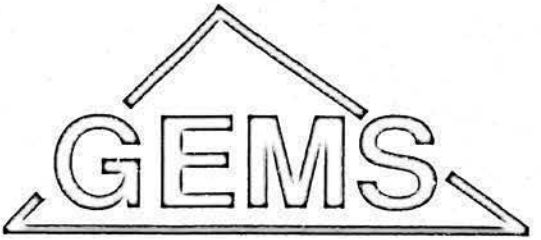
GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY



THIRTEENTH ANNUAL MEETING PROGRAM AND ABSTRACTS

FRIDAY, OCTOBER 20, 1995

SHERATON IMPERIAL HOTEL
Research Triangle Park,
North Carolina

GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY (GEMS)

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Genotoxicity and Environmental Mutagen Society

Dear GEMS Members,

It has been a pleasure to have served as President-Elect and President of GEMS over the last two years. I would like to take this opportunity to thank the Officers and the Board of Councilors for their considerable efforts on behalf of GEMS and to reflect briefly on the role and recent progress of the Society.

GEMS is a regional society formed in 1982 "for junior and senior scientists who study genotoxic effects of environmental agents in various cellular and animal systems, and apply their results to assess the health effects on man." These words from our Charter are the driving force for the planning of the annual Spring and Fall meetings and the other activities pursued by GEMS. The annual meetings are the main functions and principal means of communication and education of Society members. Over the past two years the annual meeting programs have addressed: *Mutational Processes and Human Disease* (Spring 1994), *New Topics in Genetic Toxicology* (Fall 1994) and *The Status and Future of Genetic Toxicology* (Spring 1995). The 13th Annual Fall Meeting program on *Genotoxicity Potpourri* covers perhaps the broadest range of subjects of any GEMS meeting to date: from mismatch repair to the Haw River Syndrome, apoptosis, and databases - there is truly something for everyone and this is the way it should be. If you have ideas about future topics for our annual meetings, do not hesitate to inform a GEMS Board member and/or the President-Elect who is charged with planning the programs.

Aside from the annual meetings, during the past year the Board has made a special project of co-sponsoring two Quantitative PCR Workshops together with the Perkin-Elmer Corporation, represented locally by Laura Cunningham. GEMS was represented by Board member Mike Cunningham, who chaired the Workshops. These workshops were well attended and seemed to meet a significant technical need of GEMS members and other regional scientists. The Board would be particularly interested in your views about these and possible future workshops.

Another activity initiated this year was the GEMS Outreach Committee chaired by Jack Bishop, with members Amal Abu-Shakra and Maria Donner. A lecture series is being developed wherein interested GEMS members would travel to local colleges and universities to present their research and to introduce GEMS to students and faculty. Please let one of the Outreach Committee members know if you would like to be involved in this important activity.

The Officers and the Board have worked long and well on behalf of GEMS and its membership. The very capable Officers were: President-Elect (Mike Shelby), Secretary (Diane Abernethy) and Treasurer (Diane Spencer). The continuing (1996/97) Councilors are: Mike Cunningham, Patty Poorman-Allen, Ray Tice, Jack Bishop (who ran the elections this year), Neal Cariello (who ably restructured the GEMS membership list), and Barbara Collins. The departing (1992-1995) Councilors are: Les Recio, Garret Nelson, and Rose Anne McGee. Garret and Rose Anne did great jobs with the GEMS Newsletter and Corporate Sponsors, respectively.

GEMS is extremely fortunate to have such a large group of Sustaining Members and Corporate Sponsors. They literally represent the financial backbone of the Society, since the dues collected from the membership are not adequate to support all of the programs of the Society. Please make a special effort to visit the booths of Corporate Sponsors present at the fall meeting and thank them for their support of GEMS.

In closing, I would like to acknowledge once again the outstanding personal commitment of those Officers and Board members who have served the Society this year. And thank you, the members, for the opportunity to represent you.

