metabolic processes whereas the response to LPS was centered around cell signaling, cell cycle regulation, and acute phase response. Apoptotic pathways were altered in all three treatment groups. Proteins with large abundance changes of potential toxicological significance include pulmonary surfactant protein D (elevated in all treatment groups), which is involved in resistance to infectious agents, and hepatoma-derived growth factor (elevated in smoke exposed groups) which is extracelluar with growth factor activity and also nuclear with a DNA binding domain. These results demonstrate that mass spectrometry-based global proteomics approaches can provide the resolution and sensitivity needed to identify potential biomarkers for COPD and other related diseases. (Supported by Battelle IR&D).

184 EXPRESSION OF OSTEOPONTIN BY RAT ALVEOLAR MACROPHAGES *IN VITRO*

E. Bermudez and O. R. Moss. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Osteopontin (OPN), an abundant glycosylated phosphoprotein, has been found to be associated with various disease states including pulmonary diseases. The role of OPN in the normal or diseased lung is poorly defined, however it has been shown that OPN is associated with the formation of granulomas and fibrosis. Our previous work demonstrated that the expression of OPN was elevated in rats developing fibrotic lung lesions following the inhalation of titanium dioxide particles (Tp). The expression of OPN by cells of the lung, based on in vivo evidence, has been shown to occur primarily, though not exclusively, in macrophages. The current work examines the in vitro elaboration of soluble OPN by rat alveolar macrophages (AM) treated with various agents operating via several different signaling pathways. OPN is detectable in the bronchoalveolar fluid of control rats and in medium conditioned by AM isolated from control animals. The level of expression of OPN was increased ten fold in untreated AM allowed to attach to plastic culture plates versus unattached cells, therefore the experiments were performed under conditions where attachment of the AM was abrogated. AM were exposed to Tp, silica particles (Sp), bleomycin (Bm), endotoxin (Et), epidermal growth factor (EGF), interferon (IFNγ), tumor necrosis factor (TNFα), or transforming growth factor (TGFβ1). Following various lengths of exposure the medium was removed and frozen and subsequently analyzed by ELISA for either OPN or TNFα. AM actively phagocytosed the Tp and Sp. OPN was induced by treatment with Sp and TGFβ1. Conversely, TNFα, Bm, and IFNγ treatment resulted in decreased soluble OPN. No effect on OPN expression was detected when AM were treated with Tp, EGF, or Et. TNFα expression was upregulated by Tp, Sp, Bl, Et, and IFNγ, but not by EGF. AM treated with Et or IFNγ induced the production of NO, measured as total NO_3, suggesting that iNOS was upregulated in these cells. These results suggest that the uptake of particles is not sufficient to induce OPN expression and that the regulation of OPN expression can occur via various pathways.

185 ANTIBODIES AGAINST FORMALDEHYDE-ALBUMIN CONJUGATES IN RATS TREATED WITH FORMALDEHYDE: POTENTIAL BIOMARKER OF EXPOSURE

M. Khan, H. Li, J. Wang and G. Ansari. *Pathology, University of Texas Medical Branch, Galveston, TX.*

A large human population is exposed to formaldehyde (FA) environmentally and occupationally, leading to a variety of respiratory and dermatological problems. FA covalently binds with albumin to form FA-albumin (FAA) conjugates. It is hypothesized that humoral immune response to FAA conjugates could provide biological marker of FA exposure, effective dose and susceptibility. To test this hypothesis, male SD rats were treated with FA (1/5th LD50) through drinking water for up to 6 months. Blood was collected at 3 and 6 months following FA exposure and formation of anti-FAA antibodies was measured by ELISA using synthesized rat albumin conjugates of FA as solid phase antigen. Sera from FA treated rats showed induction of antibodies to FAA in 50% of animals at 3 months. The antibody titer was significantly higher at 6 months in the serum samples of FA-treated rats which were found positive at 3 months. Greater antibody response at 6 months suggests a time-dependent formation of anti-FAA antibodies. These antibodies were highly specific for FAA and did not cross react with malondialdehyde-, 4-hydroxynonenal-, 4-hydroxyhexenal- and acrolein-protein adducts. The specificity of anti-FAA antibodies was further evaluated by inhibition studies which showed a dose-dependent decrease in binding when the serum was pre-incubated with increasing concentrations of FAA, and by Western blot analysis which showed immunoreactivity of the antibody with FAA but not with rat albumin. Furthermore, the anti-FAA antibodies (rat serum) also recognized FA-human albumin (FAHA) conjugates, but had only ~1/3rd binding affinity in comparison to FAA. These studies suggest that induction of anti-FAA antibodies has the potential to be used as biomarker of FA exposure.

