GENOTOXICITY AND ENVIRONMENTAL MUTAGEN SOCIETY

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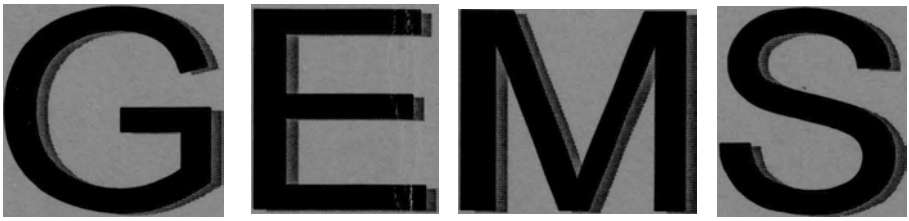

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SEVENTEENTH ANNUAL MEETING
PROGRAM AND ABSTRACTS

MONDAY, OCTOBER 25, 1999

SHERATON IMPERIAL HOTEL
Research Triangle Park, NC

GEMS

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GEMS FALL MEETING
MONDAY, OCTOBER 25, 1999
Sheraton Imperial Hotel
Page Road and I-40, RTP, NC

"CURRENT TRENDS IN GENETIC TOXICOLOGY"

8:00-8:45	Registration and Continental Breakfast
8:45-9:00	Welcome, Jack Bishop, GEMS President Introduction, Thomas Hughes, GEMS President-Elect
9:00-9:45	Errol Zeiger, OECD/NIEHS, **"Mutagenicity Tests: Promises vs. Performance"**
9:45-10:30	Talks (3) Competing for Travel Award
10:30-12:00	Exhibits, Posters, Coffee
12:00-1:00	Italian Buffet Luncheon
1:00-1:30	Charles Moreland, Vice Chancellor of Research at North Carolina State University, **"NCSU: Research at the New Centennial Campus"**
1:30-2:00	Annual Business Meeting
2:00-2:45	Talks (3) Competing for Travel Award
2:45-3:00	Break
3:00-3:45	David DeMarini, EPA, RTP, **"Mutation Spectra: Implications for Genetic Toxicology"**
3:45-4:30	Paul White, EPA, Narragansett, **"A Mass Balance of Genotoxicity in the Providence River (Rhode Island): Where Is It Coming From and What Does It Mean?"**
4:30-4:40	Award Presentations for Best Talks and Posters
4:40-5:30	Reception - Finger Foods and Cash Bar

GEMS
Genotoxicity and Environmental Mutagen Society

P.O. Box 13475, Research Triangle Park, North Carolina 27709

GEMS Members

Thank you for electing me to serve as your President/President-Elect the past two years, and as a Councilor for three years prior to that. During this time I've had the good fortune to work with and follow in the footsteps of four great GEMS Presidents (Mike Waters, Mike Shelby, Byron Butterworth and Beth George). Beth continues to provide "over-and-beyond" leadership and operational support for our annual meetings, including the present one.

Serving as Newsletter Co-Editor with your next President, Tom Hughes, from '95-'97 provided a good introduction to most aspects of the science and scientists of GEMS from Abstracts to Workshops, not to mention a lesson on the history of GEMS. The members of the Boards of Directors I've served with have been talented and dedicated individuals with lots of exciting ideas and energy. They have made my tenure of service most enjoyable and whatever accomplishments we've had these past two years I credit to them.

Marc Jackson provided a new look to our Newsletter and has passed responsibility for that effort into the capable hands of Leon King. Jeff Ross has done a very admirable job of getting our membership list current and up to date; a new list will be distributed at the Fall Meeting. Lori Phillips has been very diligent and effective in serving our Corporate Sponsors, a most critical component of GEMS, and in increasing their numbers. Jim Fuscoe and the Nominating Committee put together a great slate of candidates for us this year. Maria Donner has been a super Secretary and I am grateful to her for keeping us aware of what has been said and done over the past two years, taking care of all the Newsletter and ballot mailings, and hundreds of other Society details. My colleague, Kristine Witt, has helped keep me on the straight and narrow and has regularly pitched in to help on numerous tasks. And finally, but most significantly, Frank Stack has not only done a great job as Treasurer but, as our Webmaster, has given us a Web Site at "http://www.ncneighbors.com/312/"; if you have not already done so, do check it out!

I had great fun organizing the 1998 Spring Meeting on one of my pet topics, "Epigenetics: a wider view of genotoxicity". I was blessed with a fabulous speaker line-up and we had exceptional attendance. The organization of the 1998 Fall Meeting on "Genetic Susceptibilities Affecting the Responses of Children to Toxicants" was more challenging because of some personal family commitments and initial speaker cancellations, but the final speaker line-up for that meeting was good, too, and, I felt, well received. Tom brought us back to our roots with an excellent 1999 Spring Meeting on "Regulatory Assessment of Toxicological Data". Tom also organized a special seminar and BBQ in April, featuring Frank Johnson as speaker and Mike Kohan as chef, which was a real treat for all who attended. GEMS hosted no workshops this past year but Tom is planning on getting back to those again soon.