Mike

Michael D. Waters
President, GEMS

CHARTER SUSTAINING CORPORATIONS AND MEMBERS

ACC SALES/DIVISION OF LABREPCO, INC., 2450 Honeysuckle Road, Chapel Hill, NC 27514. Representatives: Allen L. Miller (919-967-4224) and Dan Morris (919-942-1998). *ACC Sales represents manufacturers of biotechnology equipment specializing in the areas of laminar flow, incubation, refrigeration, centrifugation, and photodocumentation.*

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MANTECH ENVIRONMENTAL TECHNOLOGY, INC. 5 Triangle Drive, Research Triangle Park, NC 27709. Representatives: Sheela Sharma (919-549-0611, x302) and Lashawn Poinsette (919-549-0611). *Develops and validates short- and long-term bioassays to identify mutagens, carcinogens, tumor promoters, reproductive toxins, neurotoxins, and chemopreventive agents. Also capable of developing biomarkers for toxic exposure to environmental agents, precancerous and cancer lesions, and tumor promoters.*

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BARTLETT ENTERPRISES, P.O. Box 16832, Chapel Hill, NC 27705. Representatives: Stephen Bartlett, 5310 Cedronella Rd., Chapel Hill, NC 27514 (919-383-2406) and Ann Williams, 239 Flemington Rd., Chapel Hill, NC 27514 (919-967-8111). *As an independent representation service for manufacturers of products and services to the biomedical research community, Bartlett provides tissue culture, prepared electrophoresis reagents, apparati, biochemicals, a broad range of lab disposables, interleukins, MABs, etc.*

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LOATS ASSOCIATES, INC., 2 North Court Street, Westminster, MD 21157. Representatives: Theresa Rippeon (410-876-8055) and Andrew Loats (410-876-8055). *Provides biomedical image analysis systems and systems integration services, including automated genetic toxicity assay scoring, automated microscopy, quantitative autoradiography (receptor, metabolic, whole-body), and nuclear medicine/3D brain imaging. Automated Genetic Toxicity Assay systems include Micronucleus Assay, Metaphase Finder, Comet and Mouse Lymphoma (colony counting). Consulting services are available for customization to individual laboratory needs.*

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NALGE COMPANY, 3621 S. Pointe Dr., Apex, NC 27502. Representatives: Steve Podolski (919–380-9007) and Robert Moore (919-380-9007). *Nalge is a leader in manufacturing disposable and reusable plastic labware and related products.*

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OXFORD LABWARE, 3000 Glen Burnie Drive, Raleigh, NC 27607. Representative: Bill Bradley (919-781-8524). *Oxford Labware, a division of Sherwood Medical, is committed to growth and to excellence in the liquid handling and in the laboratory products market. Oxford offers its own line of pipettes and dispensers.*

PGC SCIENTIFICS INC., P.O. Box 7277, Gaithersburg, MD 20898-7277. Representatives: Lora Jones (800-424-3300) and Paul Kaufman (800-424-3300). *PGC Scientifics is a leader in manufacturing and distributing products for science and industry. PGC: more than just plastics! Summary of major products and services include: molecular biology products, transgenic mouse media, tissue culture serum/serum-free media, and lab disposables.*

PERKIN ELMER, APPLIED BIOSYSTEMS DIVISION, 3729 Cambridge Road, Durham, NC 27705. Representatives: Laura Cunningham (800-248-0281, x7285) and Peter Bent (800-248-0281). *Perkin Elmer is a leading supplier of PCR thermal cyclers, DNA synthesizers and sequencers, protein sequencers, and peptide synthesizers.*

PROMEGA CORPORATION, 2800 Woods Hollow Road, Madison, WI 53711-5999. Representative: Julie Ginsler (919-782-2283). *Promega will display enzymes, vectors, and systems for gene analysis, translation, cDNA synthesis, immunopurification, genomic cloning and mapping, protein analysis, and cellular regulation.*

RJR-NABISCO, INC., Toxicology Research Division, Building 630/2, Winston-Salem, NC 27102. Representatives: David J. Doolittle (919-741-5762) and Chin K. Lee (919-741-4346). *Provides consumer products.*

SITEK RESEARCH LABS., 15235 Shady Grove Road, Suite 303, Rockville, MD 20850 Representatives: Arul Thilagar (301-926-4900) and Paul E. Kirby (301-926-4900). *SITEK Research Laboratories is a fully GLP compliant, AAALAC accredited contract laboratory. Testing services offered include genetic toxicology, general toxicology, analytical chemistry, metabolism, and pharmacokinetics. Please come see us in the exhibit area to obtain more detailed information regarding our testing services.*

