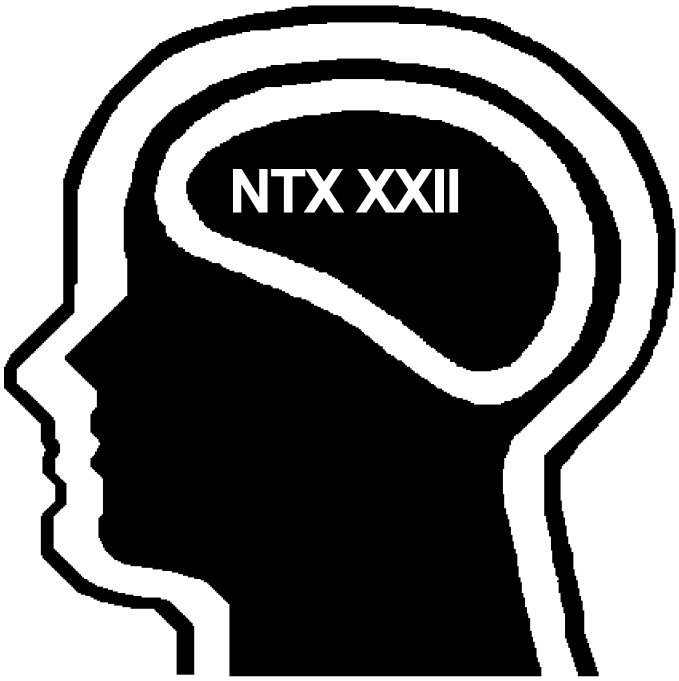
Available online at www.sciencedirect.com
ScienceDirect
NeuroToxicology 27 (2006) 875–930
NeuroToxicology

TWENTY-SECOND INTERNATIONAL NEUROTOXICOLOGY CONFERENCE

Environment and Neurodevelopmental Disorders

September 11–14, 2005 North Carolina, USA



Abstracts

0161-813X/$ – see front matter
doi:10.1016/j.neuro.2006.03.020

Openning

***SESSION I:* OPENING OF THE 22ND CONFERENCE, RECOGNITION OF SPONSORS AND ORGANIZERS**

Conference Chair: Joan M. Cranmer, Ph.D.

1

TWENTY-SECOND INTERNATIONAL NEUROTOXICOLOGY CONFERENCE: ENVIRONMENT AND NEURO-DEVELOPMENTAL DISORDERS OVER THE LIFESPAN. Joan M. Cranmer, *Departments of Pediatrics and Pharmacology/Toxicology, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, AR, USA*

The twenty-second annual meeting (NTX XXII) in this *International Neurotoxicology Conference Series* convenes September 11–14, 2005 in Research Triangle Park, North Carolina. The theme of this year's conference is *Environment and Neurodevelopmental Disorders over the Lifespan*.

This conference of internationally recognized experts, clinical and basic science researchers, clinicians, epidemiologists, neuroscientists, teratologists, regulatory scientists, public health and policy experts, health-care providers and other informed and interested individuals will provide data and information to apply to important scientific, environmental, epidemiological, clinical, methodological, policy, regulatory, risk assessment, and future funding issues.

Overall, speakers in this conference will present an integrated overview of the multidisciplinary approaches needed to understand risk factors contributing to developmental disorders and neurodegenerative diseases.

Understanding the relationship among low-level exposure to environmentally persistent chemicals, their critical molecular targets, ensuing cellular dysfunction, and defining often subtle consequences on animal and human neurodevelopment and subsequent aging is perhaps one the most challenging goals of modern toxicology.

The Conference Organizers and contributors have developed exciting symposia, platform sessions, workshops, poster presentations, student award competition, roundtable discussions, and an open public forum. Research needs will be identified for each topic. The meeting report, abstracts, session summaries, research needs, and papers rapidly will be published in *NeuroToxicology.*

NTX XXII Symposium, Workshop, Platform Session, Poster Session and Public Forum Topics Follow:

- Neurotoxicants and Learning and Developmental Disabilities: Translating the Science into Education and Public Policy.
- PBPK/PD Models for Developmental Neurotoxicology: Risk Assessment Strategies and Research Recommendations.
- Environmental Perturbations of the Immune System: Implications for Autism and Neurodevelopmental Disorders.
- Neurotoxicant Exposures in Military Deployments and Putative Associations with Neurodegenerative Diseases.
- Aquatic and Invertebrate Models of Developmental Neurotoxicity for Mechanistic and High Throughput Studies.
- Endocrine Active Compounds and their Effects on Brain Development: Integration of Methods and Approaches.
- Molecules to (Wo)man: Animals & Humans *Dissecting the Dysfunction to Look at the Whole Picture*.
- Contemporary Health Issues Associated with Over Exposure to Manganese.
- *Developmental Toxicology Technical Workshop:* Optimizing the Design and Interpretation of Epidemiological Studies for Assessing Neurodevelopmental Effects from in utero Chemical Exposure.
- Neurotoxicity of Mixtures, Solvents, and Metals in vivo and in vitro.
- Environmental Toxicants and Diseases.
- General Poster Session.
- Pre-Doctoral and Postdoctoral Student Award Competition.

A Sunday evening "*Meet, Greet & Eat*" and Tuesday social evening featuring a "*North Carolina Pig Pickin*" as well as post-conference tours of USEPA laboratories and CIIT Centers for Health Research will round out the 22nd conference.

Public Forum

***SESSION II:* NEUROTOXICANTS AND LEARNING AND DEVELOPMENTAL DISABILITIES: TRANSLATING THE SCIENCE INTO EDUCATION AND PUBLIC POLICY**

Co-Chairs: Elise Miller, M.Ed.
J. Peterson Myers, Ph.D.

Theme: *Researchers are often reluctant to become involved in educating the general public about technical subjects or the intersections of science and public policy for fear that such activities will take them away from more important work or raise questions about their objectivity. This session will highlight why researchers can and need to play an important role in helping to translate science into stronger public policy. After an overview of the science and related policies, including examples of how the precautionary principle can be implemented in this context, panelists will describe model environmental health programs initiated by learning and developmental disabilities organizations. This public forum is open not only to researchers and scientists, but to educators, administrators, parents of children with LDDs and anyone else who is concerned about environmental contributors to the apparent rise in LDDs.*

2

OVERVIEW OF EMERGING SCIENCE ON NEUROTOXICANTS IN RELATION TO LEARNING AND DEVELOPMENTAL DISABILITIES. Ted Schettler, *Science and Environmental Health Network, Newburyport, MA, USA*

Brain development begins early in fetal life and continues throughout gestation, infancy, childhood, and adolescence. Cell proliferation, migration, differentiation, synapse formation and pruning, apoptosis, and myelination progress in specific timeframes, which differ in various areas of the brain. The processes involved in brain development are under the control of genetic and environmental factors, which interact in complex ways. Pre- and post-natal nutrition, exposure to neurodevelopmental toxicants, infectious agents, and radiation are among environmental factors that can influence brain development and function. The impacts of lead, alcohol, and maternal smoking on brain development are well known, although their mechanisms of action continue to be explored. Neurodevelopmental impacts of mercury, PCBs, and some pesticides have also been described in laboratory animal models and epidemiologic studies. Interactions among nutritional factors, such as iron deficiency, social factors, such as maternal stress, and exposure to neurodevelopmental toxicants are of considerable interest because of their public health and research implications. Research study design and public health responses will need to consider these complex interactions among genetic and environmental factors. Keywords: Neurodevelopment; Neurotoxicants; Learning disabilities

3

AUTISM, GENES AND THE ENVIRONMENT. Martha R Herbert*, JP Russo, S Yang, J Roohi, M Blaxill, SG Kahler, L McCoy, DA Ziegler, E Hatchwell. *CMA & Pediatric Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA*

Autism spectrum disorders (ASD) are behaviorally defined syndromes with no clearly established biomarkers whose heterogeneous biological causes may include environmental as well as genetic factors. Candidate genes have generally been chosen from genes directly related to the central nervous system, but the apparent increase in autism incidence supports examination of environmental factors including those that may not primarily target the brain. The NIEHS has implemented the Environmental Genome Project (EGP) to study genetic susceptibility to environmental disorders and has identified a set of ''environmental response genes that may include previously uninvestigated candidate genes for autism''. *Methods:* This study utilizes bioinformatics methodologies to identify genes with functional SNPs from three environmentally relevant genome databases (NIEHS Environmental Genome Project, SeattleSNPS (inflammatory related), and Toxicogenomics) that are in linkage regions identified in published autism genome scans. We will also report the results of genotyping SNPs of TNFalpha, a gene that is found both in an autism linkage region and in all three databases. *Results:* Sixty-seven genes with functional SNPs were identified that had not previously been studied in autism. We will present this candidate gene list, selected pathway analyses oriented toward discerning relationships with metabolic and neurological abnormalities in autism, and the results of genotyping. *Conclusion:* Finding multiple overlaps between autism and environmental genomics suggests that ASD brain abnormalities could be downstream from metabolic or regulatory changes that are more widespread, or that originate in other systems (e.g. immune) and could be modulated by environmental factors. This deserves systematic investigation. Keywords: Autism, Environmental genomics and inflammation. *Funding: Cure Autism Now, Cody Autism Center.*

4

OVERVIEW OF THE LEARNING AND DEVELOPMENTAL DISABILITIES INITIATIVE. Elise Miller, Executive Director; *Institute for Children's Environmental Health, Freeland, Washington, USA*

Learning and developmental disabilities (LDDs) appear to be on the rise, affecting approximately one in six children in the U.S. under the age of 18. Emerging research suggests that certain neurotoxicants such as lead, mercury, pesticides, polychlorinated biphenyls (PCBs), brominated flame retardants and solvents can have a particularly detrimental impact on brain function and in turn lead to LDDs. Recent studies show that environment exposures can also impact the health of those who already have LDDs. LDD groups have traditionally focused on identifying kids with LDDs and getting them the services they need – something that is, of course, very important but does not address the increasing prevalence of LDDs. For this reason, the Learning and Developmental Disabilities Initiative (LDDI), was established in 2002 to encourage the LDD sector to look collectively upstream and help prevent toxic threats to child development through educational and public policy-oriented efforts. LDDI, one of the main working groups of the Collaborative on Health and the Environment (CHE), now has almost 200 organizational and individual members. Participants in LDDI include scientists, health-care providers, LDD groups, environmental health and justice advocates and community-based organizations. LDDI received the U.S. EPA's Children's Environmental Health Recognition Award in 2005 and is coordinated nationally by the Institute for Children's Environmental Health. Keywords: Learning disabilities, Developmental disabilities, Science and policy

5

AUTISM SPECTRUM DISORDERS AND NEUROTOXICITY AND ENVIRONMENTAL HEALTH ISSUES: IS THIS THE REASON FOR THE EPIDEMIC? Lee Grossman, President & CEO; *Autism Society of America, Bethesda, MD, USA*

Autism spectrum disorders (ASD) is thought to be a genetically based, neurological and lifelong condition that has reached alarming levels of incidence. Recent studies have put

the incidence at as many as 1 in 166 births will be diagnosed with ASD. This dramatic, significant and unabated rise in individuals diagnosed with ASD is creating an economic burden on society and produced a national heath crisis. Assuming the basis of ASD is genetic, what has happened recently that would so profoundly affect these large numbers to occur? Reasonable attention has been focused on the possibility of neurotoxicants and/or environmental health concerns as culprits in damaging the gene construct and ''triggering'' the symptoms known as ASD. How are the government and scientific community responding and what needs to be done?

6

THE LEARNING DISABILITIES ASSOCIATION OF AMERICA'S HEALTHY CHILDREN PROJECT. Kathy Lawson, Healthy Children Project Director; *The Learning Disabilities Association of America, 4156 Library Road, Suite 1, Pittsburgh, PA, USA*

Toxic environmental exposures pose a serious public health threat. Starting in utero and continuing through the developmental years, exposures to various environmental hazards – ranging from alcohol and tobacco to lead and pesticides – are beginning to be recognized within the scientific community as potential causes to learning problems, attention deficits and developmental delays. However, there is an immense ''disconnect'' and unacceptable delay between scientific data and public awareness and prevention. The Healthy Children Project of The Learning Disabilities Association of America was created to help fill this gap by increasing awareness and enabling women of child-bearing years, their families, and the health care professionals who serve them make more informed and safer choices that protect our children and future generations from exposure to potentially harmful toxics and health risks. This presentation will explain methods used by LDA Healthy Children Project Partners, comprised of LDA state affiliates with a particular interest in how exposure to environmental toxics relates to the increase in learning disabilities, in order to address this need to better inform the community. Keywords: Learning disabilities, Community outreach, Collaboration

7

NEUROTOIC EXPOSURES AND DEVELOPMENTAL DISABILITIES. Michele Gagnon, Director, *Environmental Health Initiative American Association on Mental Retardation, Washington DC, USA*

Mental Retardation and other developmental disabilities (DD) affect approximately 1.5 million children under the age of 18 and appear to be increasing. Mental retardation is the most common DD affecting approximately 2% of the total US population. The National Academy of Sciences predicts that 25% of developmental and neurological deficits in children are due to the interplay between chemicals and genetic factors and that 3% are caused by exposure to chemicals alone. Given that we know exposures to many neurotoxicants contribute to mental retardation and other developmental disabilities, the American Association on Mental Retardation (AAMR) has launched a national program to reduce toxic exposures that may contribute to or exacerbate cognitive problems. The main goals of the initiative are to engage the developmental disabilities sector in the growing national movement to eliminate toxics that may contribute to and worsen neurological problems and disabilities, and to promote better health by reducing toxic exposures for those living with disabilities. Keywords: Mental retardation, Neurotoxicants, Vulnerable populations

Symposium

SESSION III-A: **PBPK/PD MODELS FOR DEVELOPMENTAL NEUROTOXICOLOGY: RISK ASSESSMENT STRATEGIES AND RESEARCH RECOMMENDATIONS**

Co-Chairs: William Slikker, Jr., Ph.D.
Donald R. Mattison, M.D.