186 IDENTIFICATION OF GLUTATHIONE DEPLETION-RESPONSIVE GENES IN RAT LIVER USING THE LARGE-SCALE TOXICOGENOMIC DATABASE

N. Kiyosawa, A. Ono, T. Miyagishima, T. Urushidani and T. Nagao. *Toxicogenoimcs Project, National Institute of Biomedical Innovation, Ibaraki, Osaka, Japan.* Sponsor: T. Inoue.

Glutathione serves vital functions in detoxifying electrophiles, and evaluation of glutathione homeostasis helps assess the potential risk of acetaminophen-type hepatotoxicity. In the present study, phorone was used as a glutathione depleting agent on 6-week old male Crj:CD(SD)IGS rats (Charles River Japan). Three rats per group were administered with phorone (40, 120, 400 mg/kg, i.p.) and euthanized 3, 6, 9 and 24 h after treatment. Livers were analyzed for glutathione content and gene expression using Rat Genome 230 2.0 Array (Affymetrix, Inc.). Glutathione depletion-responsive gene probe sets (GSH probe sets) were screened from microarray data as follows: first, probe sets, whose signal values were inversely correlated with the hepatic glutathione content throughout the experimental period were selected based on Spearman's correlation coefficients. Second, probe sets, whose average signal values were greater than 2-fold (compared to their corresponding control) at 3 h after phorone treatment, were selected. Finally, probe sets whose target sequences did not show homology with known gene sequences were excluded, ending up with a total of 130 probe sets. The validity of the probe sets was examined by referring to our large-scale toxicogenomic database, which is currently under construction by the Toxicogenomics Project in Japan (TGPJ). Among the chemicals that caused liver injury to some extent, acetaminophen, bromobenzene and coumarin markedly altered the expression of GSH probe sets, whereas clofibrate, thioacetamide, phenylbutazone, glibenclamide, hexachlorobenzene, aspirin, methapyrilene, chlorpromazine, carbon tetrachloride and perhexiline maleate, did not. This result suggested that GSH probe sets would be useful for evaluation of drug-induced hepatic glutathione depletion from microarray data. In addition, the TGPJ database appeared to be an invaluable resource for validation of identified marker genes for toxicogenomic use.

187 ANALYSIS OF HYDROXYBUTENYL-VALINE ADDUCTS IN RATS EXPOSED TO BUTADIENE

N. I. Georgieva, G. Boysen, K. Jayaraj, A. Gold and J. A. Swenberg. *ESE, University of North Carolina, Chapel Hill, NC.*