SPECTRUM CHEMICAL MANUFACTURING CORP., 755 Jersey Avenue, New Brunswick, NJ 08901. Representatives: Mona Joyner (800-395-6723) and Randy Burg (310-516-8000). *Spectrum Manufacturing offers a wide range of laboratory chemicals for QC, clinical analysis, chemistry, R&D, manufacturing biotechnology, environmental and molecular biology. Our safety product line has been designed with comfort, value, and compliance in mind. Spectrum is an employee-owned company which has proven its commitment to quality with its ISO9002 certification.*

STRATAGENE, 9420 Towne Square Avenue, Suite 4, Cincinnati, OH 45242. Representatives: Jay Short (800-424-5444) and Scott Provost (800-424-5444 x5411). *Stratagene has developed the first transgenic mouse and complete system for* in vivo *mutagenesis testing. This allows investigators to detect tissue-specific (including germ cells) mutations using a short-term assay (1–2 weeks).*

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GEMS and the Board of Directors gratefully acknowledges and appreciates your continued support! You the corporate members are the backbone of our Society and you provide the Society not only with necessary financial support but with vital technical information and equipment. We the members reiterate our thanks to the corporate sponsors for their persistent efforts on behalf of the Society.

Thank you! Thank you!

GEMS
THIRTEENTH ANNUAL FALL MEETING
Friday, October 20, 1995

Program

8:00 - 8:45	**Registration and Coffee**
8:45-9:00	**Opening of the Meeting:** Dr. Michael D. Waters, EPA, President, and Dr. Michael D. Shelby, NIEHS, President-Elect
9:00 - 9:45	**DNA Mismatch Repair, Genome Instability and Cancer** Dr. Thomas A. Kunkel, NIEHS
9:45-10:30	**Haw River Syndrome, A Dominant Neurodegenerative Disease Caused by a Triplet Repeat Expansion.** Dr. James R. Burke, Duke University
10:30-12:00	**Exhibits, Posters, and Coffee**
12:00-1:00	**Lunch and Business Meeting**
1:00-1:45	**What Role Does Apoptosis Play in Tumor Development?** Dr. Gloria Preston, NIEHS
1:45-2:30	**Databases and Software for the Analysis of Mutations** Dr. Neal Cariello, Glaxo Wellcome, Inc.
2:30-3:00	**Exhibits, Posters, and Coffee**
3:00-4:30	**Presented Papers**
4:30-5:30	**Exhibits, Posters, and Reception**

ABSTRACTS
TALKS

T1 **EFFECTS OF P-CRESIDINE ON TSG-p53‰: HEMIZYGOUS P53 TUMOR SUPPRESSOR GENE KNOCKOUT MICE.** G. Lacks[1,2], J.E. French[2], and R.W. Tennant[2], [1]Integrated Laboratory Systems and [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC 27713

p53 mutations have been found in bladder cancer and single strand conformational polymorphism (SSCP) assays have been used to detect mutations in p53 among a wide range of tumor types. Mice deficient for p53 have been developed by utilizing homologous recombination methodology and introducing a neo cassette into exon 5, resulting in a truncated nonfunctional message and thereby creating a null allele (Donehower et al. 1992). Hemizygous $p53^{def}$ mice have a low frequency of spontaneously arising tumors until approximately one year of age, which allows for short-term carcinogenesis studies to be performed. Because of the hemizygous nature of the TSG-p53‰ mice, the remaining wild-type p53 allele has been used to screen for mutations using SSCP. P-Cresidine is genotoxic and a potent rodent bladder carcinogen. Previous studies have shown that bladder masses were detected with a greater frequency in p-cresidine treated TSG-p53‰ mice than the C57B/6 wild-type males and females (parent strain). Forty-one male hemizygous TSG-p53‰ mice were given either untreated (10 mice) or 0.50% p-cresidine treated feed (31 mice) for 6 months. Bladders, livers, and liver masses were collected and DNA extracted. PCR amplification of exon 5, which excludes the null allele, showed a loss of p53 wild-type exon 5 in 12/12 of the urinary bladders and 1/3 of the liver masses. Subsequent cold SSCP assay of those products showed mutations in 2/3 of the liver masses. Southern analysis data will be presented which will define the loss of the remaining wild-type allele. This and future work could have broad ramifications for the creation of a bladder tumorigenesis model, additional information as to the effects of the bladder carcinogen p-cresidine, and alterations in the tumor suppressor gene p53.

