cisplatin resistance in ovarian cancer cells. We hypothesized that NaAsO2 and hyperthermia would enhance cisplatin cytotoxicity by modifying DNA repair proteins and altering cellular platinum accumulation. Cisplatin-sensitive (A2780) and -resistant (CP70) ovarian cancer cells were treated with cisplatin, with or without 20 uM NaAsO2 at 37 or 39°C (hyperthermia) for 1 h. A2780 and CP70 cells expressed basal levels of XPC and DDB2, key lesion recognition proteins in global genomic repair, subpathway of nucleotide excision repair. Cisplatin increased the levels of these proteins. CP70 cells maintained higher levels of XPC and DDB2 with increasing concentrations of cisplatin. NaAsO2 destabilized XPC, whereas, hyperthermia stabilized it. A2780 cells accumulated ~2-fold more Pt than CP70 cells. Unlike NaAsO2, hyperthermia increased the cellular accumulation of Pt. Cell cycle analysis revealed G2/M accumulation of cells 36 h after treatment. NaAsO2 and hyperthermia stabilized p21CIP1/WAF1 over time, which correlated with decreased pRB phosphorylation at Ser807/811, suggesting cell cycle arrest. Cyclins A & B and Cdc2 proteins were stabilized following cisplatin treatment, suggesting G2 arrest. NaAsO2 decreased the expression of cyclin A and stabilized cyclin B & Cdc2, suggesting that the cells accumulating in the G2/M compartment are mitotic cells. PARP cleavage which is an early indicator of apoptosis was also evident at 36 h. In summary, these data suggest that CP70 cells have a more efficient lesion recognition system than A2780 cells. Hyperthermia increased cellular Pt accumulation and NaAsO2 altered DNA repair by decreasing the expression of XPC and causing cells to accumulate in the M phase of the cell cycle. Supported in part by NIH grants 1P30ES014443 and 5R01ES011314.

## PS 1799 THE ROLE OF PKC CONSENSUS SITES IN MTF-1 TRANSCRIPTIONAL ACTIVATION.

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Metallothioneins (MTs) belong to a superfamily of intracellular proteins that sequester environmentally toxic metals and are thought to regulate their intracellular concentration. MT expression is transcriptionally regulated by the metal-regulatory transcription factor 1 (MTF-1). MTF-1 acts by binding to short DNA sequences called metal responsive elements (MREs) found in the enhancer/promoter regions of target genes including MT-I, MT-II and ZnT1, a Zn efflux transporter. Two models exist to describe how metals regulate MTF-1 mediated transcription: One proposes that MTF-1 functions as zinc sensor, and the other that phosphorylation of MTF-1 via protein kinase C (PKC) signaling pathways controls its transcriptional activity. To examine the role of phosphorylation in MTF-1 transcriptional activation, PKC consensus sites were identified within MTF-1 using Prosite analysis and each site was systematically mutated. Lentiviruses were developed for each mutant, transduced into MTF-1 knockout mouse embryonic fibroblasts (dko7 cells) and stable cell lines expressing each construct were isolated. We exposed these cells to cadmium or zinc, and measured changes in expression of three marker genes: MT-I, MT-II and ZnT1, by real time qPCR. dko7 cells transduced with wild type MTF-1 demonstrated significant levels of metal-inducible transcription of all three marker genes examined. Surprisingly, the MTF-1-T224A mutant had MTF-1 mRNA levels similar to cells transduced with wild-type MTF-1, but did not show metal-inducible transcription of the marker genes examined. Additionally MTF-1 -/- cells expressing MTF-1-T224A are hypersensitive to cadmium exposure. These data confirm that mutation of this PKC phosphorylation site prevents metal inducible MTF-1-mediated transactivation and has detrimental effects on cell survival after exposure to metal.

