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**1934** INDUCTION OF METALLOTHIONEIN IN OREOCHROMIS MOSSAMBICUS EXPOSED TO CADMIUM STRESS.

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Metals are known to induce oxidative stress in aquatic organisms. To date numerous studies have implicated metallothionein (MT) formation in the: a) detoxification of heavy metals, b) regulation of metal-distribution and c) the tissue damage due to generation of reactive oxygen species (ROS). MT is an ubiquitous, cysteine-rich metal binding protein and its synthesis is induced by cadmium (Cd) mediated-oxidative stress, glucocorticoids and anticancer agents. In the present study, the MT in liver, kidney, brain, gill and muscle of the edible fish *Oreochromis mossambicus* (Tilapia) exposed to Cd (5mg/l) for 30 days was quantified by cadmium-saturation method. The induction of MT was in the order of kidney (97.3 µg/g) > liver (63.5 µg/g) > gill (17.2 µg/g) > brain (7.4 µg/g) > muscle (6.8 µg/g). The data show that the Cd-concentration as determined by the DEAE cellulose ion exchange chromatography was higher in kidney (11.4 ppm) followed by liver (6.6 ppm), gill (2.8 ppm), brain (2.3 ppm) and muscle (1.98 ppm). The relative migration of MT protein was measured as 0.67 for liver, kidney, gill and muscle suggesting a molecular weight of 10-12 KD whereas for brain it was found to be 0.7 suggesting 10 KD. In general, the purification of brain MT protein showed the same elution pattern as that of hepatic and renal MTs. The present study suggests that the occurrence of MT-III protein in brain of piscine model could be a growth-inhibitor factor similar to the one reported in mammalian brain.

**1935** VERY LOW DOSE MERCURY MODULATES CYTOKINE SECRETION BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS *IN VITRO* WITHOUT CHANGING CELL POPULATIONS.

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Mercury (Hg) is implicated as an immunotoxicant by studies in humans, animals, and *in vitro* cell culture systems. However, most studies have been conducted with exposures in the micromolar range, far above that found in blood under environmental exposure situations. To investigate Hg's immunotoxic effects under more environmentally relevant conditions, we investigated immune function in primary cultures of human peripheral blood mononuclear cells (PBMCs) exposed to a concentration of HgCl<sub>2</sub> up to 200 nM (which roughly corresponds to a whole blood concentration of 50 µg/L, at the upper range of occupationally exposed and fish eating populations). These concentrations, well below those in the published literature, do not induce overt cytotoxicity (as measured by MTT and trypan blue exclusion assays). Parameters of immune function were measured in the presence and absence of 50 ng/ml LPS to model Hg's effects on both resting and activated immune system. Cells were harvested after 48 hours in culture and immediately stained for flow cytometry. Cell culture supernatants were analyzed by ELISA for cytokine concentrations. In PBMCs from both male and female volunteers, dose-dependent increases in both TNF $\alpha$  and IL-6 were observed, while no changes were observed in IFN $\gamma$  levels. These changes in cytokine production were detectable under conditions where no changes were detected in cell populations as measured by flow cytometry (CD19+ B cells, CD11b+ NK cells and monocytes, and CD3+/CD4+ or CD3+/CD8+ T cells and CD4+/CD8+ T cell ratios). Additionally, there were no changes in markers of activation (CD25, CD80, CD86, CD69) for any of the sub-populations studied over the dose range of Hg used. This study shows that Hg can exert significant effects on cytokine production at levels that are both non-cytotoxic and environmentally relevant, indicating that Hg effects at low concentrations are likely reflective of changes in signal transduction pathways rather than in cell viability or subsets.

**1936** AN INTEGRATED FUNCTIONAL GENOMICS APPROACH TO ASSESS THE EFFECTS OF DIETARY EXPOSURE TO METHYLMERCURY AND SELENIUM IN JUVENILE AND ADULT MICE.

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Seafood is a good source of nutrition, yet may also be a route of exposure to contaminants such as methylmercury (MeHg). Epidemiological studies implicate the consumption of MeHg-contaminated seafood with effects on the nervous system, particularly during development. Micronutrients normally found in high levels in seafood, such as selenium have been shown to modify the toxicity of methylmercury. Selenium counteracts the toxic effects of methylmercury, possibly due to the