So, all-in-all, the past two years have been productive and enjoyable ones for me. We've gained some wonderful new members, a couple of whom are now new board members. I've enjoyed serving as your President/President-Elect and I plan to continue very active support of the outstanding upcoming leadership and participation in GEMS activities for many years to come.

Sincerely,

Jack Bishop

In Memorium

Dr. Ann Mitchell, a long-time member of GEMS and former member of the GEMS Board of Directors (1991-94) died unexpectedly Thurday, July 15, 1999. Ann, a cell biologist who earned national and international acclaim for her genetic toxicology work using the mouse lymphoma, unscheduled DNA synthesis (UDS) and cytogenetic assays, was the founder and President of Genesys Research Incorported. Ann was a highly valued and respected member of GEMS and we will miss her greatly. Several GEMS members and members of the Board of Directors attended a 'Celebration of Remembrance for Ann' on Sunday August 1 at Genesys Research Incorportated.

All GEMS members who knew Ann will miss her biting sense of humor and warm personality. Her significant support of GEMS, both as a regular and corporate member, helped make GEMS a successful scientific society. Our first place Young Investigator Travel Award at this year's Fall Annual Meeting will be given in honor of Dr. Ann Mitchell.

1999 Corporate Sponsors

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ORAL PRESENTATIONS

T1 REDUCTION IN AMES *SALMONELLA* MUTAGENICITY OF MAINSTREAM CIGARETTE SMOKE CONDENSATE BY TOBACCO PROTEIN REMOVAL. William L. Clapp, Barry S. Fagg, and Carr J. Smith. R.J. Reynolds Tobacco Company, Winston-Salem, NC 27102

Measurements of the Ames activities of smoke condensates from cigarettes, cigars, and pipe tobacco have been made and the observed mutagenicity was not correlated with either the benzo[*a*]pyrene or the N-nitrosomine content of the smoke. Pyrolysates of tobacco leaf have also been examined and increased Ames mutagenicity has been correlated with increased protein content. This result suggested that tobacco leaf proteins may be the foremost precursors of mutagens in tobacco smoke condensate. In the current study, Ames *Salmonella* assays in bacterial strains TA98 and TA100 were used to measure the mutagenic activity of cigarette smoke condensate made from tobacco with reduced protein levels. Mutagenicity in the cigarette smoke condensate of flue cured tobacco was reduced by 80% in TA98 and 50% in TA100 by protein and peptide removal via water extraction followed by protease digestion. The same process yielded reductions of 81% in TA98 and 54% in TA100 in burley tobacco. As measured by this assay, the reductions in Ames mutagenicity following protein removal suggest that protein pyrolysis products are a principal contributor to the genotoxicity of cigarette smoke condensate.

***T2 DNA REPAIR GENES--POTENTIAL CANCER SUSCEPTIBILITY GENES.** Tasha R. Smith[1], Mark S. Miller[1], Kurt Lohman[2], Jennifer J. Hu[1,2]. [1]Dept. of Cancer Biology, [2]Dept. of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC

In 1999, approximately 175,000 US women will be diagnosed with breast cancer. About 60% of breast malignancies do not have identifiable risk factors such as a first-degree relative with breast cancer. Studies suggest an association between deficient DNA repair and breast cancer with odds ratios (ORs) ranging from 1.6 to 10.0. Therefore, suboptimal DNA repair is hypothesized to be an important breast cancer susceptibility factor. DNA repair systems maintain a cell's genetic integrity and prevent DNA lesions from being replicated during DNA synthesis. DNA repair genetic polymorphisms have been identified in a normal human population. Some of the variant alleles may affect DNA repair capacity as the sites of polymorphism lead to amino acid substitutions. Thus, DNA repair genotypes have the potential to be classified as cancer susceptibility genes and may be associated with individual's risk for cancer. We have completed a breast cancer case-control study to evaluate the role of genetic polymorphisms of DNA repair genes in breast cancer risk. The study population consisted of 123 cases and 185 controls recruited at Georgetown University Medical Center from 8/95 to 11/96. The genotypes examined include five polymorphisms in four DNA repair genes: XRCC1, XRCC3, APE, and XPD. Genomic DNA isolated from whole blood was used for PCR-RFLP genotype analysis. The allele frequency of individual genotypes was not significantly different between cases and controls. However, a potential association was observed between breast cancer risk and XRCC1 (exon 6) and XRCC3 (exon 7) variants with age-adjusted ORs of 1.6 (95% confidence interval (CI)=0.8, 3.3) and 1.3 (95% CI=0.8, 2.2), respectively. For both controls and cases, the data suggested a possible linkage between the variant allele of XRCC1 (exon 10) and the wild-type allele of XRCC1 (exon 6). The most interesting finding of this study is that women with both XRCC1 (exon 6) and XRCC3 (exon 7) variant alleles have a 4.5-fold increased risk for breast cancer (95% CI=1.4, 16.0). This study supports the hypothesis that genetic polymorphisms of DNA repair genes may serve as potential biomarkers for human breast cancer risk. A larger study is warranted to confirm our findings. (*Supported by grants from ACS and NCI*)