Butadiene (BD) metabolism exhibits gender, species and concentration dependency, making difficult the extrapolation of animal results to humans. BD is metabolized mainly by P450 2E1 to 1,2-epoxy-3-butene (EB), 1,2:3,4-diepoxybutane and 1,2-epoxy-3,4-butanediol. For accurate risk assessment it is important to have an accurate measure for the internal formation of the individual epoxides and to develop scientifically based risk models. Analysis of N-terminal globin adducts is a common approach for monitoring the internal formation of reactive metabolites. We report herein the analysis of the EB derived hydroxybutenyl-valine (HB-Val) adduct by a novel LC-MS/MS method. The procedure utilizes trypsin hydrolysis of globin, immunoaffinity purification and subsequent quantitation by UPLC-ESI-MS/MS of the alkylated heptapeptide, monitoring the transition from the singly charged molecular ion of HB-Val (1-7) to the a_1 fragment. Human HB-Val (1-11) was synthesized and used for antibody production. As internal standard the stable isotope labeled rat [$^{13}C_5$ ^{15}N]-Val (1-11) was prepared through direct alkylation of the corresponding peptide with EB. Standards were characterized and quantitated by LC-MS/MS and LC-UV. The method was validated with different amounts of human HB-Val. The recovery was >75% and the CV <25%. The LOQ was set preliminary to 100 fmol/injection. Further on, globin samples from male and female rats exposed to 1000 ppm BD for 90 days were analyzed. HB-Val amounts were 268 ± 56 and 350 ± 70 pmol/g globin for male and female, respectively. No HB-Val was detected in controls. These data are much lower compared to previously reported, measured by GC-MS/MS, most likely due to (1) different internal standards, (2) higher specificity of the new method to the N-terminal valine of the alpha chain, and (3) possible degradation of HB-Val during storage(>10 years). This report demonstrates the suitability of this method to study the internal formation of EB *in vivo*.

188 BIOMARKERS OF MANGANESE EXPOSURE IN BAY BRIDGE WELDERS

R. Gwiazda[1], H. A. Roels[2], R. Park[3], R. Bowler[4], R. Lucchini[5, 1] and D. Smith[1]. *[1]University of California, Santa Cruz, Santa Cruz, CA, [2]Universite Catholique de Louvain, Brussels, Belgium, [3]NIOSH, Cincinnati, OH, [4]San Francisco State University, San Francisco, CA and [5]University of Brescia, Brescia, Italy.*

Occupational exposure to manganese (Mn) is associated with emotional disturbances and subtle motor dysfunction. Identification of biomarkers of exposure and effect are critical for the detection of individuals at risk from elevated occupational

and environmental Mn exposures. We investigated potential biomarkers of exposure in welders exposed in the construction of a section of Bay Bridge across the San Francisco Bay. Welders working in confined spaces in cofferdams were exposed to fumes with high Mn content (mean:0.209 mg/m^3) for an average duration of 15 months, usually without respiratory protection. Cumulative inhaled Mn was calculated based on the amount of time each worker spent using different welding devices and the mean air Mn concentration near each device. Blood manganese concentrations (mean 9.57 μg/L, range: 5.13-14.15 μg/L, n=42) were significantly correlated with cumulative inhaled Mn ($p<0.01$), but plasma and urine Mn were not. No association was found between blood Mn concentration and average air Mn levels. Because Mn in plasma was relatively invariant at 0.58 ± 0.13 μg/L over the whole range of blood Mn concentrations, the fraction of Mn in plasma was inversely correlated with blood Mn and ranged between 9 and 2.5% of whole blood Mn. In an animal study conducted to evaluate biomarkers of exposure, the concentration of Mn in plasma in Sprague-Dawley rats exposed i.p. 3 times/wk to $MnCl_2$ for 15 weeks was also invariant over the same range of whole blood Mn concentrations observed in the welders. However, the fraction of Mn in rat plasma varied from 20 to 50% of total blood Mn content, indicating a larger plasma Mn binding capacity in rats' blood than in human blood. These results indicate that in the case of inhalation exposure to the respirable aerosol fraction (more than 90% in welding fumes) blood Mn concentration is a more suitable biomarker of cumulative Mn exposure, than plasma or urine Mn concentration.