T2 **MUTAGENIC ACTIVITY OF RALEIGH AIR SAMPLES AT THREE DIFFERENT ELEVATIONS BEFORE, DURING, AND AFTER HURRICANE GORDON,** Fred Stratton[1], Larry Claxton[2], Viney Aneja[1], Robert C. Smart[1], and Thomas Hughes[2], [1]NCSU, Raleigh, NC 27709 and [2]EPA, RTP, NC 27711.

Diurnal ozone and volatile organic compounds (VOCs) were collected and measured at the 610 meter WRAL television tower 10 km southeast of downtown Raleigh at three different levels: Surface, Mid (240 m), and Top (460 m) in July and August of 1993 and 1994. These measurements revealed very high ozone concentrations at Mid and Top. Of the VOCs measured only arene concentrations were significantly elevated at Mid and Top and were correlated with wind direction from Raleigh. Arenes are chemically much more stable than other VOCs measured and ozone profiles varied and the combined presence of ozone, arenes and nitrooxide (NO_X) at Mid and Top suggested possible nitration of arenes. Consequently to collect nitroarenes, air samples were collected with XAD-filled canisters using high volume air samplers at the same three levels on the TV tower 3 days before, during, and after hurricane Gordon. Collected air samples were Soxhlet extracted and analyzed with *S. typhimarium* YG1021 and YG1026 (+/-S9). Due to sample size limitations of the collected air extracts, the Kado microsuspension assay (10 fold concentrate of bacteria, 5 µl of sample, 100 µl of S9/Buffer, 90 min preincubation at 37°C) was used. Mutagenicity in post-Gordon stagnant air mass samples with YG1026 was observed and the slopes (revertants/µg) were: Top (5.3) and Mid (3.3) with S9, and Top (1.7) and Mid (3.9) without S9 due to anthropogenic emissions of VOC and NO_X. The Surface level, Blank, and pre- and during Gordon samples were not mutagenic. These preliminary data suggest the possibility of long-term transport of mutagenic nitroarenes. *(This abstract does not necessarily reflect EPA policy)*

T3 INCREASED RISK FOR MYELODYSPLASTIC SYNDROMES AMONG THOSE WITH GST THETA 1 (GSTTI) GENE DEFECT, H. W. Chen[1], D. P. Sandler[1], J. A. Taylor[1], D. L. Shore[2], E. Liu[3], C. D. Bloomfield[4], and D. A. Bell[1]. [1]NIEHS, RTP, NC 27709; [2]Westat, RTP, NC; [3]UNC, Chapel Hill, NC; [4]Roswell Park, NY.

The myelodysplastic syndromes (MDS) are proliferative disorders of the bone marrow that are life threatening and often (20-40%) progress to acute myeloid leukemia (AML). Environmental and occupational exposures may increase risk of MDS. The glutathione transferases (GST) are a family of enzymes that can mediate exposure to a wide variety of cytotoxic and genotoxic agents, including those associated with increased risk of MDS. In humans, both GST Theta I (GSTTI) and GST M1 *(GSTMI)* genes have a "null" variant allele; in which the entire gene is absent. We tested to see if the homozygous null genotypes of *GSTTI* and *GSTMI* could alter risk for MDS. We have analyzed 92 MDS cases and 199 controls of similar age, race and sex for *GSTTI* and *GSTMI* null genotypes and find the frequency of the *GSTTI* null genotype to be overrepresented among MDS cases (46%) relative to controls (16%). Inheriting the *GSTTI* null genotype confers an estimated 4.3-fold risk of MDS (OR=4.3, 95% CI 2.5-7.6, $p < 0.0001$). *GSTMI* null genotype was not associated with increased risk of MDS. This suggests that the *GSTT1* null genotype enhances susceptibility to MDS, perhaps through decreased detoxification of environmental or endogenous carcinogens.