Theme: *Over the past decade regulatory agencies in developed countries have recognized that infants, children, and adolescents handle chemicals differently than adults (indeed, adults are not pharmacologically homogeneous). While recognizing those pharmacological differences has led to increased attention to preclinical and clinical data gaps, the information needed to fill those gaps can not be simply addressed by testing chemicals in pediatric populations. The concepts of efficacy and safety must be reformulated in the context of development, and new approaches for preclinical and clinical testing (including clinical trial designs) developed and validated in immature animals and humans. These testing methods, especially for the nervous system, must be grounded on an understanding of healthy developmental trajectories as well as the impact of disease and treatment on healthy development. Clearly this constraint suggests that there is little of relevance from adult pharmacology and it is necessary to develop preclinical and clinical testing for pediatric pharmacology and developmental neurotoxicology.*

8

COMPUTATIONAL TOOLS FOR COMPARISONS ACROSS STAGES OF NEURODEVELOPMENT. Julia M. Gohlke, *Environmental Systems Biology Group, Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, RTP, NC, USA*

A systems biology framework emphasizes the linkage of diverse datasets across various levels of organization including molecular, cellular, organ, and organismal. The development of computational approaches for application of this systems biology framework may ultimately provide a means for predictive models of neurodevelopmental processes. Here,

computational approaches are utilized to quantify molecular and cellular components important for the proliferation and differentiation of the developing forebrain, allowing for comparisons of susceptibility across developmental time and across molecular targets. At the cellular level, computational approaches have been developed using detailed experimental data on cell cycle and cell death rates to predict the acquisition of neuronal number in the developing dorsal forebrain. This tool allows for a direct and quantitative comparison of differential toxicant-induced susceptibility across the neurogenesis and apoptosis stages of mammalian forebrain development. Using gene expression data of components important for forebrain neuronal specification, evaluation of several approaches for quantitative data analysis at the molecular level has been performed. Gene regulatory networks (GRNs) have been built from compilation of the current literature on regulation of forebrain development using diverse experimental approaches, including transgenics, in-situ hybridization, and utilization of specific agonists and antagonists to specify interactions within the overall network. Application of a Bayesian algorithm has also been evaluated to discover the optimal GRN utilizing only microarray data from brain tissues of several transgenic mouse strains. By quantifying each network it is possible to directly compare these two methodologies. For evaluation of data at the molecular level, a literature-based approach is particularly useful in testing current hypotheses of gene regulation and refining the network structure, whereas algorithm-based approaches are particularly suited for developing hypotheses of potential novel linkages thereby directing future experimental research.

9

INCLUSION OF ''OMICS'' DATA IN MODEL DEVELOPMENT FOR THE NERVOUS SYSTEM. Rory B. Conolly, *National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC 27711, USA*

Physiologically based pharmacokinetic (PBPK) models quantify how anatomical, physiological, and biochemical factors influence the relationship between external dose and the amount of chemical reaching key target sites within the body. Systems biology and the ''omic'' technologies now provide a capability for describing at the biochemical level the pharmacodynamic relationship between a chemical at its target site and the ultimate biological effect. For example, computational models for signaling pathways such as MAPK and NF-κB have been developed. These models generate interesting dynamic behaviors including oscillations, bistability, history-dependence, and switch-like dose–response. While the available data describing the scale-up of these behaviors to all the cells in a tissue is limited, some reports suggest that signaling-mediated behaviors across large numbers of cells are coordinated. For example, the induction of hepatic CYP1A1 and 1A2 in rats exposed to TCDD spread from the central vein outwards as the dose of TCDD was increased. The interface between induced and uninduced cells was distinct, indicating that the responses of individual cells were coordinated. This behavior is consistent with bistable behavior of the network that controls CYP 1A1 and 1A2 levels in rat liver, with TCDD providing the extracellular signal that coordinates the responses of individual cells. Computational models of signal transduction pathways and other biochemical networks can thus be developed as natural extensions of PBPK models, providing more mechanistically based descriptions of the entire exposure-tissue dose–response continuum. Keywords: Systems biology, Computational modeling. *This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.*

10

PB/PK MODELING OF EARLY LIFE STAGES IN RODENTS. Hugh A. Barton, *National Center for Computational Toxicology, U.S. Environmental Protection Agency, RTP, NC, USA*

Current risk assessments based upon toxicity studies with multiple generations such as the developmental neurotoxicity (DNT), one- or two-generation reproductive/developmental, and in utero developmental studies rely on the exposure dose to the mother to define acceptable exposure levels for humans. Extrapolation of such studies to humans is dependent upon information about the window of susceptibility for observed effects, external exposures and pharmacokinetics for the compound during that window, and the toxicodynamic processes leading to the effects. Developmental processes occur in generally similar sequences across mammalian species, but birth occurs at somewhat different points during the process. Thus, some processes occur in utero or postnatally, while other processes occur postnatally in rodents and in utero in humans. If there is a well defined window of susceptibility for an effect, extrapolation across species can include describing the relevant pharmacokinetics in each species. If the critical window is not known, then based upon pharmacokinetic considerations, at least three general periods can be defined for the offspring: in utero exposure, lactational transfer, and postweaning exposure. Knowledge of the exposures and internal dosimetry during these periods can be useful for toxicity study design and evaluating uncertainty in the risk assessment using maternal exposure. Pharmacokinetic modeling, including physiologically based (PBPK) modeling, of early life stages in rodents (and humans for purposes of extrapolation) provides an approach for describing dosimetry during these periods. Types of information that are needed include quantitative descriptions of physiological parameters (e.g., body weight, body water, tissue volumes, blood flows, serum protein levels) and biochemical or chemical species parameters (e.g., metabolism, tissue distribution, lactational transfer). A range of in vivo and in vitro studies can provide useful data. Examples of the potential applicability of these approaches include organophosphate insecticides, methanol, perchlorate, and perfluorooctanoate. Keywords: Pharmacokinetics, Life

stages, Modeling. (*This work has been supported by the United States Environmental Protection Agency. While it does not necessarily represent the views of the Agency it has been subjected to Agency review and approved for presentation.*)

11

WHICH PBTK MODEL OUTPUTS SHOULD BE CONSIDERED AS INPUTS FOR PHARMACODYNAMIC MODELING OF NEURODEVELOPMENTAL EFFECTS? Dale Hattis, *George Perkins Marsh Institute, Clark University, Worcester, MA, USA*

There has been a tendency to model pharmacodynamic responses as depending on one of only two measures of delivered dose: (1) peak concentrations in some compartment in a particular ''window of vulnerability'' (C_{max}), or (2) the integrated area under a concentration $\times$ time curve (AUC) within a hypothesized time window. This talk will consider a more general formulation that allows some cumulation of internal dose X an age-specific sensitivity factor as initially explored by Luecke in the 1990s. It is important for modelers and experimentalists to make progress in understanding how specific alternative hypotheses about the mechanisms of particular developmental effects should be represented mathematically in the form of dose-time/age-response relationships. A preliminary taxonomy of mechanistic hypotheses will be offered to help structure discussion of this issue. Keywords: Modeling, PBTK, Pharmacodynamics

12

THE NEXT GENERATION OF MODELS: VISION OF THE FUTURE. Donald R. Mattison, *NIH/NICHD*

Workshop

SESSION III-B: **DEVELOPMENTAL EFFECTS ON THE IMMUNE SYSTEM: IMPLICATIONS FOR AUTISM AND NEURODEVELOPMENTAL DISORDERS**

Co-Chairs: G. Jean Harry, Ph.D.
Monica Carson, Ph.D.

Theme: *In recent years, evidence for the role of infectious and inflammatory processes as mediators of brain injury has been growing. The immune response within the brain as well as linkages between the immune and nervous system are becoming well documented. With regards to development, clinical correlations between fetal plasma cytokines and neurological outcomes in the premature infant have been established. Recent work suggests that maternal infection and inflammatory responses in the offspring are associated with increased risk for diseases such as schizophrenia and autism. Several studies have reported systemic immunologic aberrations in autism spectrum disorders (ASD) that are associated with both autoimmunity and with dysfunctional immunity such as abnormalities or deficits of function in immune cell subsets. The following sessions will focus on work underlying these hypotheses in both the human clinical setting as well as the establishment of experimental animal models. The translation of adverse effects as the result of an innate immune response in the brain following exposure to environmental agents and the contribution of the immunological competence as a factor determining susceptibility will be discussed. Such interactions may contribute to the phenotypic differences of diseases seen in the human population.*

This session is sponsored by the National Institute of Environmental Health Sciences (NIEHS).

13

MICROGLIA: A HETEROGENEOUS POPULATION OF CNS-SPECIFIC MACROPHAGES. Monica Carson, *University of California – Riverside, CA, USA*

Microglia are the resident tissue macrophage of the CNS. Their activation is an early and common feature of nearly all neuropathologies. We and others have demonstrated that microglia are phenotypically and functionally distinct from other tissue and CNS-infiltrating macrophage populations. Using both in vivo and in vitro assays of macrophage function, we demonstrate that even when found in the same CNS environment, resident microglia and CNS-infiltrating macrophages display distinct effector functions. To define these differences on a molecular level, we have used an open profiling method (TOGA) to compare microglial and macrophage gene expression. To date, we have detected over 19,000 novel and known molecules expressed in microglia (adult and neonatal), several populations of macrophages and dendritic cells. From these studies, we have identified patterns of gene expression that can distinguish microglia from other myeloid populations. Our studies also reveal that microglia are highly heterogeneous in vivo. We are currently examining to what extent microglial phenotypes are a consequence of different lineages versus different environmental cues.

14

PRENATAL EXPOSURE TO MATERNAL INFECTION AND CORTEX DEVELOPMENT. John H. Gilmore, *UNC Schizophrenia Research Center, University of North Carolina, Chapel Hill, NC, USA*

Maternal infection during pregnancy increases risk of many neurodevelopmental disorders, including autism and schizophrenia. We have hypothesized that cytokines, generated by the maternal immune system are alter cortical brain development and increase risk for neurodevelopmental disorders. In vitro, we have demonstrated that IL-1β, Il-6, and TNFα, decrease survival of cortical neurons, as well as dopaminergic and serotonergic neurons. These inflammatory cytokines also significantly decrease dendritic complexity of developing cortical neurons. Using in vivo models, we have shown that maternal infection alters cytokine and neurotrophic factor expression in the fetal brain environment. New data from our ongoing studies will also be presented.

15

AUTISM AND THE IMMUNE SYSTEM: AN OVERVIEW. Kimberly A. Stigler, *Indiana School of Medicine, Indianapolis, IN, USA*

Although autistic disorder was first described over 60 years ago, its etiology remains unknown. Investigators have long hypothesized that the disorder may have a neuroimmune pathophysiology. Early reports of an association between autism and congenital viral infections led to further research into immune-related etiologies. Studies to date have explored the role of viruses, neuroimmune factors, immunogenetics, and cellular and humoral immunity in the development of this lifelong disorder. These exploratory studies will serve to direct future research into possible immunologic factors in autism.

16

NEUROINFLAMMATORY AND NEUROGLIAL CNS RESPONSES IN AUTISM. Carlos Pardo-Villamizar, *Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Autism is a neurodevelopmental disorder characterized by marked impairment in language and verbal communication, social skills, and behavior. The etiology of autism remains unknown, despite evidence that genetic, environmental and immunological factors may play a role in its pathogenesis. Our laboratory has recently demonstrated the presence in autistic patients of neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of brains obtained at autopsy. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that MCP-1 and TGF-β1, mainly derived from activated neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique pro-inflammatory profile of cytokines, including a marked increase in MCP-1. Our findings indicate an increase in neuroglial activation and innate neuroimmune responses in brains of autistic patients, changes that suggest the presence of an active and chronic neuroinflammatory process perhaps as part of pathogenic mechanisms associated with this disorder. These findings suggest that future therapeutic strategies might involve modifying neuroglial reactions in the brains of autistic patients.

Workshop - continued

SESSION III-B: **DEVELOPMENTAL EFFECTS ON THE IMMUNE SYSTEM: IMPLICATIONS FOR AUTISM AND NEURODEVELOPMENTAL DISORDERS**

Co-Chairs: Cindy Lawler, Ph.D.
Judy van de Water, Ph.D.

This session is sponsored by the National Institute of Environmental Health Sciences (NIEHS).

17

A CASE-CONTROL STUDY OF ANTIBODIES TO CENTRAL NERVOUS SYSTEM PROTEINS AND MEASLES VIRUS IN CHILDREN WITH AUTISM. William McMahon, *Department of Neurology, University of Utah, UT, USA*

The mechanism(s) underlying the pathogenesis of autism are unknown. While genetic factors appear likely, measles and other environmental exposures have been reported to contribute to the etiology by triggering autoimmunity or through some other mechanism. The University of Utah Autism Research Program is testing antibody titers to CNS proteins as well as to measles and diphtheria toxoid, using an enzyme linked immunosorbent assay (ELISA). CNS antigens for ELISA are prepared from bovine brain and included an axolemma enriched and myelin enriched fraction, as well as purified myelin basic protein (MBP) glial fibrillary acidic protein (GFAP). The reagent for measles (Edmonston strain) for is derived from virus obtained from the American Type Culture Collection. Diptheria toxoid is obtained from List Biological Laboratories. Subjects were children from 3 years of age to 15 years of age. Autistic Disorder is diagnosed using DSM-IV criteria, parent interview using the Autism Diagnostic Interview-Revised and direct assessment using the Autism Diagnostic Observation Scale. Controls are typically developing children matched in age and sex, and show no evidence of autism. Preliminary results indicate no increase in any antibody titers in children with autism. There is no correlation between brain autoantibodies and anti-measles virus antibodies. Surprisingly, autoantibodies to MBP and GFAP tend to be lower in children with autism compared to typically developing children. Our preliminary results do not replicate published reports of increased antibodies to measles, MBP, or GFAP. We are working to increase our sample size and to add an autism subgroup with a history of regression at onset of autism.