***T3 COMPARATIVE ANALYSES OF MICRONUCLEUS AND GENE MUTATION INDUCTION IN TRANSGENIC BIG BLUE® MOUSE AND RAT CELLS AFTER EXPOSURE TO THE EPOXIDE METABOLITES OF 1,3-BUTADIENE.** G. L. Erexson and K.R. Tindall, NIEHS, P.O. Box 12233, RTP, NC 27709.

1,3-Butadiene (BD) is a by-product of the manufacture of synthetic rubber and environmental pollutant that elicits a differential carcinogenic response in rodents after chronic exposure. Mice are up to 1000-fold more sensitive to the tumorigenicity of inhaled BD than rats, thereby confounding human risk assessment. Rodent transgenic *in vivo* and *in vitro* models have been recently utilized for generating genetic toxicology data in support of risk assessment studies. However, studies have not been extended to investigate multiple endpoints of genetic damage using *in vitro* transgenic models. The goal of this study was to investigate possible differences in the production of genetic damage in transgenic Big Blue® mouse (BBM1) and rat (BBR1) primary fibroblasts exposed to the epoxide metabolites of BD. Analyses of cytotoxicity, micronucleus (MN) formation and *cII* mutant frequency (MF) were assessed after *in vitro* exposure of transgenic BBM1 and BBR1 fibroblasts to various concentrations of butadiene monoepoxide, BMO; diepoxybutane, DEB; and butadiene diolepoxide, BDE. Concentration-dependent increases in the formation of MN were observed for BMO, DEB and BDE in both BBM1 and BBR1 cells, with DEB being the most potent followed by BDE and then BMO. DEB exposure of BBM1 and BBR1 fibroblasts produced an equivalent dose-response for mutations at the *cII* locus. In contrast, the *cII* MF was significantly increased only in BBM1 cells after exposure to either BMO or BDE. In addition, apoptosis was increased in BBR1 cells but not in BBM1 cells when treated with the BD epoxide metabolites. These data demonstrate that there is a species-specific genetic response following DEB, BMO and BDE treatment. Thus, mouse and rat cells may differ in their ability to process some types of DNA damage, especially damage that results in gene mutation or apoptosis; however there appears to be no species-specific differences in the ability of the cells to process DNA damage induced by DEB, BMO or BDE that yields MN. These data may help explain the discordant tumorigenicity results observed in BD rodent carcinogenicity studies.

***T4 BIOAVAILABILITY OF THE GENOTOXIC COMPONENTS IN COAL TAR CONTAMINATED SOILS IN FISCHER 344 RATS.** N.R. Bordelon[1], K.C. Donnelly[1], L.C. King[2], D.C. Wolf[2], W.R. Reeves[1], S. E. George[2], [1]Texas A & M University, Dept. VAPH, College Station, TX. and [2] US EPA, NHEERL, Research Triangle Park, NC.

The effect of chemical aging on the bioavailability and subsequent genotoxicity of coal tar (CT) contaminated soils was evaluated in a 17 day feeding study using Fischer 344 male rats. Rats consumed a diet amended with soil, CT, and soil freshly prepared or aged for nine months with CT. The *Salmonella* histidine reversion and ^{32}P-postlabeling assays were employed to determine differences in the production of urine mutagens and DNA adducts in treated animals. Although urine was not mutagenic, all CT treatment groups induced DNA adducts in both the liver and lung. A significant decrease ($p<0.05$) in adduct levels was observed in both CT/soil treatment groups compared to CT control in liver and lung DNA after correcting for total ingested polycyclic aromatic hydrocarbons (PAHs; TIP). Adduct profiles of ^{32}P-postlabeled hepatic and lung DNA displayed several non-polar DNA adducts that comigrated with PAH-adducted calf thymus DNA standards (CT-DNA) as determined through both thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC). These results suggest that the bioavailability of genotoxic components in CT is affected by the presence of soil, but not aging as evidenced by similar levels of DNA adducts. [*Abstract does not reflect EPA policy.*]

***T5 A DIETARY ANTIOXIDANT PARADOXICALLY REDUCES TUMOR NUMBER BUT INCREASES MALIGNANCIES IN TRANSGENIC MICE TREATED TOPICALLY WITH BENZO(A)PYRENE.** Keith R. Martin[1], Carol Trempus[1], Muriel Saulnier[1], Frank W. Kari[1], J. Carl Barrett[2], and John E. French[1]. [1]Lab. Env. Carc. and Mut., NIEHS/NIH, RTP, NC; [2]Lab. Mol. Carc., NIEHS/NIH, RTP, NC.

