growth factor-*beta* 1 (TGF-β_1), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), tumor necrosis factor *alpha* (TNF-α), and *beta*-actin was investigated using reverse transcription-polymerase chain reaction (RT-PCR). Genes found to have a different pattern of expression in HD-exposed *vs.* control tissue included MMP-9, IL-6, IL-8, TNF-α, TGF-β_1 and IL-1β. Genes found to be similarly expressed in both control and HD-exposed tissue included β-actin, MCP-1, and MMP-2. These data contribute to an understanding of the chemical messengers involved in HD-induced skin injury and wound healing in the porcine model. (Supported by Battelle Memorial Institute.)

854 POTENTIAL ROLE OF HEAT SHOCK PROTEIN 60 IN CHEMICAL-INDUCED TOXICITY.

B. Billack, D. E. Heck and J. D. Laskin. *Rutgers University and UMDNJ- Robert Wood Johnson Medical School, Joint Graduate Program in Toxicology, Piscataway, NJ.*

Chaperonins, also known as heat shock proteins (HSP), are induced in cells by both chemical and physical stresses including exposure to heavy metals and ultraviolet light. Aside from their known role in the stabilization of protein folding, it has recently been demonstrated that one member of this family, HSP 60, can induce production of proinflammatory mediators including TNF-alpha and interleukin-6. In a variety of disease states, elevated serum levels of HSP 60 have been observed. Toxicants such as sodium arsenite and cadmium chloride elevate HSP 60 and its release from necrotic cells may contribute to chemical-induced inflammation. Using RAW 264.7 mouse macrophages, we found that HSP 60 induced both nitric oxide synthase enzyme expression and nitric oxide production in a time- and concentration- dependent manner. Co-stimulation with interferon-gamma resulted in a marked increase in nitric oxide production when compared to either agent alone, indicating that the JAK/STAT pathway regulates HSP 60-inducible nitric oxide biosynthesis. PD98059, a specific inhibitor of the p42/p44 MAP kinase cascade, attenuated this effect while SB203580, a specific inhibitor of the p38 MAP kinase cascade, had no apparent effect. HSP 60 also stimulated macrophages to express cyclooxygenase-2 (COX-2), a key enzyme regulating the production of inflammatory prostaglandins. We found that expression of this enzyme was inhibited in a concentration-dependent manner by phorbol ester as well as by SB203580. Taken together, our data indicate that HSP 60 acts as a potent stimulator of macrophage nitric oxide and prostaglandin biosynthesis and suggest that the induction of nitric oxide synthase and COX-2 enzymes occurs by distinct signaling pathways. (Supported by NIH grants ES 03647 and ES 06897.)

855 EFFECT OF GLUTATHIONE ON PARTICLE-INDUCED INFLAMMATION IN THE RAT LUNG.

J. M. Carter, C. E. Azuka, K. A. Singh, J. M. Gearhart and K. E. Driscoll. *Procter & Gamble, Cincinnati, OH.*

Complex interactions mediate the pathogenic effects of inhaled toxins leading to pulmonary inflammation, fibrosis and tumorigenicity. Chronic inhalation of poorly soluble particles at levels that cause persistent, marked inflammation has been associated with increased levels of adenomas and carcinomas in the rat lung. This response may be unique to the rat and has not been observed in mice, hamsters or primates. The recruitment of neutrophils during the inflammatory response and subsequent release of oxidants from these cells play an important role in the pathogenesis of lung tumors. Glutathione (GSH) may participate in detoxifying both superoxide and hydrogen peroxide. Diminshed GSH levels affect the regulation of the reductive process by the GSH redox pathway. To evaluate the role GSH plays in pulmonary defense systems, GSH levels were depleted or augmented in rats *via* i.p. administration of Buthione Sulfoxide (BSO) or N-Acetylcysteine (NAC), saline served as control. Rats from each group were either administered saline or 2mg carbon black (CB) intratracheally and sacrificed after 7 days. Bronchoalveolar lavage (BAL) was performed and BAL cells and fluid were evaluated for cell number and differential, superoxide and hydrogen peroxide production, LDH and total protein. Lung tissues were evaluated for pro- and anti- inflammatory mediators. Data from cell number and differential counts indicates the depletion of GSH affected the inflammatory process. Rats administered BSO + CB showed significant increases in cell number and % neutrophil over both control and NAC groups. BSO + CB treated animals showed increases of superoxide and hydrogen peroxide approximately 10x of rats treated with CB alone. NAC + CB rats showed no increase over control rats in superoxide and hydrogen peroxide levels even with increased %neutrophil. These results suggest that augmentation of GSH provide a measure of protection against the formation of oxidants while depletion of GSH appears to increase oxidative stress.