18

SUBOPTIMAL IGG RESPONSE TO BACTERIAL VACCINE ANTIGENS IN PATIENTS WITH AUTISM SPECTRUM DISORDER (ASD). Judy Van de Water, *Internal Medicine and M.I.N.D. Institute, UC Davis, Davis, CA, USA*

There is a growing awareness of an immunological involvement in children with ASD. Systemic immunological aberrations in ASD have been linked with both increased autoimmunity, generating antibodies that are reactive to brain or CNS proteins with the potential to inhibit or damage neuronal tissue, and secondly, with dysfunctional immunity such as abnormalities or deficits of function in immune cell subsets, that could lead to an inappropriate or ineffective immune response to pathogen challenge. To better define the humoral immune status of children with ASD, we examined by ELISA the serologic response of patients and age-matched typically developing (TD) controls to common vaccine antigens. These included Bordetella, Diptheria, Tetanus,

Measles, Mumps and Rubella. All children analyzed were vaccinated with DTaP and MMR. Based on vaccination schedules, comparisons were made between patients and typically developing controls as well as siblings and patients with MR/DD. The most striking differences were observed in patients with ASD who had a significantly lower IgG response to Bordetella ($p \leq 0.004$), Diptheria ($p \leq 0.004$) and Tetanus ($p \leq 0.004$) than TD controls. This correlated with an overall reduction in IgG, IgM and IgA levels in patients with ASD when compared to the TD and MR/DD controls. Interestingly, the siblings of patients with ASD had similarly reduced plasma levels of IgG. When the IgG response to the viral vaccine antigens Measles, Mumps and Rubella was analyzed, there was an apparent increase in reactivity in both the patients with ASD and their siblings. However, when elapsed time from vaccination was taken into account, this increase was not significant. In conclusion, while most patients with ASD were positive for the bacterial vaccine antigens analyzed in this study, their responses were significantly lower than the TD controls. This data demonstrates that patients with ASD have a suboptimal immune response when exposed to bacterial antigens.

19

MATERNAL IMMUNE STATUS DURING PREGNANCY AND CHILDHOOD AUTISM. Lisa Croen, *Kaiser Permanente Division of Research, Oakland, CA, USA*

To explore the association between maternal autoimmune and allergic diseases and childhood autism spectrum disorders (ASD), we conducted a case-control study among children born at a Kaiser Permanente Northern California (KPNC) facility between 1995 and 1999. Cases ($n = 407$) were children with an ASD diagnosis (ICD-9-CM 299.0, 299.8) recorded in KPNC outpatient databases. We randomly sampled controls ($n = 2095$) from the cohort of births without an ASD, frequency matched to cases on gender, birth year, and hospital of birth. Maternal autoimmune and allergic diseases diagnosed from 2 years preceding delivery to 2 years following delivery, as well as information on several maternal and infant characteristics, including maternal medication use in the year prior to delivery, was obtained from health plan and vital statistics databases. A similar proportion of case and control mothers had a diagnosis of any autoimmune disease in the 4-year period surrounding pregnancy (10.3% versus 8.2%, $p = 0.15$). Significantly more case than control mothers were diagnosed with psoriasis (2.7% versus 0.95%, $p = 0.004$), type 1 diabetes (1.2% versus 0.43%, $p = 0.048$), asthma (15.5% versus 10.5%, $p = 0.003$), and allergic rhinitis (20.9% versus 14.5%, $p = 0.001$). After adjusting for maternal age, race/ethnicity, education, medication use, and plurality, only one autoimmune condition, psoriasis, was significantly associated with ASDs (adjusted OR = 2.7, 95% CI 1.3–5.8). Maternal second trimester diagnoses of asthma or allergy were twice as common in cases than controls (asthma: adjusted OR = 2.2, 95% CI 1.1–4.2; allergy: adjusted OR = 2.5, 95% CI 1.2–5.2). The frequency of maternal asthma and allergies increased significantly with increasing numbers of ASD-affected children in the family (asthma: $\chi^2_{\text{trend}} = 8.36$, $P = 0.004$; allergies: $\chi^2_{\text{trend}} = 7.51$, $p = 0.006$). These results suggest that maternal immune function during pregnancy is associated with risk of ASD. To further explore this hypothesis, we are currently measuring levels of several immune markers (cytokines, chemokines, immunoglobulins, and autoantibodies) in blood specimens that were drawn during the second trimester of pregnancy. Preliminary results from this investigation will be presented.

Platform Session

SESSION IV-A: **CHILDREN'S ENVIRONMENTAL HEALTH**

Co-Chairs: Cynthia Bearer, M.D., Ph.D.
William Suk, Ph.D., M.P.H.

20

ENVIRONMENTAL ACCUMULATION AND SYNERGY OF MULTIPLE NEUROTOXICANTS AND CHILDREN'S LEARNING ACHIEVEMENT IN NEW ORLEANS, LOUISIANA, USA. Howard W. Mielke, Eric Powell, Shafania Alston, Jassma Manuel, Chris Gonzales, *College of Pharmacy, Xavier University of Louisiana, New Orleans, LA, USA*

Because of the inclusion of large quantities of metals into common products such as paint and gasoline, the urban environment has become a sink for multiple metals thereby notably altering the geochemistry of the city. Geochemical surveys show that the metals are unevenly distributed in the city and that the largest reservoir is located in relatively undisturbed soils that are commonly found on residential properties within the inner ring of communities surrounding the central business district. The purpose of this presentation is to describe the pattern of the neurotoxicants lead and mercury in New Orleans, to illustrate the association between accumulated lead in the environment and children's exposure burden, to elucidate the many potential biochemical disruptions due to the synergy between lead and mercury, to illustrate the association between seasonal variation of blood lead and soil moisture, and to show the association between multiple metal accumulation and learning achievement of elementary students (a surrogate measure of neurodevelopmental disorders) across the entire New Orleans metropolitan area. The geochemical surveys of New Orleans provide the foundation for developing a rational program for preventing children's metal exposure and thereby reduce the prevalence of neurodevelopmental disorders that are associated with metal neurotoxicants. Keywords: Lead and mercury, Urban geochemistry, Learning disorders

21

CLEARANCE OF NEUROTOXINS BY PHOSPHOLIPID EMULSION IN AUTISM AND PDD. Kane PC, Braccia D, Cartaxo A, Kane E, *Haverford Wellness Center, 2010 West Chester Pike, Suite 310, Havertown, PA 19083, USA*

Examination of RBC lipids at Kennedy Krieger Institute Peroxisomal Diseases Laboratory in our ASD and PDD subjects has revealed an accumulation of very long chain fatty acids (VLCFA), which comprise lipid rafts or ceramides indicating membrane derangement. Membrane phospholipid abnormalities with elevation of VLCFA is indicative of exposure to neurotoxins resulting in suppressed beta oxidation of VLCFA. We have embarked on a clinical treatment plan for the past 4 years of therapy including both oral and intravenous lipid therapy to address the accumulation of ceramides/VLCFA with phospholipids (IV phosphatidylcholine), methylation (IV leucovorin) and sulfation (IV rGlutathione) to address hepatic clearance of microbes, chemicals and heavy metals and to facilitate stabilization of phospholipids in cellular membranes. Weekly infusions of phosphatidylcholine are administered by Phospholipid Exchange and followed by Leucovorin (folinic acid) and rGSH Fast Push. Oral targeted treatment protocols are utilized after red cell lipid analysis has been completed. We have noted dramatic and sustained clinical improvement within the first few weeks after initiation of treatment in our patient population of 300 subjects. These results demonstrate that Lipid therapy in addition to supporting methylation and sulfation may reverse prevalent symptoms in individuals with ASD/PDD. Keywords: Methylation, Phosphatidylcholine, Autism

22

SENSITIVITY ANALYSES IN STUDIES OF CONTINUOUS OUTCOME MEASURES: THE EXAMPLE OF METHYL-MERCURY EXPOSURE AND NEUROPSYCHOLOGICAL TESTING IN CHILDREN. Michael Goodman[1], Pamela J. Mink[2], Leila M. Barraj[2], Nicole L. Britton[2], Janice W. Yager[3], Michael A. Kelsh[2], *[1]Emory University, Rollins School of Public Health, Atlanta, GA, USA; [2]Exponent, Inc., Washington DC, USA and Menlo Park, CA, USA; [3]Electric Power Research Institute, Palo Alto, CA, USA*

Regulatory decision-making is often complicated by inconsistent findings reported across epidemiological studies. One example is the apparent lack of agreement among studies of dietary methylmercury exposure and neuropsychological testing results in children. Although these inconsistencies can be explained by differences in exposure and population characteristics, it is also important to acknowledge the potential role of systematic error. We conducted sensitivity analyses to evaluate the possible role of systematic error on reported associations between low-level dietary exposure to methylmercury and neuropsychological test results in two well-known cohort studies: the Faroe Islands Study (FIS) and the Seychelles Child Development Study (SCDS). We estimated the potential impact of selection bias, confounding, and information bias on reported results in these studies. For demonstration purposes, we used the Boston Naming Test (BNT) score as the outcome variable. We found that, assuming various degrees of bias (in either direction) the corrected regression coefficients varied widely, and the results of the two studies were not necessarily conflicting. These findings illustrate the importance of formal sensitivity analyses in studies that use neuropsychological test scores as the outcome of interest. Keywords: Sensitivity analysis, Continuous variables, Neuropsychological testing

23

EFFECT OF SOLVENTS ON L1 DISTRIBUTION IN LIPID RAFTS. Ningfeng Tang, Katie Krone, Stephanie Fox, Cynthia F. Bearer, *Departments of Pediatrics and Neurosciences, Case Western Reserve University, Cleveland, OH, USA*

Inhibition of L1 cell adhesion molecule (L1) by ethanol has been implicated in the pathogenesis of fetal alcohol spectrum disorder (FASD) and fetal solvent syndrome (FSS) by the observation of similarities between patients with FASD or FSS and L1 genetic defects. L1 binds homophilically to itself. Such binding generates signal transduction leading to neurite outgrowth through an endocytic/exocytic recycling pathway in the growth cone. Ethanol has been shown to decrease L1–L1 adhesivity. Equipotent concentrations of butanol were as effective as ethanol, but pentanol had no effect. We have previously shown that ethanol inhibits L1-mediated neurite outgrowth but not *N*-cadherin mediated neurite outgrowth. Recent evidence suggests that L1 trafficking in and out of lipid raft domains is required for L1 mediated neurite outgrowth. Ethanol/solvent disruption of L1-lipid raft interaction may be a common explanation for the effect of these reagents on L1 function. Using cultured cerebellar granule neurons (CGN) from P6 rat pups, we have been able to isolate lipid rafts and assay them for protein content using western blot. CGN are serum starved for 2 h, exposed to alcohols for 1 h, and harvested for lipid raft isolation. Our results show that (1) 25 mM ethanol increases L1 distribution into lipid rafts (10% control, 49% ethanol, $p < 0.04$), ethanol has no effect on *N*-cadherin distribution and (3) an equipotent concentration of butanol mimics ethanol but pentanol had no effect (control 30%, ethanol 60% ($p < 0.02$), butanol 60% ($p < 0.003$), pentanol 30%). Preliminary data suggest that other targets of ethanol toxicity also are redistributed in lipid rats. We conclude that one mechanism by which ethanol and other solvents may act as developmental neurotoxicants is through disruption of protein–lipid raft interactions. Keywords: Fetal solvent syndrome, Lipid raft, Fetal alcohol spectrum disorder

24

SEYCHELLES CHILD DEVELOPMENT STUDY: ANALYSES OF POSTNATAL MEHG EXPOSURE. Gary J. Myers, Sally Thurston, Conrad F. Shamlaye[*], Philip W. Davidson, Sean Strain[**], Christopher Cox[***], Thomas Clarkson, *University of Rochester School of Medicine & Dentistry, Rochester, NY, USA; [*]Ministry of Health, Republic of Seychelles; [**]University of Ulster, Ireland; [***]The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA*

The Seychelles Child Development Study (SCDS) was designed to determine if prenatal exposure to low-levels of methyl mercury (MeHg) from fish consumption influences child development. The SCDS main cohort consists of over 700 children who were exposed to prenatal MeHg from maternal fish consumption and postnatal MeHg from the child consuming fish. The mother's average fish consumption was12 meals each week during pregnancy and their mean hair total mercury (T-Hg) during pregnancy was 6.9 ppm. Recent postnatal T-Hg exposure was measured at 66 and 107 months of age and was 6.5 and 6.2 ppm, respectively. Because exposure is continuous in this population, recent postnatal exposure was included as a covariate in the primary analysis at 5.5 and 9 years of age. Prenatal MeHg exposure was associated with 4 of over 60 primary endpoints evaluated through 11 years of age. One association was adverse, two beneficial and one indeterminate. However, the brain continues to undergo extensive and rapid development during the early years of life and nutrients present in fish may be essential for optimal brain development and the postnatal brain may still be susceptible to low-level MeHg exposure. We examined the relationship of the children's recent postnatal exposure measured at the 66 and 107-month evaluations to endpoints using linear or logistic regression and are examining the relationship of nutrient variables to postnatal exposure. We will discuss the metrics of postnatal MeHg exposure, the complexities of these analyses and our findings. The SCDS continues to be a prospective, double blind study with an extensive set of covariates and a predetermined primary analysis plan. Evaluation of the SCDS main cohort at age 16 years is underway. Keywords: Methyl mercury, Fish consumption, Child development

25

SENSITIVE BRAINS LASTING HARM: ENVIRONMENTAL NEUROTOXINS AND LEARNING AND DEVELOPMENTAL DISABILITIES IN CHILDREN. Kathleen Schuler, *Environmental Scientist, Institute for Agriculture and Trade Policy, 2105 First Av. S, Minneapolis, MN, USA*

This session will summarize the scientific evidence linking environmental neurotoxins and children's learning and developmental problems; prevalence and costs of these conditions; and present a primary prevention model for better protecting children from these exposures. A growing body of evidence points to children's widespread exposure to environmental neurotoxins like lead, mercury, PCBs, pesticides and other chemicals as significant contributors to the increasing prevalence and severity of learning and developmental problems. Fetuses and young children whose brains are still developing are particularly vulnerable in injury from exposure to these neurotoxins. A large body of research documents adverse effects on IQ, learning, development and behavior from prenatal and early life exposures, with some developmental problems persisting into adulthood. The prevalence of learning and developmental disabilities in children is estimated to be 17% of school age children. Children's learning and developmental problems present burdens for affected individuals and families, as well as for society and taxpayers. Preliminary estimates, as well as extrapolation from research on impacts of lead exposure, indicate that these costs are significant. Nationwide the average cost to educate children in special education programs is twice that of the average student and the numbers of children with special education needs are increasing. In addition to special education costs, IQ loss is associated with reduction in productivity and consequently lifetime earnings in adulthood. Other societal costs include: costs to social service, welfare, criminal justice and health care systems. Reducing preventable environmental contributors to these problems could help reduce the burden of these conditions on individuals, families and society.