189 DISPOSITION OF LEAD (PB) IN SALIVA, BLOOD COMPONENTS, AND TISSUES FOLLOWING REPEAT ORAL EXPOSURE IN THE RAT

W. Yantasee, R. A. Gies, H. Wu and C. Timchalk. *Pacific Northwest National Laboratory, Richland, WA.*

There is interest in evaluating saliva as a non-invasive biomatrix for assessing Pb exposure. This study was conducted to evaluate the distribution of Pb in saliva relative to other tissues (i.e. blood & bone). Male S-D rats (5-7 per group) were given oral gavage doses of Pb-acetate at 0, 1, 10 and 100 mg/kg/day, for 5 consecutive days then held for an additional 5 days before analysis. Prior to sacrifice animals were anethetized (ip; ketamine/xylazine) and administered pilocarpine (ip; 1 mg/kg) to induce salivation. Saliva was collected, animals were humanely sacrificed and samples of blood, parotid gland, and bone were collected. Samples were weighed and analyzed for Pb by ICP-MS, while saliva protein and amylase were determined spectrophotometrically. The concentration of Pb was evaluated in whole blood (WB), red blood cells (RBC), plasma, bone, parotid gland, and saliva. Saliva volume, pH, total saliva protein and amylase activity were also determined. The relative concentration of Pb followed the order: bone>>RBC>WB>saliva>plasma≈parotid. For all tissues there was a dose-dependent increase in Pb concentration; however, only the bone Pb concentration increased proportionally with dose. Relative to controls the WB Pb concentrations increased by factors of 0, 3, and 5 at doses of 1, 10 and 100 mg/kg/day, respectively. Whereas for RBCs, the Pb concentrations increased by factors of 2, 6 and 18, respectively. For the saliva, plasma, and parotid gland, the measured Pb concentrations at 1 and 10 mg/kg/day were at or slightly above the controls, but increased by factors of 2-3 following the 100 mg/kg/day dose. Under this experimental design, the Pb exposures had no appreciable impact on saliva pH, protein concentration or amylase activity. In the current study Pb was clearly quantifiable (73 ± 33 ng/ml) in the saliva when WB Pb concentration was 136 ± 24 ng/ml. Future studies need to evaluate effects of a more prolonged Pb exposure on Pb clearance and salivary gland function. (Supported by NIH/NIESH grant 1 R01 ES010976-01A2)

190 ERYTHROCYTES AS A USEFUL BIOLOGICAL MATRIX FOR ASSESSMENT OF MANGANESE EXPOSURE AMONG SMELTING WORKERS

L. Yang[1], Y. Jiang[2] and W. Zheng[3]. *[1]First Affiliated Hospital, Guangxi MED. University, Nanning, China, [2]Department of Occup Health & Toxicol, Guangxi MED. University, Nanning, China and [3]School of Health Sciences, Purdue University, West Lafayette, IN.*

Our precious human data suggest that red blood cells (RBC) may serve as a compartment in accumulation of manganese (Mn). This study aimed to explore the utility of RBC in assessing Mn exposure by determining the levels or activities of essential elements and oxidative stress markers in erythrocytes among Mn-exposed smelting workers. Concentrations of Mn, Zn, Cu, Fe, Ca, Mg, and malondialdehyde (MDA), and activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in erythrocytes were determined among 59 Mn-exposed smelting workers whose breathing zone airborne MnO2 was 0.75 mg/m3 (0.19-1.60 mg/m3) and 59 control subjects whose airborne MnO2 was 0.01 mg/m3 (0-0.03 mg/m3). Neurological exams did not detect any clinical signs and symptoms of manganism among these study participants. The mean concentrations of Mn and Ca in RBC of smelting workers were significantly higher (+33.3%) and lower (-16.8%), respectively, than those of controls. No significant difference was found in RBC levels of Zn, Cu, Fe and Mg, and RBC activities of MDA, SOD, GPX, and CAT between cases and controls. When both groups were stratified for work year as of <5 yr, 5-10 yr, and >10 yr, a significant increase in RBC Mn (+33.3%) remained evident in all 3 work year groups; yet Cu levels in RBC were significantly decreased (-11.1%) in smelting workers with over 10 yr experience and so were RBC Fe levels (-9.5%) in smelting workers with 5-10 yr experience, in comparison to the same work year control subjects. Among stress markers tested, SOD activity in RBC among 5-10 yr experience smelting workers was significantly higher (+27%) than that of controls with the same work experience. Concentrations of all six metals in the whole blood and plasma were not significantly different between smelting workers and controls. These data indicate that erythrocytes appear to be a better biological matrix than conventional blood or plasma for assessment of Mn exposure.