formation of stable adducts. A study was conducted to establish the importance of MeHg speciation, and identify the mechanisms of MeHg toxicity in a murine model. Female mice were fed MeHg (as either MeHgCl or MeHgCys) and in a separate study MeHgCys and/or selenium before mating, during gestation, and for two weeks following parturition. Neurobehavioural indices such as stress/anxiety response, reflex behaviour and locomotion were monitored in pups. Behavioural impacts were also determined in adult mice. An increased latency to movement in an elevated plus maze in MeHgCl- but not MeHgCys-exposed adults were discerned. Brains of these pups were then subjected to microarray analysis to monitor changes in gene expression. Results highlighted perturbations in a number of biological functions, befitting the widespread toxic effects of methylmercury noted in more restricted studies. Of note were alterations in genes relating to cell cycle control, cell signalling, calcium metabolism, cell adhesion, apoptosis, and oxidative stress. A number of genes were also identified that are associated with neural degenerative disorders such as Alzheimers disease, suggesting that that mechanisms of neural impairment may be conserved, independent of the causative agent. The 5 mg kg<sup>-1</sup> methylmercurycysteine treatment induced the greatest number of significantly differentially expressed genes, contrary to expectations based on previous literature findings of lowered toxicity of this methylmercury species.

**1937** NON-LINEAR EFFECTS OF METHYLMERCURY ON PRIMARY RAT CORTEX ASTROCYTES AND NEURONS: ROLE OF GRP78 AND GRP94.

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Methylmercury (MeHg) is a well-known environmental neurotoxicant. It has been suggested that MeHg exerts its neurotoxicity via the generation of reactive oxygen species and depletion of intracellular thiols. While preferentially accumulating in astrocytes, neurons are significantly more sensitive to MeHg's effects. However, the patterns and underlying mechanisms of low-level effects of MeHg in astrocytes and neurons remain unclear. Due to the crucial roles of endoplasmic reticulum (ER) chaperone proteins in protein folding, refolding and trafficking, the objective of this work was to define the differential vulnerability of astrocytes and neurons to low-level MeHg exposure with emphasis on the effects of GRP78 and GRP94, markers of ER. Primary cortical cultures of astrocytes and neurons were exposed for 0.5, 1, 2, 6, 12 and 24 hrs to 10<sup>-17</sup> to 10<sup>-4</sup> M MeHg. Cellular viability and cytotoxicity were determined by MTT and LDH leakage assays. The expression levels of GRP78 and GRP94 were assessed by western blotting. MeHg (>10<sup>-4</sup> M) was toxic to both astrocytes and neurons at all time points. However, at lower concentrations (10<sup>-13</sup>-10<sup>-7</sup> M MeHg), an opposite trend associated with cell protection, namely increased cellular viability (~120% of controls; p<0.05), was noted. Neurons and astrocytes exhibited a similar pattern of GRP78 and GRP94 protein expression levels. Significant induction of these proteins was noted at low-level (10<sup>-16</sup>-10<sup>-10</sup> M) MeHg exposure, and inhibition was associated with exposures to high-levels (10<sup>-7</sup>-10<sup>-4</sup> M) of MeHg at 6, 12 and 24 h. Our results demonstrate that MeHg induces non-linear effects in astrocytes and neurons potentially via the induction and inhibition of ER stress proteins. Accordingly, the role of ER stress signal pathways in MeHg neurotoxicity represents a fruitful area for future study (Acknowledgement: This work supported by Public Health Service of National Institute of Health Grant NIEHS ES07331).

**1938** INVESTIGATION OF CADMIUM-INDUCED OXIDATIVE STRESS AND CHANGES IN RUNX2 mRNA EXPRESSION IN THE HUMAN OSTEOBLAST-LIKE CELL LINE, SAOS-2.

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The heavy metal cadmium is an environmental and occupational toxin. Notable sources include electronic waste and cigarettes. Human exposure to cadmium has been associated with the development of bone diseases including osteoporosis, but the mechanisms by which cadmium exerts a direct effect on bone are unclear. Oxidative stress has been implied in the pathogenesis of osteoporosis, therefore one possibility is that cadmium induces oxidative stress in Saos-2 cells. Furthermore, we explore whether cadmium-induced oxidative damage leads to a decrease in the osteoblast transcriptional factor, RUNX2. This transcriptional factor is a key modulator of osteoblastogenesis and has been reported to play a protective role against osteoporosis in postmenopausal women. We hypothesize that cadmium exposure induces oxidative stress which leads to a decrease in RUNX2 mRNA expression. Saos-2 cells were treated with or without 10 µM CdCl<sub>2</sub> for 0-24 hours. Oxidative stress induced by CdCl<sub>2</sub> was assessed by measuring glutathione (GSH) depletion. RUNX2 mRNA expression was determined by RT-PCR. Cadmium exposure induced oxidative stress, in part, by a depletion of the antioxidant GSH. Ongoing oxidative stress experiments are evaluating reactive oxygen species formation and lipid