Epidemiologic studies support the protective role of dietary antioxidants in preventing cancer. However, emerging evidence suggests that in some cases dietary antioxidants actually exacerbate carcinogenesis. Our goal was to explore these paradoxical activities in a rodent model that possesses genotypic characteristics of human cancers. Therefore, we selected the $p53^{+/-}$ haploinsufficient Tg.AC (v-Ha-ras) mouse as a model, because it contains an activated, carcinogen-inducible ras oncogene and an inactivated p53 tumor suppressor gene, which are frequent genetic alterations in human cancers. These mice develop chemically-induced benign and fatal malignant skin tumors rapidly which can easily be quantified. We hypothesized that an antioxidant would elicit a disparate response on initiation and progression when compared to basal controls. Mice were fed basal diets with or without 3% N-acetyl-L-cysteine (NAC), a well-recognized antioxidant, prior to, during, and after topical application of the carcinogen benzo(a)pyrene (64 ug/mouse, 2x/week, 7 weeks). Mice continuously fed NAC demonstrated a significant 43% reduction ($p<0.05$) in tumor multiplicity compared to controls (5.9 vs. 10.4). Tumor incidence exceeded 90% for both groups. We next scored tumors (>4 mm^2) based on gross and histologic morphology and found both abundant squamous cell carcinomas and keratoacanthomas. Although total tumor yields were reduced, fatal skin malignancies were increased by 70% in animals fed NAC compared to basal-fed controls. Immunohistochemistry showed consistent and wide-spread expression of both the v-Ha-ras oncogene and p53 tumor suppressor protein. Southern analysis demonstrated no loss of p53 heterozygosity suggesting an alternate mechanism for inactivation of p53 leading to increased malignancies. In summary, our observations demonstrate that the action of a prototypic antioxidant paradoxically contributed more to tumor malignancy with increased severity than to total tumor number.

***T6 ANTIMUTAGENIC EFFECT OF CINNEMALDEHYDE DUE TO INHIBITION OF MUTATIONS AT GC SITES BUT NOT AT SITES IN SALMONELLA TA104.** D.T. Shaughnessy[1], R.W. Setzer[2], D.M. DeMarini[2]. [1]School of Public Health, UNC, Chapel Hill, NC; [2]Environ. Carcinogen. Div., US EPA, RTP, NC

Vanillin and cinnemaldehyde are dietary antimutagens that reduce the spontaneous mutant frequency in Salmonella strain TA104 (*hisG428, rfa, ΔuvrB*, pKM101) by 50%. To date, no study has ever demonstrated whether the antimutagenic effect of an agent is due to a reduction in all classes of mutations or to a reduction in selective classes of mutations. To explore this issue, we have determined the spontaneous mutation spectrum in TA104 as well as the mutation spectrum after a 50% reduction in mutant frequency due to treatment with vanillin or cinnemaldehyde. Although the spectra of the spontaneous and vanillin-treated mutants were not significantly different ($p = 0.7$), the spectra of the spontaneous and cinnemaldehyde-treated mutants were different ($p < 0.02$). Cinnemaldehyde reduced all classes of mutations at GC sites but did not affect the frequency of mutations at AT sites. The entire 50% reduction in mutant frequency by cinnemaldehyde could be attributed to the reduction of mutations at GC sites alone. Both antimutagens also caused a 50% reduction in spontaneous mutant frequency in a *uvrB*$^+$ strain, suggesting that nucleotide excision repair does not play a role in the antimutagenic effects of these two agents. Preliminary studies suggest that the antimutagenic effect is SOS-dependent. [*Abstract does not necessarily represent policy of the US EPA.*]

POSTERS

P1 RESULTS OF SHORT-TERM TESTS FOR MUTAGENIC CARCINOGENS - EVIDENCE PUBLISHED IN THE IARC MONOGRAPHS. Marcus A. Jackson[1], H. Frank Stack[1] and Michael D. Waters[2] [1]Integrated Laboratory Systems, Inc., Research Triangle Park, NC 27709 USA, [2]US Environmental Protection Agency, Research Triangle Park, NC 27711 USA