191 BRAIN MAGNETIC RESONANCE IMAGING AND BLOOD LEVELS OF TRACE ELEMENTS AMONG MANGANESE-EXPOSED STEEL WORKERS

X. Mo[1], Y. Jiang[2], L. Long[3], W. Zhao[1], X. Li[3], S. Su[4] and W. Zheng[5]. *[1]Department Neurol, Guangxi MED. University, Nanning, China, [2]Department Occ Health & Toxicol, Guangxi MED. University, Nanning, China, [3]Department Radiology, Guangxi MED. University, Nanning, China, [4]Guangxi Worker's Hospital, Nanning, China and [5]Purdue University, West Lafayette, IN.*

MRI has been suggested for diagnosis of manganism. However, the questions remain as to whether the pallidal signal intensity in T1-weighted MRI is correlated to blood Mn and to what extent the MRI can reflect the real life Mn exposure. This cross-sectional study aimed to use MRI to detect Mn exposure among workers. A group of randomly selected 18 male Mn-exposed workers of which 13 were smelting workers with a high exposure (mean airborne Mn in work place: 0.72 mg/m3; 0.07-2.93 mg/m3, same below) and 5 power distribution workers with a low exposure (0.55; 0.09-1.71 mg/m3) from ferroalloy factories, and another group of 9 male control subjects(0.01; 0-0.03 mg/m3) from non-ferroalloy factories were recruited for neurological exams, MRI procedure, and analysis of Mn in the whole blood (MnB), plasma (MnP) and red blood cell (MnRBC). No clinical symptoms and signs of manganism were observed among Mn-exposed and control workers. MRI data showed an average of 7.4% ($p<0.05$) and 16.1% ($p<0.01$) increases in pallidal index (PI) among low and high exposed workers, respectively, as compared to controls. Fourteen out of 18 Mn-exposed workers (78%) had an intensified PI, while this proportion was even higher (85%) among the high Mn-exposed workers. There was no statistically significant difference in concentrations of MnB, MnP and MnRBC between Mn exposed and control workers. However, among steel workers, the PI was significantly associated with MnRBC ($r=0.55$, $p=0.02$), less significantly with MnP ($r=0.42$, $p=0.08$), and not significantly with MnB ($r=0.09$, $p=0.72$). No association was observed between the PI and Fe levels in the whole blood, plasma or RBC. Our data suggest that the workers exposed to a high level of airborne Mn, but without clinical symptoms, appeared to display an intensified MRI signal. Thus, the MRI, as well as MnRBC, may be useful in early diagnosis of Mn exposure.

192 TOXICOGENOMICS AND BIOMARKER DISCOVERY FOR THE PREDICTION OF LONG TERM TOXICITY

H. Gmuender[1], C. Pallez[1] and A. Hohn[1]. *[1]Genedata, Basel, Switzerland, [2]Genedata, Basel, Switzerland and [3]Genedata, Basel, Switzerland.* Sponsor: H. Ahr.

Toxicogenomics is a novel approach for predicting a compound's toxicity using gene, protein or metabolite expression information. Changes to the expression profile following exposure to a drug are interpreted using knowledge of gene function and other biological information, including conventional long term toxicology results. We present evidence that toxic exposure to known genotoxic hepatocarcinogens is manifest in the expression profile following just 10 days of chronic exposure. To gain information on the toxic mode of action (MOA) we applied promoter and pathway analysis software tools for the identification and characterization of novel gene regulation mechanisms that are relevant for the cell's response to DNA damage. The results demonstrate that expression data from genes, proteins and metabolites can be used to build reference libraries of known compounds against which novel compounds can be tested. Biomarker candidates can then be deduced from such libraries and further characterized to determine the causal relationship between the biomarker and toxicity in animal models and in humans.

193 HEMOGLOBIN ADDUCT AS EXPOSURE MARKERS OF CHEMICAL SUBSTANCES

M. Ogawa, T. Oyama, T. Isse, T. Murakami, T. Yamaguchi, T. Kinaga and T. Kawamoto. *Department of Environmental Health, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan.*

Recently, a lot of new biomarkers have been studied in addition to classical biomarkers, such as chemical substances and their metabolites in blood and urine, and deviating enzymes. Among new biomarkers, hemoglobin adduct is thought to be

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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