## PS 1800 ROLE OF DIVALENT METAL IONS IN AMINOACYLASE 3 MEDIATED CATALYSIS.

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Aminoacylase 3 (AA3) is highly expressed in renal proximal tubules. The AA3 mediated deacetylation of N-acetyl-1,2-dichlorovinyl-L-cysteine (Ac-DCVC), a metabolite of a xenobiotic trichloroethylene (TCE), is involved in acute renal failure induced by TCE. Despite of ~40% identity of AA3 with the zinc containing aspartoacylase, there is no data on the metal-dependence of AA3. In this study we determined whether mouse AA3 contains a metal ion(s) and whether this metal ion(s) is necessary for the catalysis. In the absence of a high-resolution 3D structure of AA3, we performed modeling of its 3D structure to determine putative amino acid residues involved in metal binding in or near the active site. Based on the 3D model of mouse AA3, His21, Glu24 and His116 were hypothesized to be involved in the coordination of a metal ion in a putative active site. In agreement with this hypothesis, the H21A, E24A and H116A mutants of mouse AA3 were inactive. Using inductively coupled plasma mass spectrometry, 0.01-0.05 atoms of Mn, Cu, Fe or Ni, and 0.35-0.5 atoms of Zn per monomer of wild type (wt) AA3 was determined. Incubation with $Co^{2+}$ activated several times wt-AA3 and the $Co^{2+}$-activated wt-AA3 contained 1-2 atoms of Co per monomer and no Zn indicating that $Co^{2+}$ replaced $Zn^{2+}$ in the enzyme. Incubation with zinc did not change the activity of wt-AA3 despite the fact that it bound up to 4 zinc atoms per monomer. The amount of the specific metal bound to wt-AA3 was dependent on its concentration in the growth media. The H21A, E24A and H116A mutants had decreased metal content in comparison with wt-AA3. In addition to drastic increase of the rate of deacetylation of Ac-DCVC, $Co^{2+}$ significantly increased the toxicity of Ac-DCVC in the mouse proximal tubule mPCT cells expressing mouse wt-AA3. The results indicate that AA3 is a metalloenzyme and suggest that divalent metal ions may play an important role in mediating the toxicity of TCE.

## PS 1801 PROTECTIVE ROLE OF FERROUS SULFATE IN CHROMIUM TOXICITY.

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In the US, approximately 1.5 million construction workers are occupationally exposed to cement each year. Allergic contact dermatitis (ACD) is a major skin problem reported among cement workers. Hexavalent chromium [Cr(VI)], present as a contaminant in the cement, is believed to be responsible for cement ACD. A significant reduction in the incidence of cement ACD has been reported in certain European countries where the addition of ferrous sulfate (FeSO4) to cement has been mandated. However, the actual involvement of FeSO4 in counteracting the Cr(VI)-mediated cement ACD has been questioned. Presently, we have conducted in vitro cell culture experiments to investigate whether FeSO4 is capable of protecting cells of human dermal origin against Cr(VI)-induced toxicity. Human dermal fibroblasts and keratinocytes were treated with potassium dichromate alone or with potassium dichromate and FeSO4. Cytotoxicity, apoptosis, and oxidative stress were determined by MTT assay, TUNEL assay, and by electron spin resonance (ESR) analysis, respectively, in the treated cells. Exposure of the cells to potassium dichromate alone resulted in concentration-dependent cytotoxicity, apoptosis, and oxidative stress. The Cr(VI)-induced cytotoxicity, apoptosis, and oxidative stress in the dermal cell lines were significantly blocked by the addition of FeSO4 to the cell culture medium. Analysis of the global gene expression profile in the cells further confirmed the protective role of FeSO4 in Cr(VI) toxicity and provided novel insights regarding Cr(VI) toxicity as well as the protective role of FeSO4. In summary, our results demonstrated a protective role for FeSO4 in Cr(VI) toxicity suggesting that the addition of FeSO4 to cement may be helpful to prevent or at least reduce the incidence of cement ACD among construction workers in the US.
Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be constructed to represent any agency determination or policy.