ABSTRACTS
POSTERS

(* indicates competing posters)

P1 * **DETECTION OF DNA DAMAGE IN AQUATIC SPECIES USING THE ALKALINE SINGLE CELL GEL (SCG) ASSAY,** V.K. Perry and R.R. Tice, Integrated Laboratory Systems, Research Triangle Park, NC 27713

For the biomonitoring of DNA damage in aquatic species exposed to genotoxic pollutants, an optimal method should be capable of detecting a broad spectrum of DNA damage in a variety of cell types, should be adaptable to multiple species in the laboratory and the field, and should be sensitive, rapid, and cost effective. The alkaline SCG assay appears to fulfill these criteria. This assay detects, in individual cells, DNA single strand breaks, alkali-labile sites, incomplete excision repair sites, and crosslinking. Other advantages include the small numbers of cells required (i.e., only a few thousand cells), making it suitable for most aquatic species, and its speed, sensitivity, and simplicity. Here, we report on a study to evaluate the suitability of this assay for aquatic biomonitoring by assessing for DNA damage in tissues of three aquatic species exposed in the laboratory to the genotoxic agent ethyl methanesulfonate (EMS). The tissues/species investigated included blood erythrocytes, gill, liver, and brain of the freshwater fish, medaka *(Oryzias latipes);* blood erythrocytes and liver of tadpoles of the bullfrog, *Rana catesbeina*; and gill, mantle, and siphon of the freshwater mussel, *Unio complanatus*. Conditions for collecting these tissues and conducting the SCG assay were evaluated and optimized using 60 min of alkali unwinding time and 5 min of electrophoresis (25 volts, 300 mAmps) at pH>13. Tissue samples from animals (4 per dose group) housed for 24 hours in water containing EMS at three dose levels (250, 500, and 1000 mM) were collected and evaluated for DNA damage (25 cells per sample). At doses not associated with increased levels of cell toxicity, EMS induced DNA migration in all 4 tissues of medaka, in gill and siphon of mussels, and in liver of tadpoles. While more research is needed to characterize the sensitivity of the different species to different classes of environmental pollutants, the results support the conclusion that the SCG assay can be used to monitor for genotoxic pollutants in aquatic environments using a variety of aquatic species. *(Research supported by NIEHS through SBIR grant number 1-R43-ES07310-01).*

P2 * **THE POTENTIAL ROLE OF INCREASED *MYC* EXPRESSION IN THE INDUCTION OF LIVER CANCER IN F344 RATS BY NONGENOTOXIC-CYTOTOXIC CARCINOGENS,** C.S. Sprankle, M.V. Templin, J.L. Larson, and B.E. Butterworth, Chemical Industry Institute of Toxicology, Research Triangle Park, NC

Chloroform is formed as a by-product of water chlorination and paper bleaching and is present in the air above indoor swimming pools, in shower stalls, and near paper mills. High doses of chloroform induced liver cancer in male and female B6C3F1 mice when adminstered by gavage, kidney cancer in male, but not female, Osborne-Mendel rats when given by gavage or in the drinking water, and kidney cancer in male BDF1 mice but not female BDF1 mice or either sex of F-344 rat when administered by inhalation. Chloroform is not genotoxic; the close correlation between cancer induction and regenerative cell proliferation indicate that carcinogenic activity is secondary to events associated with cytolethality. These effects may alter the expression of oncogenes and other growth control genes and may play a role in tumor formation. Furan is a nongenotoxic-cytotoxic hepatocarcinogen in male F-344 rats and male and female B6C3F1 mice. Like chloroform, furan induces cell proliferation secondary to toxicity. We previously demonstrated that six weeks of daily gavage treatment with furan resulted in cytolethality, cell proliferation and large increases in levels of the *myc* oncogene mRNA in male rat liver. In this study, we examined levels of *myc* mRNA in livers of male and female F-344 rats treated by inhalation with 300 ppm chloroform for 90 days and in livers of male and female B6C3F1 mice treated with 90 ppm chloroform for 90 days to determine if the sustained cytotoxicity would be accompanied by an increase in *myc* expression similar to that produced by furan. Although the amount of toxicity and cell proliferation in the livers of the chloroform-treated animals equaled or exceeded the amount seen in the livers of the furan-treated animals, no increase in *myc* mRNA levels over control was exhibited in male or female rats or mice treated with chloroform. The lack of increase in hepatic *myc* mRNA levels during treatment with either furan or chloroform in B6C3F1 mice suggests that sustained expression of this gene is not involved in the hepatocarcinogenicity of cytotoxic carcinogens in this species. However, the increase in *myc* mRNA levels seen in male F-344 rat liver during furan treatment (which leads to cancer), but not during chloroform treatment (which does not), suggests that sustained increases in the expression of this gene may play a role in the development of liver tumors during chronic treatment with cytotoxic agents in rats.

