to hypermethylate cytosine. In this study we measured the activity of CMTase isolated from cells *in vitro* and observed that it retains methyltransferase activity in the presence of 10 mM arsenite, a concentration that is over 10,000-fold the LC_{50} for these cells. We also measured the activity of CMTase in cells grown in the presence of arsenite. We found that cells exposed to arsenite had increased levels of mRNA for CMTase as detected by RT-PCR, and the CMTase preparations isolated from arsenite-exposed cells had higher levels of activity per μg protein. These results indicate that hypermethylation of DNA seen in association with arsenic exposure may be a consequence of the resistance of CMTase toward arsenite and that arsenite somehow stimulates the production or activity of CMTase or inhibits its degradation. *This is an abstract of a proposed presentation and does not necessarily reflect policy of the U.S. EPA.*

592 5-METHYLCYTOSINE ANALYSIS OF THE DEOXYCYTIDINE KINASE PROMOTER IN CYTARABINE SENSITIVE AND RESISTANT LEUKEMIC CELLS.

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During cancer development and progression the transcriptional down regulation of genes is often associated with aberrant cytosine methylation of CpG island promoters. Because deoxycytidine kinase activates cytarabine to its cytotoxic form and its promoter is a CpG island, we hypothesized that increased levels of cytosine methylation in the deoxycytidine kinase promoter were responsible for the observed transcriptional repression of deoxycytidine kinase in cytarabine resistant HL-60 cells. To test this hypothesis the region containing the minimal promoter of the deoxycytidine kinase gene (-245 to +130 relative to the transcription start site) was sodium bisulfite modified and amplified by PCR. PCR products were cloned into plasmids. Of these recombinants, 10 independent clones of DNA derived from the individual cell lines were then sequenced. This region covered 49 CpG dinucleotides, and analysis showed that these sites were almost completely unmethylated in the cytarabine sensitive and resistant HL-60 cells. These results suggest that deoxycytidine kinase may not be a target for 5-methylcytosine-mediated gene inactivation.

593 ARSENIC AND CHROMIUM INDUCE METAL-, DOSE-, TIME-AND CELL LINE-DEPENDENT ALTERATIONS IN TRANSCRIPTION FACTOR BINDING.

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The heavy metals, arsenic and chromium, are both considered human carcinogens, although they are believed to act through very different mechanisms. Chromium(VI) acts as a classic genotoxic carcinogen, whereas arsenic(III) is considered non-genotoxic but targets a number of protein-mediated pathways. We had previously shown that chemical carcinogens preferentially alter expression of inducible relative to constitutively expressed genes. We were interested in whether these metals target specific but distinct sites within cells to modulate gene expression. We examined the effects of non-cytotoxic and toxic doses of arsenite or chromate on binding of the transcription factors, AP-1 and SP-1, in human metastatic breast cancer (MDA-MB-435) and rat hepatoma (H4IIE) cells in culture. These transcription factors were chosen because of their presence within the promoter regions of many inducible genes including those altered by chromium and arsenic treatments *in vivo*. Arsenic and chromium significantly altered binding of these factors. However, there were qualitative and quantitative differences in these effects that were metal-, dose, time- and cell line-dependent. For example, arsenic had little effect on AP-1 binding in H4IIE cells, but had a significant effect in 435 cells. In 435 cells, the low dose of arsenic (1 μM) caused an early biphasic induction event in AP-1 binding, while the lethal dose (100 μM) caused a mild suppression in binding. The low chromium dose (1 μM) caused a late AP-1 induction (12 hr) in H4IIE cells, while both low and high chromium doses caused rapid induction (1–2 hr) of AP-1 binding in 435 cells. Comparable differences were observed with SP-1 binding. These effects may play a significant role in metal-induced alterations in gene expression and may contribute to the mechanism by which these agents act as human carcinogens. (NIH CA49002 and ES07373).

594 EFFECTS OF L-CANAVANINE AND L-CANALINE ON CELL GROWTH AND THE EXPRESSION OF THE MULTIDRUG RESISTANCE GENE (MDR-1) IN CANCER AND NORMAL CELLS *IN VITRO*.

L Ogden[1], B Jin[2], W Bowen[3], E Blann[2], B D Lyn-Cook[2]. [1]*North Carolina A&T SU, Greensboro, NC;* [2]*National Center for Toxicological Research, Jefferson, AR; and* [3]*NIDDK, Bethesda, MD.* Sponsor: J D Heck.

