688 A NEW BIOMARKER FOR DETECTING THE EFFECTS OF ENVIRONMENTAL CADMIUM EXPOSURE: THE FISH IMMUNE RESPONSE. N Enane, D Bowser, K Frenkel, KS Squibb, JT Zelikoff, NYU Medical Center, Inst. of Environ. Medicine, New York, NY.

Cadmium (Cd^{+2}), a known immunomodulator shown to affect humoral, cell-mediated and non-specific immunity in mammalian systems, is an environmental health hazard and known contaminant in aquatic environments and Superfund sites. Although effects of Cd^{+2} exposure on the immune responses of mammals are well studied, little is known concerning the impact of Cd^{+2} on the immune system of those organisms directly exposed in the water. Macrophages (Mø), a pivotal cell type in the immune response of fish and mammals, are often the principal cell type altered by toxicant exposure. To determine the effects of Cd^{+2} exposure, fish or recovered peritoneal Mø, were exposed to $CdCl_2$ at relatively non-toxic levels and effects on Mø viability, morphology, phagocytosis and free radical production evaluated. Rainbow trout were used as the animal model. In vitro exposure to Cd^{+2} (10-1000 µm) reduced peritoneal Mø viability in a dose-dependent manner, and at concentrations of 31 and 62 µM altered Mø morphology, and enhanced phagocytosis and zymosan-stimulated production of hydrogen peroxide (H_2O_2). Concommitant with these effects was an increase in cellular concentrations of a metallothionein-like cadmium binding protein. In vivo water bath exposure to Cd^{+2} at 2 ppb for 17 d enhanced stimulated production of superoxide anion radical ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2), and the phagocytic uptake of opsonized latex particles. This study provides evidence that peritoneal Mø from trout are sensitive to immunomodulation by low levels of Cd^{+2}, and suggests the usefulness of trout Mø as a biomarker for detecting the effects of Cd^{+2} exposure. Supported by NIEHS Grant No. ES04895.

689 DEVELOPMENT OF A DIAGNOSTIC TEST FOR FUMONISIN TOXICOSES. W P Norred, E Wang, H S Yoo, J Showker, K Voss, T Wilson, F Ross, W Haschek, V Beasley, A H Merrill, and R T Riley. USDA-ARS, Athens, GA;, Emory University, Atlanta, GA, USDA-APHIS, Ames, IA, University of Illinois, Urbana, IL.

Fumonisins (FBs) are specific inhibitors of sphinganine (S_a) and sphingosine (S_o) N-acyltransferase. In cultured cells, inhibition of S_a N-acyl transferase results in an increase in free S_a and a decrease in biosynthesis of complex sphingolipids. Conversely, inhibition of S_o N-acyltransferase results in decreased reacylation of S_o generated via turnover of complex sphingolipids. In theory, inhibition of S_a and S_o N-acyltransferase in vivo should increase free S_a and S_o which are normally present in minute amounts in tissues and serum. A retrospective collaborative study was initiated to analyze tissues and serum from rats, pigs, and horses. The samples were from studies conducted with either FB containing culture materials or naturally contaminated corn incorporated into feed. The results indicate FB disruption of sphingolipid metabolism is easily observed in tissues and serum. In rat liver, free S_a and S_o levels were increased 65 and 8 fold, respectively, relative to controls. In horse serum, changes in free S_a and S_o were observed before liver enzymes were noticibly elevated. The most sensitive indicator is the change in the ratio of free S_a to S_o. This ratio may serve as an useful early marker for FB toxicoses.

690 PARTIAL CHARACTERIZATION OF PROTEOLYTICALLY-DERIVED PEPTIDES FROM ACRYLAMIDE ADDUCTED HEMOGLOBIN. DL Springer, CG Edmonds, DM Sylvester, C Sander and RJ Bull. Battelle, Pacific Northwest Laboratory, Richland, WA and Washington State University, Pullman, WA.

Hemoglobin adducts have been used to monitor occupational exposures to a number of compounds. The most common procedure has been to cleave the adduct from the protein and derivatize and characterize it by gas chromatography/mass spectrometry. To extend these approaches we are attempting to characterize acrylamide adducted hemoglobin using electrospray ionization mass spectrometry (ESI-MS). For this we incubated ^{14}C-acrylamide and human hemoglobin under conditions that yielded high adduct levels and separated the α- and ß-subunits using reverse phase HPLC. Under these conditions radioactivity copurified with both subunits and the adducted species were observable in the ESI mass spectrum of the isolated ß-subunit. When the ß-subunit was digested with proteolytic enzymes and the resulting peptides separated by reverse phase HPLC, radioactivity copurified with several peptides. Using ESI-MS we identified several hemoglobin derived peptides that were consistent with anticipated cleavage sites. Currently, these preparation are under further characterization by ESI-MS, which will aid in the indentification of the adducted peptides and in subsequent preparation of acrylamide-specific anti-bodies. Supported by US EPA CR-81216.

691 CIRCULATING LEUKOCYTES AS INDICATORS OF ARYLAMINE CARCINOGEN EXPOSURE. G N Levy. Dept. of Pharmacology, University of Michigan, Ann Arbor, MI.

Circulating white blood cells (WBC) are being examined as a biological monitor of exposure to arylamines and other carcinogens. DNA-carcinogen adduct formation in WBC may be a marker for the degree of in vivo exposure to carcinogens as well as an indicator of DNA adduct formation in target tissues. Controlled exposure of inbred mice to 2-aminofluorene (AF) followed by ^{32}P-postlabeling and HPLC analysis of nucleotides from WBC and the carcinogen target tissues liver and urinary bladder was used to evaluate the relationship between carcinogen exposure and DNA adduct formation. At 3 hr after a 60 mg/kg i.p. dose of AF, WBC from 7 wk old male C57BL/6J mice was adducted to a level of 24 fmol/mg. The target tissues, liver and bladder, were adducted to 180 fmol/mg and 465 fmol/mg, respectively. The adduct level in DNA from all three tissues decreased with time such that at 24 hr post-exposure, WBC DNA adducts had decreased to 7 fmol/mg, while liver and bladder DNA adducts decreased to 50 fmol/mg and 230 fmol/mg. Preliminary results thus indicate that after exposure to arylamine carcinogens WBC DNA is adducted with a time course similar to target tissues. Studies in progress relate carcinogen dose to adduct formation and consider sub-chronic exposure in addition to one time acute doses.

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