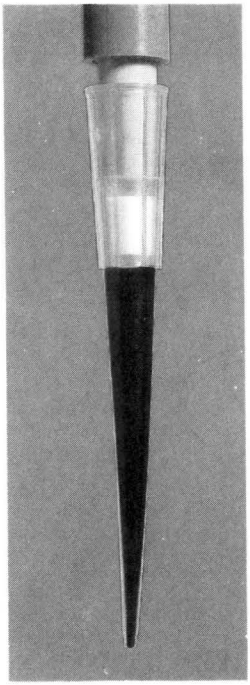


#4891 1-20µl $195.00
#4895 20-200µl $195.00

100-1000µl instrument and carousel stand to be introduced

costar
nucleporE®

For additional information and/or demonstration, contact **Ed Krehl** at **1-800-492-1110.**



USA/Scientific introduces:

AEROSEAL ADVANTAGE

FILTER PIPET TIP

Designed to fit most air displacement pipettors, AEROSEAL Tips greatly reduce the risk of sample carry-over and cross contamination. AEROSEAL Tips contain an inert filter that traps airborne aerosols and residues.

Now you can have the assurance of positive displacement pipettors with standard air displacement tip convenience. No labor intensive pistons and capillaries to assemble, AEROSEAL Tips are pre-sterilized and ready to use. AEROSEAL Tips are RNAse, DNAse, and endotoxin free.

For more information or for Free Samples, contact your local USA representative or call: 1-800-LAB-TIPS.

Known for Quality

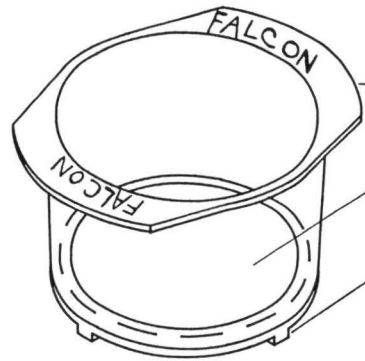
USA/SCIENTIFIC PLASTICS®

QUALITY • PRICE GUARANTEE SERVICE

P.O. BOX 3565, OCALA, FL 32678
FAX: 904/351-2057 TELEX: 5101002893
INT'L: 904/237-6288

FALCON ® CELL CULTURE INSERT

DIAGRAM OF 6-WELL INSERT DESIGN:



FEATURES:

Flanges
(Modified Hanging Design)

Cyclopore® Membrane
(Completely Transparent)

Insert Supports
(Standoff Feet That Do Not Rest On Bottom Of Well)

For More Information Contact:

CHRISTOPHER KENNEDY
Account Representative
P.O. Box 4635
Cary, NC 27519-4635
(919) 467-7074

BECTON DICKINSON

Becton Dickinson Labware
2 Bridgewater Lane
Lincoln Park, NJ 07035
(800) 235-5953
(201) 628-1144

Genetic Toxicology Testing Service

A complete genetic toxicology service for the detection of toxic, mutagenic, clastogenic, and carcinogenic activity in well-characterized test systems. An invaluable aid in product development and safety evaluations of new compound formulations and complex mixtures applicable to pharmaceuticals, medical devices, pesticides, cosmetics, industrial chemicals, petroleum products, personal-care products, and food additives.

Testing Services

- Gene Mutations in Bacteria
- Mobil Modified Ames Test
- Mutations in Mammalian Cells
- In Vitro Teratology
- DNA Damage and Repair
- Cytogenetics In Vitro/In Vivo
- In Vitro Cell Transformation

Research Services

- Mutagenicity Assessment in Transgenic Mice
- In Vitro Alternatives to Animal Testing

Additional Laboratory Services

- Acute, Subchronic, Chronic Toxicity
- Nose-only Inhalation
- Teratology/Reproduction
- Microbial Pesticide Pathogenicity/Infectivity
- Laboratory Animal Diagnostics
- Biotechnology Product Safety Evaluation
- Mycoplasma Testing
- S-9 Microsome Preparations

MICROBIOLOGICAL ASSOCIATES INC.

9900 Blackwell Road, Rockville, Maryland 20850
(301) 738-1000 • Telex: 90-8793 • FAX: 301-738-1036

NOTES

NOTES

ABB Environmental
4765 Calle Quieto
Camarillo, CA 93012
805/388-5700

5000 Executive Parkway
Suite 500
San Ramon, CA 94583
415-275-4465

This program was prepared by Marion Zeiger, Associates, Editorial Services, 1504 Lamont Court, Chapel Hill, NC 27514 (919-929-8875). Abstracts were photo offset from originals submitted by the authors. This program was printed by Colonial Press, Inc. 503 W. Franklin Street, Chapel Hill, NC 27514 (919-929-1121).