## PS 1802 3'-OH-GENISTEIN IN THE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA.

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Acute promyelocytic leukemia (APL) is caused by a unique gene translocation t(15;17), involving the promyelocytic leukemia (PML) gene that encodes a growth suppressing transcription factor and the retinoic acid receptor alpha (RARα) gene that regulates myeloid differentiation. This translocation creates a chimeric PML/RARα protein which is the key player in APL leukemogenesis. This highly lethal disease has been successfully treated with all-trans retinoic acid (ATRA). APL patients who have relapsed or who have failed to respond to standard therapy are treated with arsenic trioxide (ATO). While ATRA induces myeloid differentiation, ATO triggers apoptosis of APL cells. A recent study showed that a complete remission of APL can be achieved faster with a combined ATRA/ATO therapy as compared to monotherapy. Notably, nearly 30% of APL patients treated with ATRA and/or ATO develop a life-threatening complication known as "APL syndrome". Thus, identification of more effective drugs with fewer side effects would significantly improve the outcomes of APL therapy. Several studies have demonstrated that genistein, a soybean isoflavonoid, induces differentiation and apoptosis in human leukemia cells. Results of our preliminary studies demonstrate synergistic effects of genistein and ATRA and/or ATO on the viability of APL cells and on PML/RARα degradation. In humans, CYP1A2, CYP1A1 and CYP2E1 metabolize genistein to hydroxylated metabolites. We found that the primary metabolite of genistein, 3'-OH-genistein, is more effective than the parental compound as an inducer of cell death and PML/RARα degradation in NB4 culture. In addition, our results show that both CYP1A1 and CYP2E1 are expressed in NB4 cells and are inducible by treatment with ATO. Thus the metabolites of genistein may play a significant role in the outcomes of APL therapy.

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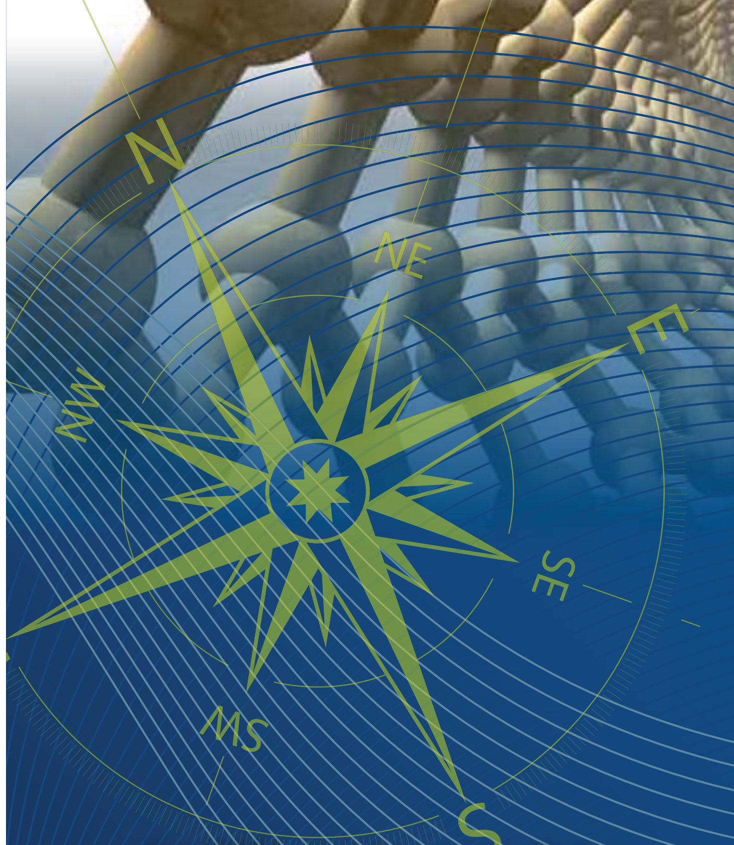


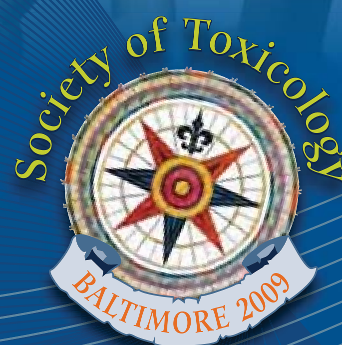


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