856 ROLE OF PEROXYNITRITE IN MEDIATING LUNG INFLAMMATION FOLLOWING OZONE INHALATION.

L. Fakhrzadeh[1], A. L. Salzman[2], G. J. Southan[2], J. D. Laskin[1] and D. L. Laskin[1]. [1]*Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Joint Program in Toxicology, Piscataway, NJ and* [2]*Inotek, Boston, MA.*

Inhalation of ozone leads to alveolar epithelial cell damage. This is associated with an accumulation of activated macrophages in the lower lungs which contribute to tissue injury. Alveolar macrophages release a number of cytotoxic mediators that have been implicated in toxicity including nitric oxide, peroxynitrite, and eicosanoids. The present studies were aimed at analyzing mechanisms regulating production of these mediators during ozone toxicity. Treatment of wild type control mice with ozone (0.8 ppm) for 3 hr resulted in increased bronchoalveolar lavage (BAL) fluid protein which reached a maximum 24-48 hr post exposure. This was correlated with increased numbers of alveolar macrophages in BAL. These cells were also found to express greater quantities of NOSII and COX-2 and produced more nitric oxide and peroxynitrite, as well as PGE2 in response to inflammatory mediators. Ozone inhalation also resulted in the appearance of nitrotyrosine, a marker for peroxynitrite mediated damage, in histologic sections of the lung. To analyze the role of peroxynitrite in toxicity, mice were pretreated with aminoethylselenourea (AESeU) (25 mg/kg, twice daily for three days), a peroxynitrite scavenger. We found that these mice were protected from ozone induced lung injury. Alveolar macrophages from these mice also produced control levels of PGE2. These data, together with our findings that transgenic mice with a targeted disruption of NOSII or overexpressing superoxide dismutase were protected from ozone toxicity demonstrate that reactive nitrogen intermediates, in particular, peroxynitrite contributes to ozone induced tissue injury. Moreover, peroxynitrite appears to regulate COX-2 activity in the lung after ozone inhalation. Supported by NIH grant ES04738 and the Burroughs Wellcome Fund.

857 ACUTE COLD-RESTRAINT STRESS LOWERS RESISTANCE OF BALB/C MICE TO *LISTERIA MONOCYTOGENES.*

L. Cao and D. A. Lawrence. *Wadsworth Center, New York State Department of Health, Environmental Health & Toxicology, Albany, NY.*