P3 * DETERMINATION OF THE MUTAGENICITY AND MUTATIONAL SPECTRUM IN HUMAN TK6 CELLS, OF 1,2,3,4-DIEPOXYBUTANE, A GENOTOXIC METABOLITE OF 1,3-BUTADIENE, A-M. Steen and L. Recio, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

We are using human TK6 cells to assess the *in vitro* mutagenicity and mutational spectrum at *hprt* of 1,2,3,4-diepoxybutane (DEB), a genotoxic metabolite of 1,3-butadiene (BD). DEB is a bifunctional alkylating agent that induced deletions in 50% of the TK6 cell *hprt* mutants analyzed *(Carcinogenesis 15,* 713-717, 1994). We have extended these studies by DNA sequence analysis of *hprt* mutants isolated following DEB exposure to determine the mutational spectrum at *hprt*. TK6 cells were exposed for 24 hr to 0, 2, 4, 6, 8, 10 mM DEB, and cytotoxicity was assessed by cloning efficiency and growth curve extrapolation. Both methods to assess cytotoxicity indicated that 4 mM DEB x 24 hr results in approximately 10% relative survival. A mutant frequency (MF) experiment was setup in parallel to collect *hprt* mutants for molecular analysis using a DEB exposure of 4 mM x 24 hr. Cloning efficiencies (CE) with and without 6-TG were calculated, assuming a Poisson distribution, to determine the *hprt* mutant frequency. A DEB exposure of 4 mM resulted in an average 5-fold increase in the MF. Six flasks (4 x 105 cells/ml in 50 ml) were exposed to a treatment of 4 mM DEB x 24 hr to collect *hprt* mutants for molecular analysis by RT-PCR of *hprt* mRNA or PCR amplification of *hprt* genomic DNA following by DNA sequencing of PCR products. After 24 hr exposure, the cells were put in fresh medium and each 50-ml flask was divided into ten 10 ml, 25cm^2 flasks at 1 x 10^5 cells/ml for a total of 60 independent flasks. The DNA sequence in *hprt* mutant DNA and RNA demonstrated 24/55 deletions (44%), 17/55 point mutations (31%), (8 at G:C and 9 at A:T base pairs), 3 insertions and one Ibp deletion. Of 55 mutants, 14 (25%) resulted in splice defects, with possible point mutations at the genomic level, 7 of those need to be further characterized. These data confirm previous studies of the mutational spectrum of DEB and also demonstrate the induction of both point mutations and deletions by DEB. Both deletions and point mutation in cellular oncogenes and tumor supressor genes have been observed in BD-induced tumors.

P4 * GENOTOXICITY EVALUATIONS OF DRINKING WATER CHLORINE DISINFECTION BYPRODUCTS, A. Afshari, J. Fuscoe, M. George[1], A. DeAngelo[1], B. Collins, R. Tice[2], and J. Allen, Mutagenesis and Cellular Toxicology Branch, [1]Carcinogenesis and Metabolism Branch, Environmental Carcinogenesis Division, NHEERL, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711; [2]Integrated Laboratory Systems, RTP, NC 27709

The Disinfection By-Products Research Team within NHEERL is investigating the potential carcinogenicity of contaminants in drinking water produced by chlorine disinfection. Major disinfection by-products from the chlorination of drinking water include dichloroacetic acid (DCA) and potassium bromate ($KBrO_3$). These chemicals are known to be carcinogenic in rodents, yet little genotoxicity data are available to assess the possible role of chromosome damage in this process. We have used the micronucleus assay to investigate the genotoxic effects of DCA and $KBrO_3$. This assay can detect chromosome breakage and/or malsegregation caused by exposure to the test agents. Mice were exposed to these compounds in drinking water, available *ad libitum* for up to 78 weeks. Micronucleus induction in blood erythrocytes was evaluated following staining with propidium iodide and an anti-kinetochore antibody to determine centromere status. Our results show that at the highest dose of DCA tested, a small but significant increase in the frequency of micronucleated cells was detected in the normochromatic erythrocyte (NCE) population following exposure for longer than 10 weeks. In addition, a small but statistically significant dose dependent increase in the frequency of micronucleated polychromatic erythrocytes (PCEs) was observed after a 9 day chronic exposure. $KBrO_3$-exposed mice showed a doubling in the frequency of micronucleated cells as compared with the negative control group. Furthermore, our data suggest that both DCA and $KBrO_3$ function as clastogens since the overwhelming majority of the micronuclei observed did not stain with the anti-kinetochore antibody, and thus presumably lack centromeres. This suggests that the micronuclei induced by these chemicals represent chromosome fragments. *(This is an abstract of a proposed presentation and does not necessarily represent EPA policy).*

P5 COMPARATIVE MUTAGENICITY OF A STANDARD REFERENCE MATERIAL IN THE SALMONELLA ASSAY, Thomas Hughes, Larry Claxton and Joellen Lewtas, EPA, MD68, RTP, NC 27711.

