



## CEEH Pilot Projects Program Abstracts from Year 13 (April 1, 2007 to March 31, 2008)

**Neurotoxicity of PBDEs in a Genetic Model of Glutathione Deficiency** • Lucio G. Costa, PhD, Professor, Department of Environmental and Occupational Health Sciences, University of Washington

Polybrominated diphenyl ethers (PBDEs) are a group of flame retardants widely used in a variety of consumer products. Levels of PBDE in the environment and in human tissues have been increasing in the USA in the past few decades; particularly high levels are found in infants and toddlers because of exposure through maternal milk and household dust. The greatest concern for potential adverse health effects of PBDEs relates to their developmental neurotoxicity, whose underlying mechanisms are not known, though some preliminary evidence suggests that PBDEs may cause oxidative stress. The aim of this proposal is to investigate the *in vitro* neurotoxicity of PBDEs in a genetic model of glutathione (GSH) deficiency. Neurons isolated from mice lacking the modifier subunit of glutamate cysteine ligase (GCLM) have very low GSH levels and are more susceptible to neurotoxicants that increase oxidative stress. *Gclm* (-/-) mice represent a model for the relatively common human C588T *GCLM* polymorphism, in which individuals carrying the T allele have low GSH levels and are less capable of coping with oxidative insults. Neurotoxicity of various PBDEs will be assessed *in vitro* in cerebellar granule neurons isolated from *Gclm* (+/+) and (-/-) mice. The possible protective role of antioxidants will be determined. The ability of PBDEs to induce reactive oxygen species and lipid peroxidation will be assessed, and the possible protective role of antioxidants toward PBDEs will also be determined. These pilot experiments will allow the initial testing of the hypothesis that genetic polymorphisms leading to low GSH levels may increase susceptibility to PBDE-induced neurotoxicity by enhancing oxidative stress. The *Gclm* null mouse thus represents a useful model for *GCLM* polymorphisms, amenable to further *in vitro*, as well as *in vivo* studies.

**Genetic Polymorphisms as Modifiers of the Effects of PCB and Dietary Trans Fat Exposure on the Risk of Endometriosis** • Victoria L. Holt, PhD, Professor, Department of Epidemiology, University of Washington and FHCRC

Endometriosis is a common gynecological disorder that affects up to 10% of women in their reproductive years. Two novel environmental exposures, dietary intake of *trans* fat and exposure to polychlorinated biphenyls (PCB) via consumption of contaminated fish and animal products, are potential risk factors for endometriosis. We propose to examine variability in response to these

environmental exposures through the investigation of the interaction with genetic polymorphisms involved in estrogen and progesterone biosynthesis and metabolism. Specifically, we will focus on potentially functional polymorphisms in the estrogen receptor alpha (*ESRI*) and the progesterone receptor (*PGR*) genes as well as tagSNPs characterizing the *CYP17a1* gene. We hypothesize that gene variants that alter the estrogen/progesterone ratio or increase the circulating levels of unopposed estrogen will increase the risk of endometriosis among the subset of women exposed to PCBs and *trans* fat.

We propose a study design that maximizes available information regarding genetic variability in the estrogen/progesterone pathways by examining non-synonymous SNPs and other candidate polymorphisms as well as common haplotypes with tagging SNPs. This study provides a unique opportunity to study risk factors for endometriosis, a disease affecting approximately 5 million U.S. women, while offering a good setting to test hypotheses regarding hormone-mediated factors due to the existence of a population-based sample of premenopausal women with available genomic DNA samples. By combining environmental risk factors with genetic polymorphisms related to hormone biosynthesis and metabolism we will attain a comprehensive picture of the causal mechanisms of endometriosis, and perhaps identify subgroups towards whom prevention programs should be targeted.

### **Pre- and Postnatal PCB Exposure and Antibody Responses in Slovak Infants •**

Stephen Marc Schwartz, PhD, Professor, Department of Epidemiology, University of Washington and FHCRC

The goal of the proposed research is to better understand the potential immunotoxic consequences of developmental polychlorinated biphenyl (PCB) exposure. Only a handful of epidemiologic studies have directly assessed immune function in infants following *in utero* and lactational exposures to PCBs, and even fewer have assessed the impact of these exposures on infant and child vaccinations. In the proposed research, we will determine the association between PCB exposure and immune responses to Bacillus Calmette-Guerin (BCG) vaccination in 6-month-old infants. PCB exposure has already been estimated from measurement of specific congeners in blood from 1000 pregnant mothers, umbilical cord blood from infants, and blood from children at 6 months of age. Measurements of the immune response of interest—post-vaccination IgG and IgA antibody levels to BCG, quantified by ELISA—will be performed with the funding provided by the grant, and serve as a biomarker for immunologic function in this study. Multivariate models incorporating data from PCB measurements, antibody response, maternal questionnaires, medical chart abstraction (mother), and from pediatricians' records will be fit to test the specific aims. This collaborative, multidisciplinary project draws on the expertise of investigators in environmental epidemiology (Drs. Schwartz, De Roos, and Hertz-Picciotto), immunotoxicology (Drs. Lawrence and Silverstone), and functional proteomics (Dr. Farin) at several

U.S. universities. Results from this study will provide much-needed clinically relevant outcome measures of immune function in relation to pre- and postnatal PCB exposure.

**Genetic Polymorphisms, Mercury Exposure and Porphyrinuria in Children with Autism** • James S. Woods, PhD, Research Professor, Department of Environmental and Occupational Health Sciences, University of Washington

Autism, a serious developmental disorder, afflicts one in 166 children in the United States. Environmental factors are hypothesized in the etiology of autism, and inheritance studies suggest that such factors impact principally persons who are genetically susceptible to this disorder. One such factor, mercury, has been linked to increased risk of autism, although genetic factors affecting this susceptibility have not been defined. We have shown that mercury in any form impairs the heme pathway enzyme, coproporphyrinogen oxidase (CPOX), producing a change in the urinary porphyrin excretion pattern that is both specific and selective for mercury exposure. Additionally, we identified a novel polymorphism (CPOX4) in the human CPOX gene that is associated with impaired neurobehavioral functions, exacerbates mercury neurotoxicity, and produces an atypical change in the porphyrin excretion pattern in persons with mercury exposure. This precise change in the porphyrin excretion pattern has been independently observed in several populations of autistic children. These findings suggest that CPOX4 may increase the risk of developing autism by increasing sensitivity to mercury-mediated neurologic deficits. The proposed project will test the hypothesis that autistic children have a greater prevalence of CPOX4 than observed among neurotypical control subjects and that the altered porphyrin excretion pattern observed among autistic children associates with CPOX4 and mercury exposure. The study will be conducted in collaboration with the UW Autism Center, which will provide DNA and urine sample from autistic children. Genotyping for CPOX4 will be conducted by the CEEH Functional Genomics Core. The proposed research directly addresses CEEH programmatic goals by focusing on a gene-environment interaction that may play a role in the etiology of autism and will establish both theoretical and collaborative grounds for an NIEHS grant application to define how CPOX4 increases susceptibility to autism in relation to mercury exposure.