26

PREVENTING NEURODEVELOPMENT DISORDERS: THE CDC SHOULD LOWER THE BLOOD LEAD ACTION LEVEL FROM 10 TO 2 mcg/dL. SG Gilbert, *Institute of Neurotoxicology and Neurological Disorders, Seattle, WA, USA*

The Centers for Disease Control and Prevention (CDC) set the current blood lead action level for children at 10 mcg/dL in 1990. Recent studies provide evidence that the neurobehavioral effects of lead in children occur at even lower levels of exposure. New data as well as summaries of past studies indicate that there is a greater loss of IQ when childhood blood levels increase to 10 mcg/dL, than when above 10 mcg/dL. The Environmental Protection Agency has refused to establish a reference dose because some of the ''health effects associated with exposure to lead occur at blood levels as low as to be essential without a threshold''. Currently, the CDC estimates that 310,000 U.S. children aged 1–5 years have blood lead levels greater than the CDC recommended level of 10 mcg/dL of blood. Children continue to be exposed to lead from the dust of homes, lead based paint in and around homes, drinking water, and contaminated dirt often from old lead smelters. Furthermore, there is no safety factor built into the CDC action level. The persistent and irreversible damage to child's intellectual abilities does not receive the same level of concern. Landrigan and others have estimated that the direct and indirect costs of elevated blood lead levels to society are US$ 43.4 billion. Children do not give their consent to be exposed to lead, we have a duty and ethical responsibility to

ensure that children develop in an environment that allows them to reach and maintain their full potential. This presentation will review the neurobehavioral effects of low level lead exposure and examine the scientific and ethical arguments for lowering CDC blood lead action level. Keywords: Lead, Neurodevelopment, Children

Symposium

SESSION IV-B: **NEUROTOXICANT EXPOSURES IN MILITARY DEPLOYMENTS AND PUTATIVE ASSOCIATIONS WITH NEURODEGENERATIVE DISEASES**

Co-Chairs: Susan P. Proctor, D.Sc.
COL Karl E. Friedl, Ph.D.

Theme: *Topics presented in this session will include epidemiologically focused research on neurotoxicant exposures and putative associations with neurodegenerative diseases. This session will feature presentations of on-going projects sponsored by the US Army Military Research and Materiel Command, Military Operational Medicine Research Program and the US Army Research Institute of Environmental Medicine.*

This session is sponsored by the US Army Research Institute of Environmental Medicine (USARIEM) and the Neurotoxin Treatment Research Program of the US Army Medical Research and Materiel Command (USAMRMC).

27

OVERVIEW: NEUROTOXICANT EXPOSURES IN MILITARY DEPLOYMENTS AND PUTATIVE ASSOCIATIONS WITH NEURODEGENERATIVE DISEASES. Susan P. Proctor, Karl E. Friedl, *U.S. Army Research Institute of Environmental Medicine, Natick, MA, USA*

Long-term health risks associated with military deployments and occupational exposures have become a focus of attention since the 1991 Persian Gulf War, with emphasis on neurological and neurodegenerative disease outcomes. A major research initiative, the Neurotoxin Exposure Treatment Research Program (NETRP) has targeted the association of environmental exposures and Parkinson's Disease (PD) as a neuroepidemiology study model that may also provide important insights to a variety of stressors (e.g., toxicological exposures, head impact, traumatic stress, radiofrequency radiation), singly and in combination, and neurodegenerative processes. In this session, four neuroepidemiological approaches to the investigation of militarily relevant chemical exposures will be presented, including studies of military service and neurodegenerative disease rates (for ALS and PD); PD associations with environmental pollutants in native populations; PD and occupational exposures in electrical workers; and solvent effects in military personnel. These studies explore the basis for further investigation of disease risks and protective countermeasures of soldiers and other relevant groups.

28

PROSPECTIVE STUDY OF MILITARY SERVICE AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS AND PARKINSON'S DISEASE. MG Weisskopf, E O'Reilly, ML McCullough, EE Calle, MJ Thun, M Cudkowicz, A Ascherio, *Departments of Environmental Health, Epidemiology, and Nutrition, Harvard School of Public Health, Boston, MA, USA; Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA; Epidemiology and Surveillance Research, American Cancer Society, Atlanta, GA, USA; Neurology Clinical Trial Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*

Two recent studies have suggested that the risk of Amyotrophic Lateral Sclerosis (ALS) is increased among Gulf War veterans. Our investigation sought to determine whether military service prior to the Gulf War is associated with an increase in risk for ALS and Parkinson's Disease (PD). We prospectively assessed the association between ALS mortality and self-reported military service in the American Cancer Society's Cancer Prevention Study II (CPS II) cohort, a cohort that includes over 500,000 men surveyed by questionnaire in 1982. ALS mortality was assessed via linkage with the National Death Index. Our original analyses (Neurology, 64:32, 2005) included ALS deaths ($n = 280$) up to 1998. We have now extended the follow-up to 2002 (ALS deaths = 513). Mantel-Haenszel relative risks (RR) adjusted for age and smoking were calculated and Cox proportional hazards models stratified on single year of age were used when adjusting for additional variables. Men who served in the military (69%) had a significantly increased ALS mortality (RR = 1.5; 95% CI: 1.2–2.0; $p = 0.0009$) compared to those who did not serve. The increase in ALS mortality was similar among men who served in the Army or National Guard (RR = 1.6), Navy (RR = 1.7), or Air Force (RR = 1.5). The association between military service and incidence of PD was assessed among men participating in the CPS-II Nutrition cohort, a prospective investigation initiated in 1992 among a subset of participants in the original CPS-II cohort. The occurrence of PD in the CPS-II Nutrition cohort was assessed in 2001 using a mailed questionnaire. We documented 266 incident cases of PD (by contacting the treating neurologist and/or reviewing the medical records) among 65,867 men. We found no association between military service and PD incidence. The age- and smoking-adjusted RR was 0.96 (95% CI: 0.72–1.29) for men who served in the military compared with those who did not. Similar null results were obtained for men who served in the Army, Navy, or Air Force. In summary, we confirmed our original finding that men who served in the military have an increased risk of ALS. This increase appeared largely independent of the branch of service. In contrast, military service was not related to risk of developing PD. Keywords: Epidemiology, Prospective studies, Mortality.

29

POLYCHLORINATED BIPHENYLS, ORGANOCHLORINES AND PARKINSON'S DISEASE (PD) RISK: A CASE CONTROL STUDY IN ALASKA NATIVES. CM Tanner[1], G Webster Ross[2], Brian Trimble[3], Monica Korell[1], Marion Lee[4], Samuel Goldman[1], Thomas Gordon[3], Donato DiMonte[1], Robert Abbott[2,5], *[1]The Parkinson's Institute, Sunnyvale, CA, USA; [2]Pacific Health Research Institute, Honolulu, Hawaii, USA; [3]Alaska Native Health Service, Anchorage, AK, USA; [4]University of California-San Francisco, San Francisco, CA, USA; [5]University of Virginia, Charlottesville, VA, USA*

Certain persistent organic pollutants (POPs) have been proposed to increase the risk of developing Parkinson's disease (PD). We are conducting a case control study of PD among Alaska Natives to determine the association of exposure to polychlorinated biphenyl (PCBs) residues, organochlorine pesticides and methyl mercury with PD. The hypothesis is that increased exposure to these compounds will be associated with an increased risk of PD. Indirect support of this hypothesis is provided by a recent report from Greenland, finding Parkinson's disease prevalence to be high in Inuit people, possibly due to exposure to persistent organic pollutants (POPs). Exposure may be especially high in Inuit who consume a traditional diet, high in marine mammals, with potential bioconcentration of POPs. Specific PCB congeners have been associated with PD in the Greenland Inuit population. Alaska Native populations have a similar traditional diet to Greenland Inuit, providing an ideal setting for a second investigation of this association. We will use direct measurements of exposure, as these compounds are persistent in body tissues. In addition, lifelong exposure will be estimated by structured interview, including a dietary history with specific attention to intake of fish, marine mammals and wild game, known sources of bioconcentration of these environmentally persistent compounds. Additional study hypotheses will investigate factors inversely associated with PD risk, implying potential beneficial effects, including recognized factors such as nicotine and caffeine, as well as novel factors associated with the traditional lifestyle. The project is being conducted in two phases. Phase 1 is a developmental period. During this time, the specific aspects of the study design are being established and the necessary approvals for the research are being obtained. During Phase 2 the study will be conducted. An update of this on-going work will be presented. Keywords: Parkinsonism, Toxicants, Epidemiology

30

POLYCHLORINATED BIPHENYLS ALTER DOPAMINE FUNCTION IN OLDER CAPACITOR WORKERS. RF Seegal, *Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences, University at Albany, Albany, NY, USA*

Polychlorinated biphenyls (PCBs) alter central nervous system (CNS) function, including reductions in central dopamine (DA) function, in laboratory animals. However, their role as a human neurotoxicant is less clear because most environmental exposures to PCBs are via complex mixtures of many additional neurotoxicants. An opportunity exists to minimize this problem by studying the neurological and neuropsychological consequences of occupational exposure to PCBs in an aging population of former capacitor workers. Furthermore, using β-CIT SPECT imaging we can determine PCB-induced changes in basal ganglia dopamine transporter densities and the relationship of these changes with measures of CNS function. Preliminary results suggest that PCB body burdens are statistically associated with reductions in basal ganglia DA densities in women, but not in men. The importance of these findings is strengthened by other recent epidemiological findings of increased Parkinson's disease associated mortality only in highly exposed female capacitor workers. Potential mechanisms for the association between PCB exposure, gender and altered basal ganglia DA function will be discussed. Supported by the Neurotoxin Research Program of the U.S. Army Medical Research and Materiel Command grant # DAMD17-02-0173 to RFS. Keywords: Polychlorinated biphenyls, Dopamine, Occupational exposure

31

SHOAMP: THE STUDY OF HEALTH OUTCOMES IN AIRCRAFT MAINTENANCE PERSONNEL. C D'Este, J Attia, A Brown, P Schofield, M Tavener, R. Gibson, K Horsley[*], [*]*on behalf of the SHOAMP team*, *Royal Newcastle Hospital, Newcastle, New South Wales, AU*

The Study of Health Outcomes in Aircraft Maintenance Study (SHOAMP) was an epidemiological study conducted in response to health concerns, including memory loss, fatigue, and other neurological problems, by workers in the F-111 aircraft Deseal Reseal (DSRS) Program. Workers had a variety of exposures including solvents and jet fuel. Consenting subjects from the F-111 DSRS group ($n = 659$) and two comparison groups – technical personnel at a different Air Force Base ($n = 600$) and non-technical personnel at the same base ($n = 495$) – completed a mailed Postal Questionnaire and/or had a physical examination. Data were collected on a variety of outcomes including general health and wellbeing, neurological outcomes, male sexual function, mental health, and cognition and memory. Linear or logistic regression analyses were conducted to compare adjusted outcomes across the three groups. The F-111 DSRS group had statistically significantly higher proportions of self-reported sensory and motor neuropathic symptoms, higher self reported physician diagnosis of erectile dysfunction, depression and anxiety, executive functioning, psychomotor speed, quality of life, and slightly higher colour vision deficits. There were no differences in olfaction, neuropathy, or attention/working memory tests. Although there are uncertainties in the interpretation of the study results due to such factors as uncertain sampling frames, potential survivor bias, low participation rates, and multiple

comparisons, the results indicate an association between F-111 DSRS involvement and a lower quality of life and more common erectile dysfunction, depression, anxiety, and subjective memory impairment. There is also evidence, albeit less compelling, of an association of DSRS with neuropsychological deficits. Keywords: Solvents, Neurological health, Epidemiology

Symposium

SESSION EVENING-A: **AQUATIC AND INVERTEBRATE MODELS OF DEVELOPMENTAL NEUROTOXICITY FOR MECHANISTIC AND HIGH THROUGHPUT STUDIES**

Co-Chairs: Edward D. Levin, Ph.D.
Jonathan Freedman, Ph.D.

Theme: *Fish and invertebrates offer useful models complementary to the classic mammalian and in vitro models of neurotoxicity. These simple but functionally intact systems provide visual access during the process of development that is unavailable in mammals. The aquatic and invertebrate models provide the anatomic and temporal integrity of the whole animal unavailable with in vitro preparations. Elegant genetic methods are available for sophisticated studies of the molecular bases of developmental neurotoxicity. Rapid assessment techniques using C. elegans and zebrafish embryos are being developed for high through-put screening studies. Reliable behavioral assays are being constructed to determine the functional consequences of neurodevelopmental insults. These newer complementary models can provide important information for initial triage of the multitude of chemicals to be tested in the necessary but more expensive and time consuming mammalian studies as well as identifying important molecular targets for the toxicodynamic effects of toxicants on neurodevelopment.*

32

DEVELOPMENT OF MEDIUM-THROUGHPUT TOXICITY SCREENS USING *C. ELEGANS*. WA Boyd, SJ McBride, JR Rice, JH Freedman, *Duke University Durham, NC, USA*

Recently, the Toxicology Program (NTP) and other regulatory agencies have recognized the need for alternative toxicological methods and models to decrease the time and expense of current toxicity testing protocols. The numerous advantages of using *C. elegans* as a model organism are well documented. Recently, these advantages have led to a rise in the use of *C. elegans* as a toxicity testing organism. Through collaboration with the NTP, we are developing the means to screen potential neurological and developmental toxicants.