Canavanine [L-2-amino-4-(quanidinooxy) butyric acid] is a structural analogue of arginine that was originally isolated from the jack bean, *Canavalia ensiformis*. Canavanine can also be synthesized from many leguminous plants. Canavanine differs structurally from arginine in that oxygen replaces the terminal methylene group of arginine. Investigators have shown that canavanine inhibits the growth of human pancreatic cancer cells. Our study demonstrates that canavanine and it's metabolite, canaline, inhibits the growth of the rat glioma cell line, C6. Further studies, using reverse transcriptase (RT-PCR), indicated that canaline inhibited the expression of the multidrug resistance (mdr-1) gene, which is found at high levels in C6 cells. When examining the effects of canavanine or canaline on normal pancreatic acinar cells *in vitro*, there were no effects on cell growth. These studies indicate that canavanine and canaline have selective toxic effects for cancer cells which could be attributed to down-regulation of the mdr-1 gene.

595 THE EFFECT OF MULTIPLE ORAL ADMINISTRATION ON THE FORMATION AND DISAPPEARANCE OF 4,4'-METHYLENE-BIS(2-CHLOROANILINE)-DNA ADDUCTS IN RAT LIVER.

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The ^{32}P postlabeling assay using nuclease P1 enhancement procedures was utilized to investigate the formation and disappearance of DNA adducts in rats after multiple oral administration of 4,4'-methylene-bis(2-chloroaniline) [MOCA], a probable human carcinogen. Adult male rats were dosed with 7.5 mg MOCA/kg body weight for up to 28 days. Groups of rats were sacrificed every 7 days for the 4-week treatment period and for an additional three weeks after treatment cessation. Two adducts, A and E, were observed in liver DNA of the rats from all treatment groups. The major adduct, A, had similar chromatographic properties to *N*-(deoxyadenosin-8-*yl*)-4-amino-3-chlorobenzyl alcohol, which is the same adduct that has been previously identified in target site DNA from both human uroepithelial cells treated with MOCA and in exfoliated urothelial cells from a worker accidentally exposed to MOCA. The minor adduct, E, has not been identified. Levels of both A and E increased in a dose-dependent manner over the 28-day treatment period. After 7 days, adduct levels were 7,627 ± 2,585 and 1,279 ± 354 (relative adduct level ± SE x 10^7 nucleotides) for A and E, respectively. By 28 days, the levels of A and E had increased to 13,071 ± 3,585 and 4,423 ± 1,238, respectively. Adduct levels for A and E rapidly decreased after the cessation of dosing. Seven days after the treatment period had ended, the levels of A and E were 3,134 ± 638 and 319 ± 77, respectively. However, detectable adduct levels remained 21 days after the dosing period had been completed. The half-life of the adducts were determined to be 4 days for A and 2 days for E. The development of a mathematical model to assess exposure based on DNA adduct levels has been initiated. In summary, this study shows that relatively high levels of DNA adducts are formed with repeated low level exposure to MOCA and that quantifiable levels of these adducts remain at the target site for at least 3 weeks following the exposure period. In addition, this study provides additional evidence that the major adduct, A, may be useful for occupational biomonitoring.

596 HRAS MUTATIONAL ANALYSIS OF CHEMICALLY INDUCED MOUSE SKIN AND LIVER TUMORS.

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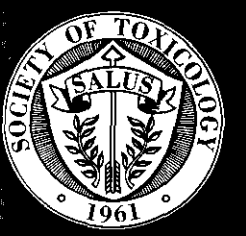
7H-Dibenz(c,g) carbazole (DBC) is a potent skin and liver carcinogen and benzo(a)pyrene (BaP) is a potent skin carcinogen following topical application to back skin of mice. These compounds are found in complex mixtures produced during incomplete combustion of fossil fuels. The objective of this study was to elucidate the mechanism of action of these two compounds by assessing the HRAS mutational spectra in a sensitive mouse. BaP (50 nmoles) and DBC (50 or 100 nmoles) were applied topically to backs of 36 female Hsd:ICR(Br) mice (12 per group) twice weekly up to 70 weeks. After sacrifice, BaP skin and DBC skin and liver tumors were removed and DNA isolated.



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 36th Annual Meeting of the Society of Toxicology, held at the Cincinnati Convention Center, Cincinnati, Ohio, March 9-13, 1997.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 371.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 395.

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