Stress has been implicated in the pathophysiology of infectious and autoimmune diseases. Studies have suggested that stress can impair host defense, but the mechanisms involved have not been well delineated. We evaluated acute cold-restraint stress (ACRS) in combination with *L. monocytogenes* (LM) infection. BALB/c mice (10~12-wk, male) were individually restrained (R) at 4°C for 1h, and control (NR) mice were undisturbed until infection. Within 30 min of initiating ACRS, the serum corticosterone increased dramatically and remained elevated until 1h after ACRS. LM caused a significant decrease in food/water intake (FWI), but not in body weight (BW); ACRS caused significant BW loss and reduced FWI, which worsened with LM ($5\text{-}6\cdot10^3$/mouse iv). The viable LM burdens in both spleen and liver were significantly higher in R mice compared to NR mice throughout the entire study period (9 days). In NR mice, the LM burden peaked on day 2 then gradually decreased, while in R mice, it continued rising until day 3, then started to decrease. This suggests ACRS causing delayed resistance, affecting the transition from innate to acquired immunity. During the same time period, the higher LM burden was associated with higher serum and splenic IL-6 levels in R mice, indicating a stronger inflammatory reaction. Interestingly, the levels of well-known macrophage (Mϕ) activator IFNγ were also higher in serum, spleen and liver in R mice; the splenic IFNγ level increased suddenly on day 3 in R mice, whereas similar to NR mice before and after. The paradoxically high IFNγ levels with high LM burdens suggest that ACRS may induce a hypo-responsiveness of Mϕs to IFNγ. Stressed mice displayed significantly less resistance to LM and enhanced sickness, which was associated with heightened cytokine levels. The physical and emotional stress appears to lessen IFNγ-induced killing of LM, suggesting that environmental stresses can substantially modify susceptibility to infections. (Supported by NIEHS ES03778).

858 INHIBITION OF LPS-INDUCED TNF-α EXPRESSION BY PHENOLIC ANTIOXIDANTS.

K. Kinneer-DeFede[1], J. Ye[2], M. Luster[1] and Q. Ma[1]. [1]*NIOSH, TMBB/HELD, Morgantown, WV and* [2]*NIOSH, PPRB/HELD, Morgantown, WV.*

Phenolic antioxidants exhibit anti-inflammatory, anti-atherosclerotic, and anti-carcinogenic activities in animals. The broad range of biological effects of the phenolic antioxidants suggests multiple mechanisms exist for their action. Tumor necrosis

factor alpha (TNF-α) is an important regulator of immune and inflammatory responses and tumor growth, thus, is involved in many biological, as well as pathological processes. Bacterial endotoxin LPS stimulates production of TNF-α, a process essential to LPS- elicited inflammation and sepsis. Previous studies suggest that certain phenolic antioxidants protect against LPS toxicity. However, the mechanism of such protection is not known. In this report, we examine the effect of phenolic antioxidants on LPS-induced expression of TNF-α in macrophage cells. We found that tert-butylhydroquinone (tBHQ), a prototype of phenolic antioxidants, totally inhibits LPS-induced production of TNF-α protein. To further investigate the molecular mechanism of this inhibition, we generated a stable reporter cell line that expresses luciferase under the control of a TNF-α promoter region. Our results show that LPS induces luciferase production and tBHQ totally blocks this induction in the cell. This inhibition is both time- and dose-dependent. Furthermore, diphenols such as para-hydroquinone, catechol, and resorcinol also inhibit LPS-induced luciferase expression. The inhibition correlates with the redox potential of these chemicals, which implicates redox cycling as a mechanism for the inhibition of TNF-α. Our current goal is to identify the molecular target and pathway for the regulation of TNF-α production by phenolic antioxidants.

859 THE ARYL HYDROCARBON RECEPTOR IS ESSENTIAL FOR THE RESOLUTION OF SOME FETAL VASCULAR STRUCTURES.

G. P. Lahvis and C. A. Bradfield. *University of Wisconsin, McArdle Laboratory for Cancer Research, Madison, WI.*

The aryl hydrocarbon receptor (AHR) has been extensively studied for its role in regulating xenobiotic metabolism and the toxicity of dioxins. A developmental role of this protein has been suggested by the observations that mice harboring Ahr null alleles display disproportionately smaller livers, reduced fecundity and decreased body weights. Upon investigating the liver phenotype, we found that the 25% decrease in organ size is directly correlated with a 30% reduction in hepatocyte size. We also found evidence to indicate that smaller hepatocyte size is the result of massive portosystemic shunting in null animals. Colloidal carbon uptake and microsphere perfusion studies indicate that 56% of portal blood flow bypasses the liver sinusoids. Latex-corrosion casts and angiography indicate that shunting results from patent ductus venosus. Intravital microscopy shows persistence of anastomotic sinusoidal architecture in the liver and of hyaloid arteries in the eyes. These data provide the identity of the first mammalian gene that is both causally related to congenital portosystemic shunting and required for the resolution of fetal vascular structures.