The purpose of this research was to determine accurate and reproducible slope values (S) in revertants/uG for a diesel extract in the Salmonella mutagenicity assay. The diesel extract was a standard reference material (SRM 1975) that was Soxhlet extracted with dichloromethane from 500 lbs of particulate material obtained from filters in operating forklifts. The SRM 1975 will be available from the NIST in 1996, along with the mutagenicity data generated here and a comprehensive analytical chemistry analysis. Mutagenicity parameters were: TA98, TA100, TA98NR, TAlOONR (nitroreductase (NR) gene deficient), YG1021, YG1026 (NR gene addition), YG1024, YG1029 (acetyltransferase (AT) gene addition), and YG1041 and YG1042 (contain both NR and AT genes); 10 dose levels in the linear portion of the dose response curve; duplicate plates per dose; S9 at 1.1 mg of protein/plate; plate incorporation assay for all strains and Kado microsuspension assay for TA98, TA100, YG1021, YG1026. Results suggested: (1) mutagenic activity without S9 correlated with the presence or absence of the NR or AT genes; slope values (revertants/uG) were TA98NR(128), TA98(445), YG1021(605), YG1024(2096), YG1041(11,443); (2) the Kado microsuspension assay was 2-fold more sensitive than the plate incorporation assay (e.g., YG1021, -S9, S = 605 vs. 1317); (3) the addition of S9 significantly reduced the mutagenic activity of SRM 1975 (e.g., YG1024, plate, S = 2096 vs. 268); (4) the addition of the NR and AT genes increased the mutagenic activity of the SRM 1975 89-fold from TA98NR(S=128) to YG1041(S=11,443); (5) the TA98 series listed above was 4-20 fold more sensitive than the TAl00 series in measuring the mutagenic activity of the diesel extract; (6) YGl041 was sensitive to nitroarenes at the 10 nanogram level. *(This abstract does not necessarily reflect EPA policy)*

P6 FIBER INDUCED DNA DAMAGE AS AN INDICATOR OF CARCINOGENICITY, P.W. Andrews[1], B. Libbus[1,2], M. Vasquez[1], and R.R. Tice[1], [1]Integrated Laboratory Systems, Research Triangle Park, NC 27709 and [2]Genetic Research, Inc., Chapel Hill, NC 27516

The carcinogenicity of asbestiform fibers constitutes a major health concern. Furthermore, the use of man-made fibers is increasing and the potential risk of such fibers pose needs to be evaluated. A major need is to develop a sensitive *in vitro* assay for determining the relative genotoxicity of fibers. The alkaline Single Cell Gel (SCG) assay was used to detect DNA damage (DNA single strand breaks, alkali-labile sites) induced in cultured rat mesothelial cells (SFM-1) exposed for 2 hours at 37°C to two naturally occurring fibers (crocidollte, amosite), a glass wool (kawool), two man-made vitreous fibers (MMVF-10, MMVE-22), or four refractory ceramic fibers (RCF 1,2,3,4). Fibers were prepared according to UICC procedures, weighed, washed in acetone overnight, and then resuspended in PBS and elutriated using a 21 gauge needle. Fibers were incubated overnight in culture medium containing 10% serum and then added, at the appropriate concentration, to cultured cells. Four to six concentrations ranging from 0.0001 to 10.0 mg/cm^2, at full-log steps, were used with the highest dose such that cell viability was not reduced below 80% of control levels. After treatment, cells from duplicate cultures were collected by scraping and processed for SCG analysis using 20 min of unwinding and electrophoresis at pH>13. Twenty-five cells were scored per sample for the extent of DNA migration. Kawool, amosite, and crocidolite induced a significant, dose-dependent increase in DNA migration beginning at 0.01, 0.1, and 0.01 mg/cm^2, respectively. The dispersion (variance/mean) of DNA migration was greatly increased among cells treated with amosite, indicating that the distribution of damage was very heterogeneous. The ceramic fibers, RCF 1, 2, 3, and 4 induced a significant, dose-dependent increase in DNA migration beginning at 0.01 mg/cm^2 and plateauing at all subsequent doses. However, the significantly increased dispersion of DNA migration at 0.001 mg/cm^2 indicated the presence of significant damage even at that dose range. In contrast, MMVF-10 and MMVF-22 were largely ineffective in inducing DNA migration in SFM-1 cells over the same dose range. These results demonstrate the utility of the SCG assay for evaluating the ability of fibers to induce DNA damage *in vitro*. *(Research supported by NCI through SBIR Grant No. 1-R43-CA65273-01)*