In 1969, the International Agency for Research on Cancer (IARC) initiated the Monographs Programme to evaluate the carcinogenic risk of chemicals to humans. Results from short-term tests (STT) for genetic and related effects were first included in the IARC Monographs beginning in the mid-1970s based on the observation that most carcinogens are also mutagens, although not all mutagens are carcinogens. Such tests provide vital information needed for identifying and understanding mechanisms involved in carcinogenicity. Growing evidence suggests that the mutagenic events involved in carcinogenesis are produced by one of two broad modes of action. The first may be mediated by the covalent binding of a chemical or its metabolites to DNA or chromatin, or by interference with DNA-related processes, such as spindle function or transcription, thereby directly affecting the integrity of the genome (i.e., the structure or content of DNA). The second process may involve chemical alterations in homeostasis mediated via tissue necrosis, apoptosis, or cellular turnover leading indirectly to the expression of mutations in DNA. The STT results for agents compiled in the EPA/IARC Genetic Activity Profile (GAP) database over the past 15 years are summarized and reviewed here with regard to the agent=s IARC carcinogenicity classifications through IARC Monographs Volume 69. The role of STT data in the overall classification of specific compounds in recent Monographs is discussed. Data for three genetic endpoints (gene mutation, chromosomal aberrations, and aneuploidy) are analyzed and the utility of these data in identifying putative mutational mechanisms of carcinogenicity are presented. [*This is an abstract of a presentation and does not necessarily reflect U.S. EPA policy.*]

***P2 BENZO[A]PYRENE TREATMENT OF THE ESTUARINE FISH, *FUNDULUS HETEROCLITUS*, COLLECTED FROM A PCB-CONTAMINATED SITE RESULTS IN DECREASED HEPATIC BAP-DNA ADDUCTS COMPARED TO REFERENCE SITE.** M. Kohan[1], D. Nacci[2], M. Pelletier[2], L. Coiro[2], and S.E. George[1]. [1]Environmental Carcinogenesis Division, US EPA, Research Triangle Park, NC. and [2]Atlantic Ecology Division Laboratory, US EPA, Narragansett, RI.,

We have examined populations of an estuarine fish, *Fundulus heteroclitus*, resident of a highly contaminated site for their ability to metabolize Benzo[a]pyrene(BaP). The northern most portion of New Bedford Harbor (MA, USA) has been designated a Superfund site because of high concentrations of PCBs and PAHs (2100 and 170 ug/g dry weight, respectively). Mean PCB levels of 324 ug/g dry weight have been observed in fish livers from the Superfund site (Hot Spot, HS). In comparison, the mean concentration of PCB in livers from fish from a reference site removed from HS, West Island (WI), was 2.4ug/g dry weight. The fish from the HS site appear to be resistant to the toxic effects of their environment. It is known that the fish from the HS site show non-responsiveness to AH receptors agonists as well as poor inducibility of P450 enzymes, i.e., CYP1A. Reduced BaP metabolism and DNA adduct formation should occur in fish from the HS site. To test this, we injected BaP (5mg/kg and 50mg/kg or corn oil) into male fish from the HS site and WI site. Ten days after treatment, the fish were sacrificed and the livers removed. The ^{32}P postlabeling assay was used to detect BaP-DNA adducts. Total mean DNA adducts for low and high dose for the WI site was 78 and 2,623 nmoles adducts/mole DNA, respectively and for the HS site is 16 and 279 nmoles adduct/mole DNA, respectively. The WI site had a significantly higher level of DNA adducts than the HS site at the higher dose ($P<0.05$). At the low dose, the two sites were not significantly different in DNA adduct formation. The total mean BaP-DNA adduct formation was higher in fish from the WI site compared to HS site. These results show a possible adaption by the HS site fish to reduce toxicity and could offer protection from tumor formation. [*Abstract does not reflect EPA policy.*]

***P3 SCGE AND ANTIOXIDANT CAPACITY IN MARINE MUSSEL: A FIELD STUDY IN A BRACKISH ENVIRONMENTS OF THE ITALIAN COASTS.** G. Frenzilli[1], F. Regoli[2], V. Scarcelli[1], G. Winston[3] and M. Nigro[1], [1]Dipartimento Morfologia Umana e Biologia Applicata, Pisa University, Italy, [2] Istituto Biologia e Genetica, Ancona University, Italy, [3] Department Toxicology, Raleigh, NCSU