860 MATERNAL AND FETAL RESPONSES OF EGF KNOCKOUT MICE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD).

P. L. Bryant[1], A. R. Buckalew[2] and B. D. Abbott[2]. *[1]UNC, Environment Sciences & Engineering, Chapel Hill, NC and [2]USEPA, Reproductive Toxicology Division, NHEERL, Research Triangle Park, NC.*

TCDD is teratogenic in C57BL/6N mice producing cleft palate and hydronephrosis. Previous studies show that exposure on gestational day (GD) 12 to 24 µg TCDD/kg results in high incidences of both defects without significant maternal or fetal toxicity. The teratogenic responses were linked to altered proliferation of epithelial cells and disrupted expression of epidermal growth factor (EGF) and the EGF receptor. The present study evaluates the importance of EGF in the pathogenesis of these responses. Pregnant wild type (WT) and EGF knockout mice were exposed to 24 µg TCDD/kg on GD12 and killed on GD17.5. Data evaluated included: maternal and fetal body weights, liver weights and liver/body weight ratios; placental weight, embryo/fetal survival, sex of fetuses, cleft palate and hydronephrosis. Renal pelvic space dilation was scored from 0-4 and scores of 2 or higher were considered hydronephrotic. The maternal and fetal weights and survival were not affected by TCDD in WT or EGF null. Liver weight and liver/body weight ratios increased in TCDD-exposed WT and EGF null dams and fetuses. TCDD-exposed WT fetuses had 18%±6.9 incidence of cleft palate (litter mean±SEM) and the rate was significantly lower in the EGF null (6.1%±2.8). In contrast, the incidence (98%±2) and severity (3.7±0.08) of hydronephrosis was increased in the TCDD-treated, EGF null litters relative to WT (75%±6, 2.4±0.21). These data suggest that EGF mediates the effects leading to cleft palate, as in the absence of EGF the response is muted. In the urinary tract, in the absence of EGF, responsiveness increased and this may be related to effects of TCDD on the expression of TGF- in ureteric epithelial cells. It is clear that the expression of EGF during fetal development is a factor in the responsiveness to TCDD in both the palate and urinary tract. Disclaimer: This abstract does not necessarily reflect EPA policy.

861 2,3,7,8-TETRACHLORODIBENZO-*p*- DIOXIN (TCDD) INHIBITS CORONARY ANGIOGENESIS AND MYOCYTE PROLIFERATION DURING EMBRYONIC DEVELOPMENT.

I. D. Ivnitski and M. K. Walker. *University of New Mexico, College of Pharmacy, Albuquerque, NM.*

TCDD is a ubiquitous environmental pollutant that causes developmental toxicity in fish, birds, and mammals. Since exposure of the chick embryo to TCDD results in adverse effects on the cardiovascular system, the chick embryo represents an in vivo model that can be used to elucidate the fundamental mechanism of TCDD-mediated cardiovascular toxicity. Fertile chicken eggs were injected with 5 µl corn oil (control) or 0.24-0.40 pmol TCDD/g in corn oil prior to incubation. To evaluate TCDD's effects on differentiation of coronary arteries, paraformaldehyde-fixed, paraffin-embedded sections of day 10 and 12 chick embryo hearts were stained with anti-smooth muscle alpha actin. TCDD caused a significant decrease in the number of coronary arteries manifested most dramatically on incubation day 10, while some decrease was observed also on day 12. We then tested the hypothesis that this reduction resulted from apoptosis induced by TCDD. Apoptotic cells were detected by TUNEL in control and TCDD-treated hearts on incubation days 6 and 8, when the heart remodeling and coronary angiogenesis occur by an apoptotic process. TCDD increased the number of apoptotic cells in the dorsal mesocardium on day 6, representing a population of cells believed to contribute to cardiac coronary and septal morphogenesis. Since disruption of equilibrium between cell proliferation and cell death may play a key role in inhibition of coronary angiogenesis, we investigated proliferation rate by BrdU incorporation in chicken embryo hearts on days 8, 10, and 12 following TCDD treatment. TCDD significantly inhibited myocyte proliferation on incubation days 8 and 10. Such cooperative events as apoptosis induction and proliferation inhibition may represent potential mechanisms of TCDD-mediated cardiovascular toxicity. Future research will focus on identifying the specific cell population undergoing TCDD-induced apoptosis. Supported by NIH R01 to MKW.