P7 * **GENETIC INSTABILITY AND RISK ASSESSMENT: A STUDY BY THE SCGE TECHNIQUE,** A. Gurugunta, M. Jaiswal, N. Rajeswari, and Y.R. Ahuja, Mahavir Hospital, 10-1-1, Hyderabad-500004, India.

Precancerous lesions of cervix which are commonly designated as dysplasia present a complex problem because of their biological behavior. Increased genetic instability, either inherent or induced by some external mutagen has been considered as a primary event or a predisposing factor to neoplastic transformation. In an effort to evaluate a possible correlation between genetic instability and susceptibility towards cervical cancer, the single cell gel electrophoresis (SCGE) was performed.

Among precancerous and cancerous individuals, genomic instability was observed in cervical epithelial cells as well as in peripheral blood leucocytes, which is not the target tissue. A stepwise increase in the mean basal DNA damage with inter and intra individual variability was seen from controls to patients with carcinoma of the cervix via precancerous individuals. Susceptibility towards mutagens (crystal violet and MNNG) increased and repair capacity decreased with the progression of the disease. Also, risk assessment was done by giving predictive value for each precancerous individual. Risk prediction with a single parameter is not enough. In combination with morphological, biochemical, and cytogenetic parameters, the SCGE assay may serve as a novel tool to predict the fate of cervical dysplasia. Detailed follow-up studies are required to confirm these contentions.

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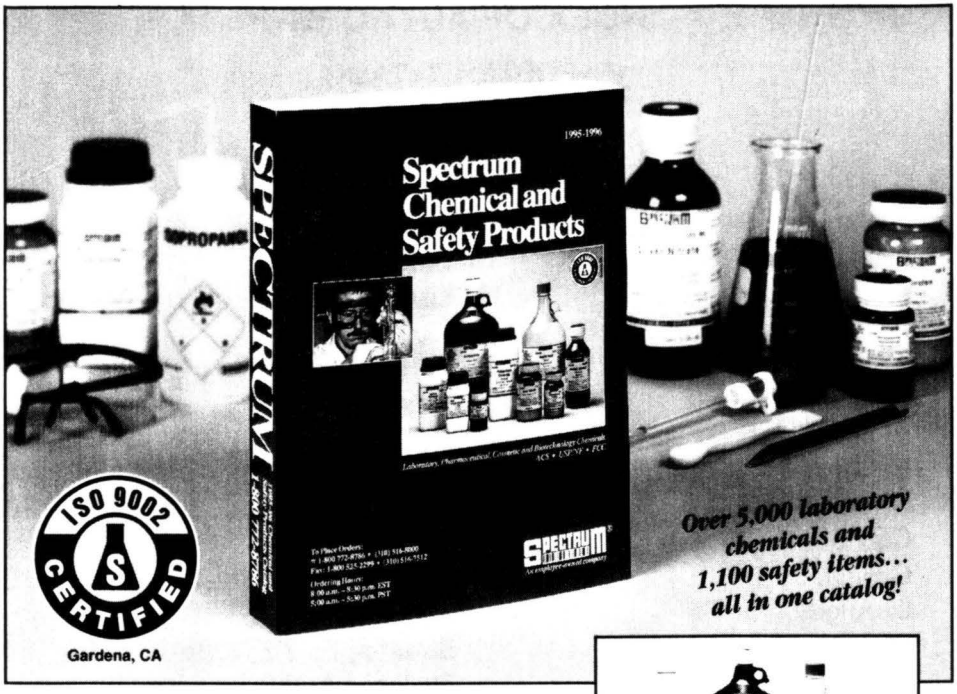
(P=POSTER, T=TALK)



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