Brackish ecosystems are prone to the human impacts, particularly along the highly anthropized coasts of the Mediterranean region. Thus, the development of molecular and cellular biomarkers to be used for the early detection of adverse biological effects is relevant for the conservation of such endangered environments. In the present investigation, the DNA integrity and the responses of the oantioxidant defence system were investigated in mussel from the Orbetello Lagoon, a highly eutrophicate brackish basin (Weaster Italian Coasts). SCGE (both at pH=12.1 and pH=13) was used for detecting DNA damage in isolated gill cell, while the Total Oxyradical Scavenging Capacity Assay (TOSCA) was used for investigate the susceptibility to peroxyl and hydroxyl radicals in the digestive gland. The results showed that DNA integrity in mussels from the lagoon was significantly lower than in specimens collected at an external control site. However, a marked spatial variability of DNA damage was apparent, with mussels from the more internal sites showing higher damage than specimens living by the canals connecting the lagoon with the sea. The amonut of DNA damage did not vary betwee the two pH value used for the assay, suggesting that alkali lablile sites did not contribute significantly to the DNA alterations. A significant negative correlation was seen between tail lenght and oxyradical scavenging capacity. This finding suggests that mussels from the lagoon were subjected to a oxidative stress, and consequently that the DNA damages were mediated by oxyradicals toxicity. The high load of dissolved organic matter, possibly producing hydrogen peroxyde by photochemical degradation in the water column, and the high fluctuations of dissolveg oxygen have been hypothesized as main responsible for the intense prooxidant challenge to which the species living in eutrophicated coastal lagoons are exposed.

P4 IS GENOTOXICITY A POTENTIAL MODE OF ACTION FOR THE CARCINOGENICITY OF BROMATE? K. Harrington-Brock, D. C. Collard, and R. M. Zucker, US EPA, Research Triangle Park, NC USA 27711

The EPA Office of Drinking Water is currently performing a risk assessment analysis of bromate, an ozonation disinfection by-product. Possible exposure to chlorination disinfection by-products in finished drinking water has heightened concern for public health safety. As a consequence, many utilities are using ozonation as an alternative treatment method. However, very little is known about the health risk associated with the disinfectant by-products of ozonation. The occurrence of bromate in ozonated drinking water ranges from 5-100 µg/L, in areas of the U.S. where source waters contain enhanced levels of bromide. Potassium bromate is a reported rat (kidney, mesothelium, thyroid) and mouse (kidney) carcinogen. We have evaluated the mutagenic potential of potassium bromate (CAS # 7758-01-2) and sodium bromate (CAS # 7789-38-0), both ozonation disinfection by-products, at the *Tk* locus of mouse lymphoma cells. To gain additional information on bromate, we have measured the capability of potassium bromate to induce apoptosis and specific cell cycle perturbations using flow cytometry. Both sodium and potassium bromate demonstrated a positive mutagenic dose response and were active within the same concentration range (660-2653 µM). The lowest effective concentration (660 µM) was the same for both compounds and occurred with minimal cytotoxicity (Survival 70-80%). A significant induction of small colony mutants was observed indicative of a clastogenic mode of action for bromate. Flow cytometric analysis was performed on DNA samples stained with propidium iodide 2 h post-treatment. A dose dependent increase of G_2/M cells was observed. Few cells with a DNA content lower than that of G_1 cells (i.e. apoptotic cells) were present. The most conspicuous cell cycle perturbation observed 2 h post-treatment was a mid-S block. These results indicate that apoptosis is not present 2 h post-treatment, but normal cell-cycle kinetics is interrupted. The positive mutagenic response observed indicates that bromate has the potential to induce tumors by a genotoxic mode of action. [*Abstract does not reflect EPA policy.*]

***P5 STUDIES ON THE GENOTOXICITY OF DIBROMOACETONITRILE: SEPARATION AND QUANTITATION OF ^{32}P-POSTLABELED DNA ADDUCTS IN RAT LIVER, LUNG, BLADDER, KIDNEY AND COLON FOLLOWING AN *IN VIVO* EXPOSURE**, L.D. Adams[1], L.C. King[1], J. Allison[1], M. George[1] and T. DeAngelo[1]. U.S. EPA, NHEERL, Environmental Carcinogenesis Division, Biochemistry and Pathobiology Branch, RTP, NC

The formation of DNA adducts of dibromoacetonitrile (DBAN), a direct-acting genotoxic agent detected in drinking water, has been investigated. In a time course study, male F-344 rats were treated with DBAN (7.2 mg/kg and 3.4 mg/kg PO) and killed at 0, 4, 12, and 24 hr after treatment. Liver, lung, bladder, kidney and colon were collected and stored at -80°C until the time of extraction of DNA for adduct analysis. The ^{32}P-postlabeling assay, thin-layer chromatography (TLC), and reverse-phase high pressure liquid chromatography (HPLC) were used to separate and quantitate the level of DNA adducts in these organs. Single oral doses (7.2 or 3.4 mg/kg) of DBAN induced a time-dependent increase in DNA adduct levels. The maximum extent of DNA adduct formation at 7.2 mg/kg was observed at 24 h in the following tissues; colon>lung>liver>kidney>bladder. Similar extents of DNA adduct formation were found in the tissues at 3.4 mg/kg of DBAN, with the maximum levels also observed at 24 h. DNA- adducts were detected in all tissues examined. Based on comparisons of relative retention time (RRT) of the reaction products of DBAN with 2'-deoxyguanosine-3-monophosphate (3'-dGMP) and 2'-deoxyadenosine-3-monophosphate (3'-dAMP): the major adduct and one of the minor adducts were identified as reaction products of DBAN with 3'-dGMP. The remaining minor adduct was identified as a reaction product of DBAN with 3'-dAMP. These results provide additional evidence of the genotoxicity of DBAN and its potential human health hazard in drinking water. [*This is an abstract of a proposed presentation and does not necessarily reflect EPA policy*]