Sublethal toxicity endpoints including growth, reproduction, movement, and feeding are automated using two liquid handling robotic workstations; a Complex Object Parametric Analyzer and Sorter (COPAS) BIOSORT for dispensing and analyzing nematode length and fluorescence; and an imaging workstation for motion tracking and multidimensional image analysis. To optimize rate at which chemicals can be screened, 96-well plate formats are used for sample preparation, dispensing of test organisms, and quantification of specific toxicological endpoints. As nematodes are dispensed, the time of flight, extinction, green, and red fluorescence are measured for each nematode by the BIOSORT. Nematodes are exposed to toxicants over specific developmental stages for each test: L1s for 72 h growth, L4s for 48 h reproduction, and 3-day-old adults for 4 or 24 h movement and feeding assays. After toxicant exposures for growth, reproduction, or feeding, the samples are aspirated with the COPAS and the same four parameters are measured. Movement tracking and image capturing of nematodes are also performed after toxicant exposures. For each test, the effective concentration that results in a 50% reduction in response relative to controls (EC50) is then calculated. Many chemicals have been tested and used as model test chemicals for development of the screens. Cadmium data will be presented as illustrations of the methods and results of each assay. Keywords: *C. elegans*, Neurotoxicants, Medium-throughput screens

33

A NOVEL C. ELEGANS MODEL FOR DETERMINING METAL-INDUCED DOPAMINE NEURODEGENERATION AND ALTERNATIONS IN NEURODEVELOPMENT. R Nass, *Departments of Anesthesiology and Pharmacology, and Center for Molecular Neuroscience, Vanderbilt University Medical Center, Nashville, TN, USA*

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease characterized by the selective loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc). Although the origin of pathogenesis of the DA neurons in idiopathic PD remains unclear, corollary evidence suggests both genetic and xenobiotic contributions. In some familial forms of PD, alleles of alpha-synuclein have been linked to the disease, and epidemiological studies involving exposure to heavy metals (e.g., Fe^{3+}, Al^{3+}, Cu^{2+}, and Mn^{2+}) show an increased risk for PD. Furthermore, accumulation of these heavy metals has been found in the SNpc in PD brains. In rodents and man, DA transporters (DATs) constitute the molecular gateway through which many exogenous neurotoxins (e.g., 6-OHDA, methamphetamine, and MPP^{+}) enter these neurons and affect lesions reminiscent of the DA neuron pathology of PD. DATs can also physically interact with alpha-synuclein, and pharmacological blockade of DAT can protect DA neurons from endogenous and environmental toxins. We have previously shown that in the nematode *C. elegans* the DA neurons can be selectively damaged by exposure to 6-OHDA. We now show that exposure to Fe^{3+}, Al^{3+}, Cu^{2+}, Mn^{2+} causes DA neuron specific degeneration. Furthermore, 6-OHDA toxicity is amplified by chronic exposure to these metals. We also show that heavy metals can amplify the neurotoxicty

conferred by alpha-synuclein. This system will allow us a facile test to examine the role that these metals and xenobiotics play in the degeneration and the development of these neurons.

34

STRATEGIES TOWARDS USING ZEBRAFISH AS A COMPLEMENTARY NEUROTOXICOLOGICAL MODEL. Elwood Linney, *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA*

Zebrafish continues to be an excellent model for studying the development of the vertebrate nervous system. Some of its practical advantages include ease of obtaining large numbers of embryos, fast development of the nervous system and its functional usage, a small size that allows one to use a variety of microscopy approaches including fluorescent and confocal examination, and the sequencing of the whole genome by the Sanger Centre. As a complement to mammalian models, its development outside the mother allows one to visualize the development of the nervous system and with the use of fluorescent, transgenic lines one can visualize specific parts of the nervous system as it is formed. While gene knockout technology has not as yet been developed, gene expression can be selectively knocked-down throughout embryonic development through the use of gene specific anti-sense morpholinos.

These aspects of the system and the technologies developed around it will be discussed and specific examples of strategies of their useage will presented to provide insight into some of the advantages of this vertebrate model system. *(Supported by the Duke Superfund Basic Research Center ES10356 and the Duke Toxicogenomics Consortium Group ES11375).*

35

NEUROBEHAVIORAL CONSEQUENCES OF NEURODEVELOPMENTAL TOXICITY ZEBRAFISH. Edward D. Levin, *Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC, USA*

Zebrafish offer useful models complementary to the classic mammalian and in vitro models of neurotoxicity. This is particularly true for developmental neurobehavioral toxicology in which intact organisms are essential for determining behavioral function. Zebrafish provide both the functional integrity for behavioral analysis as well as continual visual access during early development and the elegant molecular tools available with which to dissect developmental models permit in depth mechanistic studies of the bases of functional impairments. In collaboration with the laboratory of Dr. Elwood Linney. We have found that early exposure to the organophosphate insecticide chlorpyrifos causes persisting deficits in delayed spatial alternation choice accuracy, indicative of a long-lasting cognitive impairment. We have also found significant differences in spontaneous swimming behavior soon after hatching which may be an early indicator of later cognitive impairment. We are developing higher through put systems for assessing cognitive function of both adult and newly hatched zebrafish. The development of higher through put behavioral assessments in zebrafish will provide the opportunity to quickly determine the functional toxicity of a wide range of toxicants and mixtures. It will also facilitate the discovery of molecular processes underlying these impairments. Keywords: Zebrafish, Chlorpyrifos, Nicotine. *(Supported by the Duke University Superfund Basic Research Center P42 ES010356.)*

Symposium

SESSION EVENING-B: **ENDOCRINE ACTIVE COMPOUNDS AND THEIR EFFECTS ON BRAIN DEVELOPMENT: INTEGRATION OF METHODS AND APPROACHES**

Co-Chairs: Eva Polston, Ph.D.
Robert Handa, Ph.D.

Theme: *Throughout an animal's lifetime, steroid hormones have profound effects on brain function. Because the brain is sensitive to low concentrations of steroids and steroid-like compounds, there is growing concern that low levels of endocrine-active compounds (EACs) in the environment may exert toxicological effects in the brain. In contrast to necrosis-inducing neurotoxins that cause histopathological damage, the effects of EACs are likely to result in subtle and specific alterations of neuronal function. This workshop will present a multifaceted approach through which changes in the developing and adult brain can be assessed. Talks will focus on cellular/molecular, neuroanatomical, and functional approaches for detecting perturbations in hormone-sensitive neuronal systems.*

36

ENDOCRINE ACTIVE COMPOUNDS AND THEIR EFFECTS ON BRAIN DEVELOPMENT: INTEGRATION OF METHODS ANDAPPROACHES. David C. Dorman, *CIIT Centers for Health Research, Research Triangle Park, NC, USA*

It is well established that the development of a variety of brain structures (i.e., sexually dimorphic nuclei [SDN]) is under hormonal control. One of the better understood SDN is the medial preoptic area (SDN-POA) of the hypothalamus. The SDN-POA is present in multiple species including the rat, guinea pig, monkey, and human. SDN development typically occurs during periods when the reproductive tract and the brain develop simultaneously. Decreased estrogen levels in certain brain structures will promote neuronal apoptosis, resulting in a decreased size of the SDN. In the male fetus, testosterone delivered to the brain is metabolized by aromatase to estrogen. Estrogen in the male brain is protective and will inhibit apoptosis, resulting in an increased size of the SDN. Other SDN brain regions (e.g., anteroventral periventricular (AVPV)

nucleus in the rat hypothalamus) demonstrate an opposite trend with larger volumes present in females when compared to male rats. Because the SDN are sensitive to low concentrations of steroids and steroid-like compounds, there is growing concern that low levels of endocrine-active compounds (EACs) in the environment may exert toxicological effects in the brain. The effects of EACs may be detected at the cellular or molecular, neuroanatomical, or behavioral level. This workshop will present a multifaceted approach through which these EAC-induced changes in the developing and adult brain can be assessed. Keywords: Anti-androgen, SDN-POA

37

PRENATAL EXPOSURE TO FENITROTHION: ARE CHANGES IN THE SDN-POA A CONCERN? Melanie F. Struve, Katie J. Turner, Patricia K. Dodson, David C. Dorman, *Division of Biological Sciences, CIIT Centers for Health Research, Research Triangle Park, NC, USA*

Fenitrothion is an organophosphate insecticide that also has antiandrogenic properties. In vitro, fenitrothion has been shown to antagonize androgen receptor binding. In vivo, conflicting reports describe the presence or lack of effects on male rat reproductive organs. Pregnant Crl:CD(SD)BR rats were orally dosed with corn oil or fenitrothion in corn oil at 20 or 25 mg/kg/day from gestation day (GD) 12–21. These doses were selected to demonstrate maternal toxicity at the highest dose level. Flutamide, a potent nonsteroidal androgen receptor antagonist, was used as a positive control in an additional nine litters. Pups were dosed subcutaneously with 1% methylcellulose or 100 mg/kg flutamide in methylcellulose on postnatal days (PND) 1, 5, 15, and 20. Offspring were euthanized after reaching sexual maturity (females 60–65 days old and males 96–105 days old). Brains from two males and two females per litter were evaluated for SDN-POA volumes. Although the SDN-POA volume was decreased in the male rats exposed postnatally to flutamide, similar changes were not observed in rats exposed prenatally to fenitrothion. Surprisingly, there was a dose-related increase in the SDN-POA volume in males and a dose-related decrease in volume in females exposed to fenitrothion. Transient changes to the reproductive system, including reduced anogenital distance in PND1 males and increased retention of areolae in PND13 male offspring were observed in these animals but did not persist until adulthood. Clinical observations consistent with inhibition of cholinesterase activity (tremors, decreased body weight gain) were observed in the pregnant females dosed with fenitrothion, as was a decrease in the number of live pups born to those animals. Based on the transient nature of the reproductive alterations seen, and the atypical change in the SDN-POA volumes, inhibition of cholinesterase activity remains the critical endpoint for risk assessment. Keywords: Anti-androgen, SDN-POA, Fenitrothion, Flutamide. *Supported by the American Chemistry Council, project SFFDDO033.*

38

ESTROGEN RECEPTOR SIGNALING IN SEXUAL DIFFERENTIATION OF THE BRAIN: CAN WE TEACH AN OLD DOGMA NEW TRICKS? Robert J. Handa[1], Wilson, C.J. Chung[1], Russell S. Thomas[2], Melvin E. Andersen[2], *[1]Department of Biomedical Sciences, Neuroscience Division, College of Veterinary Medicine, Colorado State University, Fort Collins, CO; [2]Division of Computational Biology and Functional Genomics Research Program, CIIT, Research Triangle Park, NC, USA*

Endocrine active compounds influence the sexual differentiation of the brain. In rodent models, sexual differentiation of the brain is dependent upon estrogen receptor (ER) signaling. Defeminization of the male brain is a consequence of the neural aromatization of circulating testosterone to estradiol and subsequent activation of ERs. In contrast, it has been thought that circulating estrogen does not act on the brain of developing female rats due to the high levels of serum alpha fetoprotein during neonatal life. Alpha fetoprotein, a liver derived serum protein, binds estrogen with relatively high affinity and is believed to sequester estrogen and block its access to the brain.

The medial preoptic area of the rodent brain has conspicuous sex differences in the expression of progesterone receptor (PR). Males express high levels of PR due to the presence of intracellular estradiol; females do not. Thus, the induction of PR by estrogen (or EACs) is a convenient and sensitive biological reporter for examining estrogen sensitivity. Moreover, estrogen is known to act through two ER subtypes, estrogen receptor alpha and beta, but the role of each of these in sexual differentiation of the brain has not been established. We have found that subcutaneous injections of relatively low levels of estrogen on post-natal days 2–4 to neonatal female and gonadectomized male rats induces PR immunoreactivity in the medial preoptic area and arcuate nucleus. These responses suggest that alpha fetoprotein does not completely sequester estradiol from the brain and therefore, subtle changes in circulating estrogen during neonatal life may have long-lasting effects on sexually differentiated brain structure and function. Furthermore, the induction of PR by estradiol was mimicked by ERalpha agonists, but not ERbeta agonists, implicating ERalpha as an important receptor for sexual differentiation of the brain. Pharmacokinetic studies of estrogen in neonatal female rats should provide important information on dose-dependence of altered brain development in the females. In addition, the consequences of PR upregulation by estradiol on adult behavioral sequelae need to be determined.