862 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ON CARDIAC NA+/K+ ATPASE.

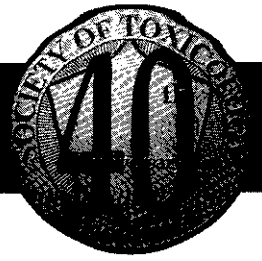
C. J. Reamer[1], R. J. Sommer[1] and M. K. Walker[2]. *[1]Bates College, Department of Biology, Lewiston, ME and [2]University of New Mexico, College of Pharmacy, Albuquerque, NM.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a ubiquitous environmental pollutant, causes cardiac malformations and functional deficits in the chick embryo and our previous research has demonstrated that TCDD alters the ECG in manner consistent with reduction of Na+/K+ ATPase. To investigate the effects of TCDD on Na+/K+ ATPase expression, fertile chicken eggs were injected with corn oil (control) or 0.24 pmol TCDD/g prior to incubation. Embryos collected on incubation days 6, 8, 10, and 12 were paraformaldehyde fixed, paraffin embedded, sectioned and stained for Na+/K+ ATPase alpha1 subunit. Embryo hearts were also collected on day 10, microsomal protein isolated and analyzed for Na+/K+ ATPase alpha1 protein expression and Na+/K+ ATPase enzyme activity. Initial IHC results on days 6 and 8 indicate that Na+/K+ ATPase alpha1 is expressed in the myocardium of atria and ventricles, but not in the mesenchyme of the atrio-ventricular cushion. Myocardial staining intensity on day 6 varied from light-to-moderate and on day 8 was moderate with less variation than on day 6. TCDD exposure did not appear to alter the Na+/K+ ATPase alpha1 expression on either day. Western blots showed that Na+/K+ ATPase alpha1 expression was reduced in day 10 hearts from TCDD-treated embryos with expression at 44 ± 11% of control. Similarly, Na+/K+ ATPase enzyme activiry (inhibited by ouabain) was reduced ; in TCDD-exposed hearts Na+/K+ ATPase enzyme activity was 0.039 ± 0.005 micromol ATP dephophorylated/min/mg compared to 0.070 ± 0.008 in controls ($p < 0.01$). However, total cardiac ATPase activity was also reduced (control, 0.16 ± 0.01; TCDD 0.08 ± 0.007; $p < 0.002$), suggesting that other cardiac ATPase enzymes are also effected by TCDD. Additional IHC analysis of day 10 and 12 hearts samples will confirm whether TCDD treatment also alters the spatial expression of the Na+/K+ ATPase alpha1. Supported by NIH R01 to MKW and the Surdna Foundation to RJS.

863 ANALYSIS OF THE EFFECTS OF LEAD EXPOSURE ON DEVELOPMENTAL GENE EXPRESSION IN THE RAT HIPPOCAMPUS USING MICROARRAY TECHNIQUES.

S. Bakheet, M. Bookland, W. Wei and N. H. Zawia. *University of Rhode Island, Biomedical Sciences, Kingston, RI.*

The deleterious effects of environmental metals such as lead (Pb) may be mediated through alterations in the regulation of gene expression. Previous studies from our lab indicate that the neurotoxiciry of Pb is partially mediated through disruption of



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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, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

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