P6 STUDIES ON BENZO[*C*]CHRYSENE-DNA ADDUCT FORMATION BY RECOMBINANT HUMAN CYTOCHROME P450 1A1 AND P450 1B1 MICROSOMES, J. Allison [1], L.C. King[1], L.D. Adams[1], D. Desai[2] and S. Amin[2] [1] U.S. EPA, NHEERL, Environmental Carcinogenesis Division, Biochemistry and Pathobiology Branch, RTP, NC [2] American Health Foundation, Valhalla, NY.

The role of recombinant human cytochrome P450 1A1 and P450 1B1microsomes in the metabolic activation of benzo[c]chrysene (B[c]C) and formation of DNA adducts was investigated *in vitro* using calf thymus DNA in the presence of epoxide hydrolase. The ^{32}P-postlabeling assay, thin layer chromatography (TLC) and reverse phase high pressure liquid chromatography (HPLC) were used to isolate and characterize the DNA adducts. We found that P450 1A1 and P450 1B1 in the presence of epoxide hydrolase, catalyzed the formation of B[c]C-1,2-diol-3,4-epoxide (B[c]C-DE)-DNA adducts and two unidentified adducts at an equal rate (2.78 fmoles/h/nmole P450 versus 2.92 fmoles/h/nmole P450 and 1.98 f-moles/h/nmole P450 versus 1.30 fmoles/h/nmole P450), respectively. By chromatographic comparisons with adduct standards, the principal adduct was identified as products of the interaction of B[c]C-1,2-diol-3,4-epoxide with 2-deoxyadenosine. These results provide additional evidence that the major B[c]C -DNA adduct detected in vivo was not from a fjord-region (B[c]C-9,10-diol-11,12-epoxide)-DNA adducts but from a bay region adduct derived from B[c]C-1,2-diol-3,4-epoxide. [*This is an abstract of a proposed presentation and does not necessarily reflect EPA policy*]

An asterisk by the abstract title indicates that a presenter is in competition for the Best Talk or Best Poster Award. This year there are two "Best Talk" awards. The First Place recipient will receive $1000 to be used to defray costs associated with attendance at the Environmental Mutagen Society Meeting in New Orleans, LA. The Second Place winner will receive $750 to be used towards EMS expenditures. Best Poster recipient is awarded $100.

P7 INDUCIBLE HEAT SHOCK PROTEIN (HSP70-1) PROTECTS MCF-7 CELLS FROM THE CYTOTOXIC AND GENOTOXIC EFFECTS OF ARSENITE

J.A. Barnes[1], D.J. Dix[2], J.B. Garges[2], B.W. Collins[3] and J.W. Allen[3]. [1]National Research Council, [2]Gamete and Early Embryo Biology Branch, Reproductive Toxicology D-ivision,[3]Genetic and Cellular Toxicology Branch, Environmental Carcinogenesis Division, U.S. EPA, NHEERL, RTP, NC

Heat shock proteins (HSPs) belong to the highly conserved family of stress proteins and are induced following exposure to arsenic. Elevated HSPs protect against cellular damage from heat but it is unclear wether HSP induction alters the damaging effects of environmental chemical contaminants. Therefore, this study was designed to investigate the effects of sodium arsenite (As) in MCF-7 cells that overexpress inducible HSP70 (HSP70-1). A tetracycline-controlled expression system was developed to regulate HSP70-1 levels. Clone14 (C14) cells exhibited the highest induction of HSP70-1 in the absence of doxycycline ("ON") and HSP70-1 could be down-regulated to control levels in the presence of doxycycline ("OFF"). In order to examine the cytotoxic and genotoxic effects of sodium arsenite, C14ON and C14OFF cells were exposed to either 0 (control), 5 μM or 10 μM As. For cytotoxicity studies, cells were exposed for 24 hours and assayed for viability and apoptosis using acridine orange and ethidium bromide staining. Both C14ON and C14OFF cells showed a d-ose-dependent decrease in cell viability and increase in apoptosis in response to arsenite. However, cells expressing elevated HSP70-1 were significantly less affected than cells that did not overexpress HSP70-1. The genotoxicity of arsenite was evaluated using a cytokinesis-block micronucleus (MN) assay. MN were induced in both C14ON and C14OFF cells in response to As. However, induction levels of MN were significantly lower in HSP70-1 overexpressing cells as compared to cells that expressed control levels of HSP70-1 ($p < 0.05$). Kinetochore immunostaining of MN revealed that both kinetochore positive (K+) and kinetochore negative (K-) MN were elevated in response to As. Interestingly, K+ MN were more elevated over control levels than were K- MN. Collectively, these data demonstrate a protective effect of HSP70-1 expression on the cytotoxic and genotoxic effects of arsenite in MCF-7 cells. [*This abstract of a proposed presentation does not necessarily reflect EPA policy.*]