To determine potential gene targets for estrogen receptor during brain development, we have utilized an Affymetrix gene array platform. Neonatal female and gonadectomized male rats were treated with modest levels of estradiol (10 and 100 ng/kg BW) and gene expression profiles of the medial preoptic area were compared to oil treated controls. Only 74 genes (of over

31,000) were significantly changed by neonatal estradiol treatment in both males and females. These genes provide potential targets for estrogen action in the developing brain and the pattern of gene alterations by estrogen should be useful in determining the genomic events that underlie sexual differentiation of the brain. Keywords: Estrogen receptor, Progesterone receptor, Sexual differentiation, Gene array. *Supported by the American Chemistry Council, project END0017.*

39

SEX AND THE BRAIN: EVALUATING SEX DIFFERENCES IN NEUROENDOCRINE AND BEHAVIORAL CIRCUITS. Eva K. Polston, Anne E. Fortino, Heather B. Patisaul, *Division of Biological Sciences, CIIT Centers for Health Research, Research Triangle Park, NC, USA*

Developmental exposure to steroid hormones orchestrates sexual differentiation of the brain. Perinatal exposure of the male brain to high levels of testosterone-derived 17β-estradiol masculinizes hormone-sensitive forebrain areas. The female brain, which is exposed to only low levels of hormone, remains unmasculinized. Under normal conditions, this process of brain differentiation produces permanent neuroanatomical sex differences in the circuits that control gonadotropin secretion and male and female sexual behavior. The developing brain is sensitive to low concentrations of steroids, so it is possible that exposure to endocrine-active compounds (EACs) in the environment may influence the process of sexual differentiation. To address this possibility, we examined the development of the anteroventral periventricular nucleus of the hypothalamus (AVPV), a sexually dimorphic nucleus that regulates luteinizing hormone secretion, following neonatal exposure to high doses of genistein (GEN) or bisphenol-A (BIS). Newborn rats were given four subcutaneous injections of sesame oil (control), 250 μg GEN or 250 μg BIS at 12 h intervals over postnatal days 1 and 2. Neonatal exposure to either GEN or BIS eliminated the sex difference normally seen in the numbers of dopaminergic neurons in the AVPV. GEN, but not BIS, also abolished the normal sex difference in AVPV volume. In both cases, the compounds appeared to prevent brain masculinization in males, such that the AVPVs of treated males were indistinguishable from those of control females. Neither GEN nor BIS treatments influenced the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA), a brain area involved in the display of male sexual behavior. Our results suggest that both naturally occurring and synthetic EACs can have anti-estrogenic effects in the developing brain and may disrupt normal processes of brain masculinization. We also demonstrate that the effects of EAC exposure during the critical period for brain sexual differentiation are compound- and brain site-specific. Keywords: Sex difference, AVPV, SDN-POA, Genistein, Bisphenol-A. *Supported by the American Chemistry Council, project IMFOB0034.*

40

BEYOND THE BRAIN: HOW EAC'S AFFECT NEUROENDOCRINE SYSTEMS AND COMPLEX BEHAVIORS. Heather Patisaul, *CIIT Centers for Health Research, Research Triangle Park, NC, USA*

The evaluation of a given compound's activity in the brain often focuses on changes in gene expression or protein synthesis within a neuronal system. When seeking to determine whether or not a compound is potentially deleterious, these effects are only part of the picture. It is critical to determine whether or not alterations seen on a genomic or cellular level translate to physiological and behavioral changes on the individual level. Changes in behavior, particularly reproductive behavior, may effectively decrease the fertility of an individual even if the gonads are fully functional. I have found that short-duration exposure to soy phytoestrogens during adulthood can affect a number of behaviors related to reproduction and anxiety in male and female rodents. Consumption of a commercially available soy supplement for 5 days significantly suppressed both sexual receptivity (28% decrease) and proceptivity (60% decrease) in hormone-replaced ovariectomized females. Pacing behaviors were also abolished with soy intake, suggesting that the soy supplement decreased both sexual interest and performance in female rats. Soy phytoestrogens also affect anxiety. Open arm activity on an elevated plus maze was increased during the proestrus but not the diestrus phase of the estrus cycle in intact female rats. Interestingly, an opposite effect was seen in male suggesting that these compounds are anxiolytic in proestrus females, but anxiogenic in males. Exposure to soy phytoestrogens and other EDCs in the neonatal period can also affect reproductive fitness in adulthood. Studies in our and other laboratories have found that neonatal exposure impacts a range of adult neuroendocrine and behavioral endpoints including sexual behavior and estrous cyclicity.

Using behavioral and neuroendocrine assays to determine the reproductive toxicity of a compound has several advantages. These assays are non-lethal, easily repeatable, and simple to conduct and manipulate. By identifying behavioral and neuroendocrine parameters that are sensitive to EAC exposure, we can then isolate the neural systems that may be affected and identify the mechanisms by which behavioral or neuroendocrine function was disrupted. In doing so, we can focus our attention on systems that we know affect the fitness and fertility of the animal. Keywords: Lordosis, Anxiety, Soy, Estrogen receptor, Genistein, Hypothalamus. *Supported by NSF STC Center for Behavioral Neuroscience IBN-9876754 and the American Chemistry Council, project IMFOB0034.*

41

DOES SEX MATTER? MALE BRAINS, FEMALE BRAINS, AND ENVIRONMENTAL EXPOSURES. Bernard Weiss, *Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*

Sex differences are recognized in several aspects of environmental health and toxicological research. One example is the standard bioassay for assessing whether or not a particular chemical poses a cancer risk, which requires both males and females. Reproductive endpoints, especially in males, are the focus of considerable contemporary attention. Chemicals whose toxic targets include the brain have not received an equivalent emphasis on sexually dimorphic responses. Behavior and brain anatomy are sexually dimorphic in humans and animals. Such differences are important in evaluating the risks of environmental exposures because they may reflect disturbances of neurobehavioral development. In our experiments, we administered dioxin to pregnant rats at doses producing environmentally relevant body burdens. We then tested cognitive performance (schedule-controlled operant behavior) in the offspring. Offspring from rat mothers that had not been given dioxin showed the usual pattern of sex differences, such as higher response rates in males. Offspring from exposed mothers reversed the typical difference. Males reduced their response rates while females increased their rates. When we looked at brain structure, we found that male rat brains became more symmetrical, the female norm, while female brains showed a slight tendency to become less symmetrical, the male norm. Endocrine-active chemicals such as phthalates, bisphenol A, vinclozolin, and others also induce marked sexually dimorphic responses. These, and other findings, support the use of sex differences as endpoints for neurodevelopmental risk assessment. Keywords: Sexual dimorphisms, Operant behavior, Brain development

Symposium

SESSION V: **MOLECULES TO (WO)MAN: A. ANIMALS**

Dissecting the Dysfunction to Look at the Whole Picture

Co-Chairs: Isaac N. Pessah, Ph.D.
Richard F. Seegal, Ph.D.

Theme: *Invited Speakers in this Symposium will present an integrated overview of the multidisciplinary approaches needed to understand risk factors contributing to developmental disorders and aging. Goal: To provide mechanistic data that will aid in the interpretation of epidemiological data and in understanding the role that environmental agents play in inducing central nervous system dysfunctions. Identifying the principal molecular targets that are responsible for producing toxicosis has been a cornerstone of risk assessment. Prominent examples include the activity of dioxins at the AhR, anticholinesterase activity of organophosphates and carbamates, and the interaction of pyrethroids with sodium channels. Understanding the relationship among low level exposure to environmentally persistent chemicals, their critical molecular targets, ensuing cellular dysfunction, and defining often subtle consequences on animal and human neurodevelopment is perhaps one the most challenging goals of modern toxicology.*

42

GENETIC AND EPIGENETIC MECHANISMS CONFERRING SUSCEPTIBILITY TO ENVIRONMENTAL AGENTS. I.N. Pessah, *U.C. Davis Center for Children's Environmental Health and The M.I.N.D. Institute, University of California, Davis, CA, USA*

We are entering the post genome era with the vast majority of human genes sequences accessible on the web. Over the past decade the application of molecular genetics has led to the unraveling of the etiologies of many of the single gene disorders that lead to debilitating neurodevelopmental and neurodegenerative disorders. Missense mutations within genes coding for voltage- and ligand-gated ion channels serve as prime examples. Typically the ''channelopathy'' resulting from such a mutation produces recognizable phenotypes whose primary etiology can be traced to a single dysfunctional protein. More common disorders such as autism, schizophrenia, and Parkinson's do not show simple patterns of inheritance. This overview will focus on the challenges in understanding complex gene–environment interactions that contribute to autism. Autism is a heterogeneous syndrome defined by deficits in social reciprocity and communication, and by unusual restricted, repetitive behaviors. Based on our current knowledge multiple genes (greater than 2 and as many as 10) contribute to autism. One model postulates that some forms of autism are caused by an increased ratio of excitation/inhibition in several central processes that can be caused by combinatorial effects of genetic and environmental variables. Recent research has identified chromosomal aberrations, gene–gene interactions (epistasis), and epigenetic (DNA methylation and acetylation) mechanisms that may deregulate GABAergic neurotransmission in the autistic brain. Xenobiotic agents that directly target GABAergic neurotransmission (e.g., lindane and fipronil) and persistent organic pollutants (e.g., PCBs and mercury) that target related downstream signaling events will be presented in a mechanistic framework that further deregulates genetic liabilities present in the autistic brain. A heuristic hypothesis will be presented that can test if some children with autism represent a highly susceptible population to common environmental chemicals that alter the ratio of central excitation/inhibition of sensory, mnemonic, social, and emotional systems.

43

PCBs, METHYLMERCURY AND DOPAMINE: FROM TISSUE CULTURE TO HUMANS. Richard F Seegal, *Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences, University at Albany, Albany, NY, USA*

Extrapolation of findings from in vitro studies, to those conducted in the intact organism (either animals or humans), are often questioned. We will discuss results from both in vitro and in vivo studies of polychlorinated biphenyls (PCBs) and methylmercury (MeHg) that illustrate the value of in vitro studies in predicting neurological change in humans. Using an in vitro preparation of rat striatal synaptosomes we show that PCBs and MeHg synergistically alter both tissue and medium concentrations of dopamine (DA). This synergism provides a potential explanation for differences in behavior between children from the Faroe and Seychelle Islands: Faroe Island children developmentally exposed to PCBs and MeHg show behavioral deficits while children from The Seychelles, developmentally exposed only to MeHg, do not. Furthermore, using a neuronal cell line (N27) we show that PCBs induce oxidative stress and apoptotic cell death, while exposure of adult non-human primates to PCBs reduces basal ganglia DA concentrations and the number of tyrosine hydroxylase positive neurons in the substantia nigra. These laboratory studies demonstrate the neurotoxicity of PCBs and provide the basis for a hypothesis that similar changes in DA function may occur in humans. Indeed, preliminary studies carried out in former workers, occupationally exposed to PCBs at levels at least 100× greater than seen in the general population, demonstrate a negative relationship between PCB body burden and basal ganglia DA neuronal densities. Thus, in vitro studies, particularly when confirmed in vivo, may not only predict the consequences of human exposure to environmental neurotoxicants but may also aid in understanding the mechanisms by which these changes occur. Supported in part by U.S. Army grant # DAMD17-02-0173, NIEHS grant # ES11263 and U.S. EPA grant # R829390 to RFS. Keywords: Environmental contaminants, In vivo–in vitro comparisons, Neurotoxicity

44

DEFINING MOUSE BEHAVIORS RELATED AUTISM. Jacqueline N. Crawley, *National Institute of Mental Health, Bethesda, MD, USA and Neurodevelopmental Disorders Research Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA*

Autism is a major mental illness with a strong genetic basis. The defining symptoms are (1) deficits in reciprocal social interaction, (2) impaired social communication, and (3) stereotyped, ritualistic, repetitive behaviors or narrow, restricted interests. Genes linked to autism are being identified. Targeted mutations in mice are progressing, to test hypotheses about these genes in mediating similar behaviors in mice. To evaluate the phenotypes of emerging mutant lines of mice, we are designing behavioral tests for mice that have conceptual analogies to the defining symptoms of autism. The core deficit in reciprocal social interaction is being modeled with mouse social tasks. Our teams at the University of North Carolina and NIMH designed and built a new automated three-chambered apparatus for quantitating social behaviors in mice. First results show high levels of social approach and normal preference for social novelty in six inbred strains of mice. Sociability scores were similar with automated and observer-scored methods, in juveniles and adults, in males and females, and with repeated use of the same individuals. Three inbred strains displayed unusually low levels of social approach. Repetitive ritualistic behaviors are being evaluated by training mice to form a spatial habit for a food reinforcer in a T-maze, and for an escape platform in the Morris water maze, and then changing the location of the reinforcer, to model the symptom of resistance to change in routine. Behavioral assays with face validity to some of the core symptoms of autism can be applied to test hypotheses about toxicological causes of autism. *Supported by STAART U54 MH66418 and the NIMH Intramural Research Program.*

45

MURINE MODELS OF SOCIAL BEHAVIOR: GENE TOXICANT INTERACTIONS. Robert F Berman, *Center for Children's Environmental Health and the Murine Behavioral Assessment Laboratory, UC Davis, Davis, CA, USA*

The possible effects of neonatal exposure to environmental toxins on brain development and social behavior are of concern in view of epidemiological data suggesting an increased incidence of neurodevelopmental disorders, including autism. In order to systematically investigate the possible involvement of neonatal xenobiotic exposure in abnormal development, we have developed and applied a battery of behavioral tests and histological procedures for use in mice. This battery incorporates established neurobehavioral toxicological tests of development, including developmental markers, and tests of sensory and motor performance. A novel and important feature of our assessment procedures is its focus on tests of social recognition and social interactions, as well as sensory gating, fear and anxiety. Histological procedures include unbiased stereological quantification of neuronal numbers in the hippocampus as well as assessment of gross morphological features. Data will be presented demonstrating the application of this test battery to assess complex social behaviors in the Homer 1 knockout mouse. In addition, the results of an extensive series of studies examining the effects of neonatal exposure to thimerosal in mice will be presented. The strategy for assessing toxin exposure in mice on brain development and expression of complex behaviors (e.g., social interactions) will be discussed. Keywords: Thimerosal, Mice, Behavior. *Supported by NIEHS 1 P01 ES11269 and the UC Davis M.I.N.D. Institute.*

46

CHOLINERGIC INVOLVEMENT IN NEUROCOGNITIVE FUNCTION: FROM ZEBRAFISH TO HUMANS. Edward D. Levin, *Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC, USA*

Translation among complementary model systems requires the determination of common mechanisms of effect. Cholinergic systems have been widely shown to be critical

for cognitive function in human and rodent models. Cholinergic systems are critically involved both in neurodevelopmental processes laying down the brain systems essential for cognitive function and in the expression of those systems in adults performing cognitive tasks. This dual phase involvement of cholinergic system involvement in cognitive function has been well documented in rodent models. For example, rats exposed during early development to the organophosphate insecticide chlorpyrifos showed learning and memory impairments on the radial-arm maze when they became adults. Other studies have demonstrated the involvement of both nicotinic and muscarinic cholinergic receptor systems in the expression of learning and memory function with acute manipulations in adult rats. For example, we and others have found that nicotine significantly improves working memory function in rats. It would be beneficial to use zebrafish models to screen for neurocognitive effects of drugs and toxicants. We have developed methods for assessing learning and memory in zebrafish. Using the delayed spatial alternation test in adult zebrafish, we have found that exposure to chylopyrifos during early development causes persisting choice accuracy deficits. Acute nicotine administration in adult zebrafish cause an improvement in choice accuracy with an inverted U-shaped dose effect function much as is seen in rodents, monkeys and humans. Cholinergic systems appear to be critically involved in both the early development and later expression of neural systems underlying cognitive function in a variety of species including fish, rats, monkeys and humans. Keywords: Zebrafish, Acetylcholine, Cognition. *(Supported by the Duke University Superfund Basic Research Center P42 ES010356.)*

Symposium

SESSION V: **MOLECULES TO (WO)MAN – B. HUMANS**

Dissecting the Dysfunction to Look at the Whole Picture

Co-Chairs: Susan L. Schantz, Ph.D.
S. Jill James, Ph.D.

47

APPLYING DATA FROM ANIMAL MODELS TO EPIDEMIOLOGICAL RESEARCH. Susan L Schantz, *University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Results from toxicological research in animal models can provide valuable insights into what types of functional effects might be expected in human populations, particularly if careful thought is given to the following two issues: (1) designing the animal studies to model actual human exposure scenarios as closely as possible and (2) selecting outcome measures that have clear human counterparts. In our research, we used fish contaminant data from the Wisconsin Department of Natural Resources to formulate an experimental PCB mixture that is representative of the PCB mixture found in fish actually being consumed by the human population we are studying in northeastern Wisconsin. Female Long-Evans rats were exposed to the PCB mixture beginning 4 weeks prior to mating and continuing through weaning. Male and female offspring from each litter were evaluated on an extensive battery of tests of cognitive, motor and auditory function. The results of these animal studies are be used to guide the selection of appropriate tests for assessing neurodevelopment in infants exposed to PCBs through maternal consumption of contaminated fish in the epidemiological study. For example, auditory testing of rats using distortion product otoacoustic emissions (DPOAEs), which measure cochlear function, revealed a decrease in the amplitude of the response and an increased in the threshold for a response across a range of frequencies. Auditory brainstem response (ABR) thresholds were also increased across a range of frequencies. These data were used to design an auditory testing protocol for newborn babies, including assessments of DPOAEs and ABRs across a range of frequencies. Keywords:

48

OXIDATIVE STRESS IN CHILDREN WITH AUTISM: METABOLIC BIOMARKERS AND GENETIC POLYMORPHISMS. S.J. James, S. Melnyk, S. Jernigan, *Department of Pediatrics, School of Medicine, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR, USA*

Autism is a complex neurodevelopmental disorder with a reported prevalence of 1 in 1000 children in the US. Although both genetic and environmental factors are thought to contribute to the development of autism, none have been reproducibly identified. The possibility that autism has a metabolic basis has not been well-explored, despite the fact that chronic biochemical imbalance is often a primary factor in the development of complex disease. The metabolic phenotype of an individual is a reflection of the combined influence of endogenous and exogenous factors on genotype. As such, it provides a window through which the interactive impact of genes and environment may be viewed and relevant susceptibility factors identified. New evidence indicates that children with autism have a severely abnormal metabolic profile indicating a significant deficit in antioxidant and methylation capacity. Baseline levels of methionine transmethylation and transsulfuration metabolites were initially measured in plasma from 20 autistic children and 33 age-matched control children. These preliminary results were recently confirmed in a larger cohort of 95 autistic and 75 control children and indicated a two-fold reduction in the ratio of reduced to oxidized glutathione (GSH/GSSG redox ratio). Metabolic precursors for glutathione, methionine and cysteine, were also lower suggesting inadequate GSH synthesis in the autistic children. In addition, the ratio of plasma *S*-adenosylmethionine to *S*-adenosylhomocysteine (SAM/SAH ratio), an index of cellular methylation capacity, was decreased by 30%. These results are of concern because they indicate a significant decrease in antioxidant (↓GSH/GSSG) and methylation (↓SAM/SAH) capacity and an increase in oxidative stress (↑GSSG). The

abnormal levels of metabolites that regulate these pathways provide a rationale for the selection of functional candidate genes that could interact to promote the metabolic imbalance. Polymorphic variants in transcobalamin II (TCII), methylenetetrahydrofolate reductase (MTHFR), catecholamine-*O*-methyltransferase (COMT), glutathione-*S*-transferase (GST) M1/T1 and APO E4 were evaluated in 360 autistic children and 205 controls. The frequency of MTHFR 677CT/1298AG heterozygosity, TCII 776GG, COMT 1947GG and the GST M1/T1 double null genotypes were increased in the autistic children relative to controls. We hypothesize that an increased vulnerability to oxidative stress (environmental and/or intracellular) may contribute to the development and clinical manifestations of autism. Keywords: Autism, Glutathione, Polymorphisms

49

DEFINING THE AUTISM AND BROAD AUTISM PHENOTYPES. Joe Piven, *Neurodevelopmental Disorders Research Center, University of North Carolina, Chapel Hill, NC, USA*

Autism is a neurodevelopmental disorder defined by the presence of social and language characteristics, ritualistic-repetitive behaviors and a characteristic course. Family and twin studies show strong evidence for genetic factors underlying this condition, although there is evidence to also suggest the importance of environmental factors in some cases. While the defining features of autism are behavioral, studies in autistic individuals and their family members indicate the segregation of several neuropsychological characteristics that suggest 'intermediate phenotypes' in this condition. In this presentation data from family genetic and neuroimaging studies showing clinical characteristics that may index genetic liability to autism will be reviewed. While the phenotype in autism has long been defined on the basis of clinical characteristics associated with impairment, future studies aiming to gain insight into the underlying pathogenesis of autism should rely on behavioral, neuropsychological and biological phenotypes that have been shown to be associated with the underlying genetic liability to this condition. Keywords: Autism, Phenotype, Genetics

50

FROM ANTIQUITY TO THE 21st CENTURY: THE PAST, PRESENT AND FUTURE OF LEAD TOXICITY. Herbert L Needleman, *Department of Psychiatry, University of Pittsburgh School of Medicine. Pittsburgh, PA, USA*

Scientific understanding of childhood lead toxicity has traversed five phases: physicians initially doubted the existence of the disease in children. After its impact in children was accepted, it was widely believed was that there were only two outcomes: death or complete residua-free recovery. Long-term effects were demonstrated in 1943, but were thought to be seen only in children who displayed frank signs of encephalopathy. In the current fourth phase, the effects of asymptomatic lead exposure on IQ scores in children are generally accepted. More recent studies demonstrate effects of lead on attention, aggression and delinquency, and indicate that these are more important and sensitive effects than cognition. Bone lead levels levels in 301 12-year-old Pittsburgh school children were significantly related to attentional dysfunction and delinquent behavior after covariate adjustment. High lead subjects also reported more delinquent behaviors on the Self Report of Delinquency. We next conducted a case-control study of 195 arrested and adjudicated as delinquent males and 145 non-delinquent controls. After covariate adjustment, the odds ratio for delinquent behavior in relation to high bone lead level was 4.0 (CL 95%: 1.4–11.1). At least three ecological studies support the above findings; they report close correlations between either sales of leaded gasoline or air lead levels and homicide rates. Lead is primarily sequestered in bone, and bone demineralizes with age. The recirculation of lead with age raises the question of its role in dementia. Lead exposure and Alzheimer's disease share similar risk factors: race and urban residence. Autopsy of a 40-year-old male who had experienced severe lead toxicity as a child disclosed both neurofibrillary tangles and amyloid plaques. Most recently rats given small doses of lead at 1 day of age had elevated Amyloid precusor protein levels at 20 months of age. An epidemiological design to examine the association between prenatal lead exposure and Alzheimers' disease will be presented. Keywords: lead, neurodevelopment, delinquency.

51

UPDATE ON THE NATIONAL CHILDREN'S STUDY. Carole A. Kimmel, *Consultant, National Children's Study Program Office, National Institute of Child Health and Human Development, NIH, DHHS, Bethesda, MD, USA*

The National Children's Study is the largest and longest research effort of its type planned for studying the effects of environmental factors on children's growth, development, and maturation. The study, which is co-sponsored by the Department of Health and Human Services (NICHD, CDC, NIEHS) and the U.S. Environmental Protection Agency, will enroll women through a household-based probability sample, and will include those women who are contemplating pregnancy as well as those who become pregnant during the enrollment period. Approximately 100,000 children will be followed from birth to adulthood for a number of measures of growth and development, including neurobehavioral development, and their environments will be evaluated in detail. For this study, environment is defined broadly to include chemical, physical, biological (including genetic), and psychosocial factors. Using a hypothesis-driven approach, the study will explore the effects not only of environmental pollutants, but also of factors such as the built environment, the role of infection and inflammation, the impact of the media, parenting, family structure, education, health care delivery, and how these may interact with genetic or other environmental

factors to impair or improve the health and well-being of children. The study will evaluate the impact of such exposures and their timing during development on pregnancy outcomes, growth, physical and psychosocial development, asthma, injuries, and obesity. Implementation of the National Children's Study is underway. NICHD, which has the lead for the study, will award contracts for the Coordinating Center and Vanguard Centers for the study this fall. The Study Plan, published in November 2004 (www. nationalchildrensstudy.gov), outlines the basic study design for enrollment and the locations of the vanguard and other sites for the study, which are geographically and demographically dispersed throughout the United States. The first efforts of the centers will be to engage communities in partnership for enrollment and participation in the study as well as for long-term follow-up throughout the life of the study. A number of environmental and biological samples collected for the study will be stored for future research efforts. The goal of the National Children's Study is to provide the data necessary for reducing disease and disability and for improving the lives and well-being of the nation's children.

Poster Session

SESSION VII: **GENERAL POSTER SESSION**

See pages 26–44 for Abstracts presented from Poster. Poster abstracts are numbered from P-73 to P-134.

The poster session is a highlight of this conference series. It has proven to be an effective venue for informal, in-depth discussion and collaboration building – as well as an important networking opportunity for all participants. Papers on any aspect of neuroscience, toxicology, children's environmental health, public health and policy are welcome! Judging and selection of Student Awardees will be made during the poster session.

Symposium

SESSION IX-A: **CONTEMPORARY HEALTH ISSUES ASSOCIATED WITH OVER EXPOSURE TO MANGANESE**

Co-Chairs: Michael Aschner, Ph.D.
Thomas Gunter, Ph.D.

Theme: *This multidisciplinary session will address contemporary research issues associated with the health effects of manganese (Mn) both in humans and animal models. Speakers will discuss recent findings on the specific cellular, molecular, and physiologic mechanisms by which manganese mediates its adverse effects. Speakers will also note factors, such as age, pre-existing disease, and genetics, as conditions that might predispose individuals to enhanced susceptibility to manganese toxicity. The session will span studies in various tissue culture models to non-human primates, incorporating diversity of techniques, from molecular biology to imaging.*

This session is sponsored by the Manganese Health Research Program (MHRP).

52

FACTORS THAT INFLUENCE THE PHARMACOKINETICS OF INHALED MANGANESE. DC Dorman, MF Struve, *CIIT Centers for Health Research, Research Triangle Park, NC, USA*

Manganese (Mn) is an essential trace metal that at high doses is also associated with a neurological disorder in humans that mimics Parkinson's disease. Increased Mn deposition within dopaminergic brain structures that regulate motor activity is a key first step in the pathogenesis of Mn neurotoxicity. Our recent work provides additional evidence that the dosimetry of Mn is heavily influenced by the route of exposure. Mn absorption from the gastrointestinal tract ans subsequent elimination in the bile (i.e., net absorption) is influenced by the amount of Mn in the diet. The dosimetry of inhaled Mn is influenced by the exposure concentration, duration of exposure, the age of the animal, particle size, and particle solubility. Species differences in brain delivery exist between rodents and nonhuman primates. Our laboratory has also demonstrated that inhaled Mn is absorbed by the olfactory epithelium and subsequently undergoes transport via the olfactory nerve to the olfactory bulb. Ongoing studies are exploring cellular mechanisms involved in the uptake and delivery of inhaled Mn via the olfactory nerve.

53

MANGANESE TRANSPORT IN THE CNS. VA Fitsanakis[1], KM Erikson[2], M Aschner[1,3,4], *[1]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN; [2]Department of Nutrition, University of North Carolina, Greensboro, NC; [3]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN; [4]The Kennedy Center, Vanderbilt University Medical Center, Nashville, TN*

Manganese (Mn) plays an important role in many biological processes. It is a component or co-factor of many enzymes involved in the metabolism of fats and proteins, and is utilized by various antioxidant enzymes such as superoxide dismutase (MnSOD) and glutamine synthetase. Though essential, Mn is neurotoxic at high levels and has been associated with the development of Parkinson's disease. Currently, the mechanisms responsible for transporting Mn across the blood–brain barrier (BBB) are unknown. Using rat brain endothelial 4 (RBE4) cell monolayers cultured in the presence and absence of astrocyte conditioned media (ACM), we examined the effects of temperature-, energy-, proton (pH)-, iron (Fe)- and sodium (Na^+)-dependence on Mn transport. Our results suggest that Mn transport is temperature-, energy- and pH-dependent, but not Fe- or Na^+-dependent. The presence of ACM in endothelial cell cultures also decreased the permeability of these cells to Mn, reinforcing the use of ACM or astrocyte co-cultures in studies examining metal transport across the BBB. Additional studies were designed to examine the effect of dietary iron (Fe) and manganese levels on the concentrations Mn in five various brain regions. Because divalent metal transporter has been implicated

as a transporter of brain Fe and Mn and, another goal of the study was to measure brain regional changes in transporter levels using Western blot analysis. As expected, there was a significant effect of Fe deficiency ($P < 0.05$) on decreasing Fe concentrations and increasing Mn concentrations throughout the brain. Transporter protein in all regions increased due to ID compared to control levels ($P < 0.05$). Keywords: Manganese, Transport, Iron. *(Supported by NIEHS 10563.)*

54

CHARACTERIZATION OF WELDING FUMES AND THEIR POTENTIAL NEUROTOXIC EFFECTS. JM Antonini, DB Miller, JP O'Callaghan, *Health Effects Laboratory, NIOSH, Morgantown, WV, USA*