***P8 MODULATION OF MITOMYCIN C CYTOTOXICITY BY OLTIPRAZ**, Debbie Sachs, Mark Miller, Ph. D. and Ronald Fleming, Pharm. D., Wake Forest University School of Medicine, Winston-Salem, NC 27157.

An at-risk genotype for the phase II detoxification enzyme, NAD(P)H: quinone oxidoreduc-tase 1 (NQO1), has been shown to be induced by the dithiolthione oltipraz. Oltipraz may be able to modulate greater intracellular cytotoxicity of mitomycin C through the induction of NQO1. To determine whether an unfavorable NQO1 genotype may be phenotypically altered by pharmacologic modulation to increase mitomycin C efficacy, two human cancer cell lines (MCF-7 and Lovo) were studied using an optimal oltipraz dose of 100μM. Effects on NQO1 enzyme activity after treatment with oltipraz were assayed using a spectrophotometric method and compared to baseline enzymatic activities in non-treated cells. NQO1 activity in oltipraz-treated cells increased two- to three-fold. In additional studies where pre-treatment of the cells with 100μM oltipraz over an optimal induction time course of 48 hours was followed by a 24-hour treatment with 0.5ug/mL mitomycin C, the number of colonies counted in flasks containing MCF-7 cells was reduced by one-half when compared to cells treated with mitomycin C alone. Lovo cells undergoing the combined oltipraz/mitomycin C treatment had colony numbers reduced by nearly 100% compared to treatment with mitomycin C alone. The human cancer cell lines, MCF-7 and Lovo, appear to exhibit an increased mitomycin C cytotoxicity when pre-treated with oltipraz. This may translate for cancer patients with an at-risk NQO1 genotype as an opportunity to increase mitomycin C sensitivity and a therapeutic improvement in mitomycin C cytotoxicity.

***P9 MICE CHRONICALLY EXPOSED TO POTASSIUM BROMATE IN DRINKING WATER REVEAL INCREASED FREQUENCIES OF MICRONUCLEI IN ERYTHROCYTES.** A. Lori[1], B. Collins[2], A. Afshari[2], M. George[2], A. DeAngelo[2], J. Fuscoe[2], J. Allen[2]. [1]Univ. of Pisa, Pisa, Italy; [2]Environmental Carcinogenesis Div., NHEERL, US EPA, RTP, NC

A potential risk to human health associated with the use of ozone for water disinfection is the generation of potassium bromate ($KBrO_3$) in finished drinking water. Chronic exposure to $KBrO_3$ in drinking water has been shown to be carcinogenic in both rats and mice. $KBrO_3$ is known to be genotoxic in conventional bacterial and mammalian cell (e.g., micronucleus) assays; however, to our knowledge, there have been no studies of such effects in relation to chronic exposure to this substance in drinking water. We are evaluating changes in gene expression and chromosomal constitution in mice exposed to $KBrO_3$ in drinking water and herein present our results from micronucleus analyses. Male, 1 mo. old, B6C3F1 mice were subjected to 0, 0.08, 0.04, or 0.8 g/L concentrations of $KBrO_3$ in drinking water for 8 weeks or for 78 weeks. Chromosome damage was assayed by a peripheral blood micronucleus assay used in conjunction with anti-kinetochore antibody staining to determine the presence or absence of a kinetochore and thereby distinguish between aneugenic and clastogenic mechanisms of derivation. Our results indicate that mice chronically exposed to $KBrO_3$ in drinking water over either treatment period exhibit significant dose-dependent increases in erythrocyte micronuclei. The lowest dose was positive only in normochromatic erythrocytes from the 8 weeks trial period (1.6- fold over negative control levels; $p= 0.02$). The highest dose induced roughly 2- to 4- fold increases in kinetochore-negative types of micronuclei in both polychromatic and normochromatic erythrocytes over both exposure periods. Kinetochore-positive types of micronuclei were rarely observed and were not elevated in any treatment groups over control levels. We conclude that $KBrO_3$ under the described chronic exposure conditions is clastogenic. [*This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*]

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