Serious questions have been raised regarding a possible causal association between neurological effects in welders and the presence of manganese in welding consumables. The objectives of the study are to: (1) construct an automated, computer-controlled welding fume generation system to simulate real workplace conditions and (2) examine the potential neurotoxic effect of manganese in rats after pulmonary exposure to different welding fumes. The system is comprised of a programmable six-axis robotic welding arm and a water-cooled arc welding torch. A flexible trunk has been attached to the robotic arm of the welder and is used to collect and transport fume from the vicinity of the arc to the animal exposure chamber. Of the metals measured, manganese comprised ~10–15% of the stainless and mild steel fume generated as determined by inductively coupled plasma atomic emission spectroscopy. Size distribution analysis indicated the mass median aerodynamic diameter of the generated particles to be approximately 0.24 μm. As determined by scanning electron microscopy, the generated aerosols were mostly arranged as chain-like agglomerates of primary particles. These fume characterization studies have indicated that particle morphology, size, and chemical composition are comparable to welding fume generated in the workplace. Initial animal inhalation studies are underway. Sprague–Dawley rats are being exposed to 15 or 40 mg/m^3 of welding fume for 3 h/day for 10 or 30 days. After exposure, manganese concentrations will be determined in a number of discrete brain regions. Neurotoxicity will be detected and quantified by measuring the increased expression of glial fibrillary acidic protein and using silver degeneration staining technology. Because dopaminergic systems have been implicated as targets of manganese exposure, levels of dopamine and tyrosine hydroxylase, biomarkers of dopaminergic neuronal damage, also will be measured. With the development of this novel system, it will be possible to establish an animal model using controlled welding exposures to investigate the possible mechanisms by which welding fumes may affect the central nervous system. Keywords: Welding fume, Manganese, Neurotoxicology

Disclaimer: The finding and conclusions of this abstract have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

55

DISCOVERY OF BIOMARKERS OF MANGANESE EXPOSURE IN HUMANS. Wei Zheng, *School of Health Sciences, Purdue University, West Lafayette, IN, USA*

The lack of a reliable biomarker for manganese (Mn)-induced Parkinsonism has hampered the diagnosis and therapeutic intervention of Mn neurotoxicity. We have determined airborne levels of Mn during welding practice and serum concentrations of possible biomarkers, aiming to establish the relationship between long-term, low-level exposure to Mn and altered serum levels of Mn, Fe, Cu, Zn, and Pb as well as proteins associated with Fe metabolism in career welders. Mn-exposed group consisted of welders who have engaged in electric arc weld in a vehicle manufacturer, while the control subjects were employees in the same factory but not in the welding profession. Average serum levels of Mn and Fe among welders were significantly higher than those of controls ($p < 0.01$). Among the welders, serum concentrations of both metals were highest in the youngest age group (≤30 years), an evidence of relationship to recent, active exposure. While serum Cu concentrations in welders were unchanged, blood Pb concentrations and serum Zn levels in welders were significantly increased and decreased, respectively, compared to controls. Concentrations of serum ferritin and transferrin were increased among welders; however, serum transferrin receptor levels were significantly decreased in comparison to controls. Linear regression revealed a positive association between serum Fe and ferritin levels and welder's employment years; yet serum transferrin receptor levels were inversely associated with serum Mn concentrations. Biochemical assays showed that the activity of erythrocytic superoxide dismutase (SOD) in welders was reduced by 24% compared to controls ($p < 0.05$), while the levels of serum malondialdehyde (MDA) were increased by 78% ($p < 0.05$). These findings suggest that occupational exposure to the welding fume among career welders disturbs the homeostasis of trace elements in the systemic circulation and induces oxidative stress. Although the feasibility of using proteins associated with Fe metabolism as the biomarker for Mn exposure remains to be further explored, serum Mn may serve well as a reasonable biomarker for assessment of recent exposure to airborne Mn. Keywords: Biomarker, Iron, Ferritin. *(Supported by NIH/NIEHS Grant ES-08146.)*

56

NEUROCHEMICAL CHANGES IN THE LIVING NON-HUMAN PRIMATE BRAIN FOLLOWING MANGANESE EXPOSURE. Tomás R. Guilarte, *The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA*

Occupational exposures to manganese (Mn) are known to cause a Parkinsonian-like syndrome in humans and non-human primates. The precise molecular mechanism(s) by which Mn

produces these neurological effects are not known but dopaminergic dysfunction has been proposed as an underlying pathology. A limited number of studies on humans and non-human primates exposed to Mn have assessed the integrity of the dopaminergic system in the caudate/putamen (C/P) using in vivo brain imaging techniques such as Positron Emission Tomography (PET). The majority of PET studies have measured either dopamine transporter (DAT) levels or fluoro-dopa accumulation in the C/P as markers of nigrostriatal dopaminergic neuron integrity. The findings have been equivocal in that some studies demonstrate significant deficits in DAT while fluoro-dopa studies seem to demonstrate an intact dopaminergic system. No previous study has measured the effect of Mn exposure on in vivo dopamine release (DAR) following Mn exposure.

The present work is part of an ongoing multidisciplinary study prospectively examining the effects of chronic Mn exposure on behavioral, in vivo neuroimaging and pathological endpoints in non-human primates. Cynomolgus macaques ($n = 4$) exposed to the lowest level of $MnSO_4$ (cumulative doses of Mn ranged from 152 to 172 mg/kg BW, i.v.) over approximately 9 months exhibited marked reductions in DAR in the C/P. In vivo DAR was measured by PET following continuous infusion of [11C]-raclopride, a D2 receptor ligand, with a dopamine-releasing dose of amphetamine (2 mg/kg), 40 min post initiation of [11C]-raclopride infusion. A dose-dependent reduction in DAR in Mn-exposed animals was measured in the absence of a change in [11C]-raclopride binding to dopamine receptors or [11C]-methylphenidate binding to DAT from baseline levels. These findings suggest that at this level of Mn exposure there was a significant decrease in DAR in the absence of a change in dopamine terminals in the C/P. The lack of a change in C/P DAT levels from baseline was confirmed by ex vivo [125I]-RTI-121 quantitative autoradiography when compared to Mn-naïve animals ($n = 3$). The current study demonstrates that Mn exposure produces dysfunction of pre-synaptic dopaminergic neurons in the C/P and it may help explain the Mn-induced Parkinsonism known to occur in exposed subjects. To our knowledge, this is the first report of Mn-induced reductions in brain DAR measured in vivo by PET. *[This work is supported by grant ES010975 to TRG.]*

Developmental Toxicology Technical Workshop

SESSION IX-B: **OPTIMIZING THE DESIGN AND INTERPRETATION OF EPIDEMIOLOGICAL STUDIES FOR ASSESSING NEURODEVELOPMENTAL EFFECTS FROM IN UTERO CHEMICAL EXPOSURE**

Chair: Roger Ladda, M.D., *Hershey Medical Center*

Session Theme and Description: *While many epidemiologic studies of children's environmental health have been completed, and more are being planned, a comprehensive critical examination of the methodologies commonly used in past studies has not been conducted. In fact, in some of the completed studies (e.g., those related to pharmaceuticals and environmental chemicals such as lead, methylmercury, and PCBs), the authors have acknowledged the limitations of existing methods. Currently, there is a great deal of interest in conducting additional epidemiologic investigations into environmental chemicals and children's health. For example, the proposed National Children's Study (NCS) is likely to investigate environmental and other factors influencing the health and development of children in utero, through birth, childhood, and into young adulthood.*

Therefore, as new studies are being planned, this is an appropriate time to determine whether existing methods as they have been practiced will serve future studies, especially those designed to assess the potential impacts at current exposures. In short, such an examination serves to identify the key methodological factors that ultimately determine the value and strength of future research. Thus, investigators designing new studies will benefit from this thoughtful examination as they develop future study designs and analyze resulting study data. The outcome of this session will provide valuable input not only to the design of future investigations, but also metrics whereby scientists and others can judge the adequacy of reported studies. However, the scope of the session will focus only on scientific methodological issues (i.e., the development of 'best practices' for future study design, conduct, reporting, and interpretation); that is, specifically, it will not include an evaluation of conclusions or findings from previous epidemiological studies of environmental health.

The Expert Panel assembled for this session will address a series of topics with related questions prior to, and during, the session. These topics and questions include:

Study Design:

- What is the best experimental design and methodology to assess the likelihood that in utero exposure to an environmental chemical can result in adverse neurodevelopmental effects in newborns that continue into childhood?
- What are the statistical issues that must be addressed to conclude with adequate confidence that an in utero exposure to a specific environmental chemical can result in adverse neurodevelopmental effects?

Measurement Tool:

- What specific measurement tools/tests are best suited and validated for assessing the variety of potential neurodevelopmental and behavioral deficits? What is the known sensitivity, specificity and predictive value of each endpoint being measured? How reproducible is/are the measurement(s)?

- Are there particular sampling strategies or data collection methods that are especially relevant to detecting potential neurodevelopmental effects from in utero exposure? What sampling and analysis strategies can be employed to avoid Type II (failure to detect a real effect) errors?
- What is the relationship between the estimated window of exposure and the nature of a potential effect, and how might this affect the selection of tests?
- How might data and methods from the field of molecular epidemiology be used to enhance traditional epidemiologic approaches?

Exposure Assessment:

- Which specific measurement tools and biomarkers are best suited and validated for assessing the nature, extent, and patterns of in utero exposure to a particular environmental chemical? How might these differ from tools and biomarkers used to assess post-natal exposure?
- To avoid exposure misclassification or misleading estimates when assessing potential exposure, how frequently should exposure be estimated (i.e., what temporal units should be used for serial exposure measurements)? If appropriate temporal units are chemical- or tissue-specific, what data or criteria should be used to determine the optimal units? How should critical timeframes – critical windows of vulnerability in neurodevelopment – be taken into account when designing an exposure assessment approach?
- How should potential aggregate exposure from multiple routes (inhalation, ingestion, dermal) be addressed? How should potential cumulative exposure to multiple chemicals be addressed? How can trends in exposure and trends in neurodevelopmental outcomes be assessed?
- How long should the subject be followed with appropriate studies (e.g., school age, puberty, reproductive ages)?

Participant Selection:

- What is the most appropriate and valid way to select and follow exposed and control groups for studies attempting to demonstrate an association between in utero exposure to a specific environmental chemical and adverse neurodevelopmental effects?

Confounders:

- What guidance can be offered with respect to selecting and measuring potential confounders? What criteria should be applied when selecting control variables for inclusion in a multivariate analysis? How should potential mediating factors be identified and analyzed?
- Are there cultural aspects to neurodevelopmental tests that should be considered before use?

Reporting:

- How is clinical significance versus population significance defined and reported?
- How do researchers address the issue of labeling of children based on study results and how should the study results be reported to parents?

Research Needs and Recommendation:

- What are the key needs for future research? What are the primary uncertainties and gaps in our knowledge that should be addressed with future research?

Expert Panel of Participants

Robert W. Amler, *New York Medical College, Valhalla, NY, USA*.
Stanley Barone, Jr., *NCEA/ORD, Neurotoxicology Division, US EPA*.
Aysenil Belger, *Department of Psychiatry, UNC at Chapel Hill, NC, USA*.
Cheston M. Berlin, Jr., (Steering Committee), *Children's Hospital, Milton S. Hershey Medical Center, Hershey, PA, USA*.
Christopher Cox, *Johns Hopkins University, Baltimore, MD, USA*.
Harry Frank, *The University of Michigan, Ann Arbor, MI, USA*.
Michael Goodman, *Emory University School of Public Health, Atlanta, GA, USA*.
Jean Harry, *National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA*.
Stephen R. Hooper, *University of North Carolina School of Medicine, Chapel Hill, NC, USA*.
Roger Ladda, *Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA, USA*.
Judy S. LaKind, (Steering Committee), *LaKind Associates, LLC, Hershey Medical Center, Penn State College of Medicine, University of Maryland School of Medicine, USA*.
Paul H. Lipkin, *The Johns Hopkins University School of Medicine, Baltimore, MD, USA*.
Lewis P. Lipsitt, *Brown University, Providence, RI, USA*.
Matthew N. Lorber, *National Center for Environmental Assessment, US EPA*.
Ann M. Mason (Steering Committee), *Research Foundation for Health and Environmental Effects, Arlington, VA, USA*.
Gary Myers, *University of Rochester Medical Center, Rochester, NY, USA*.
Larry L. Needham, *Division of Environmental Health Laboratory Sciences, Centers for Disease Control and Prevention, Atlanta, GA, USA*.

Theodore D. Wachs, *Purdue University, West Lafayette, IN, USA*.
Janice W. Yager (Steering Committee), *Electric Power Research Institute, Palo Alto, CA, USA*.

Platform Session

SESSION IX-C: **NEUROTOXICITY OF MIXTURES, SOLVENTS, AND METALS IN VIVO AND IN VITRO**

Co-Chairs: Evelyn Tiffany-Castiglioni, Ph.D.
Virginia Moser, Ph.D.

Theme: *Session IX-C will present an overview of the neurotoxicity of mixtures and multiple agents, including insecticides, metals, and solvents. Topics will include both in vivo and in vitro approaches for understanding the mechanisms and toxicologic interactions of organophosphate (OP) compounds in vivo and in vitro. The influence of age (young versus adult) as well as dose sequence on the outcome of OP pesticide mixtures will be described. State-of-the-art physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling approaches will be illustrated for OP compounds. Talks will also focus on in vitro models for screening and investigating interactions of complex mixtures and multiple sequential agents, as well as neurotoxicity of solvents and copper. The focus of in vitro studies will be mechanisms and relevance to neurologic disease.*

57

COMPARISON OF THE NON-ADDITIVE INTERACTIONS OF AN ORGANOPHOSPHORUS PESTICIDE MIXTURE IN ADULT AND PREWEANLING RATS. VC Moser, *NTD/NHEERL/ORD, US EPA, RTP, NC, USA*

Critical features of risk assessment include the evaluation of risk following exposure to pesticide mixtures as well as the potential for increased sensitivity of the young. This research tested for interaction(s) using a mixture of five organophosphorus (OP) pesticides (chlorpyrifos, diazinon, dimethoate, acephate, and malathion) in both adult and preweanling (17 days old) rats using a fixed-ratio ray design. The pesticide ratio was based on the relative dietary exposure estimates. Neurochemical (blood, brain cholinesterase [ChE] activity) and behavioral (motor activity, gait score, tail–pinch response score) endpoints were assessed following acute oral exposure. Dose–response data were collected for each OP alone, which were used to build an additivity model to predict the effects of the pesticide mixture along a ray of increasing total doses. The mixture (''full'' ray) data were similarly modeled and statistically compared to the additivity model along the ray. To evaluate the influence of malathion, a second pesticide mixture was tested without malathion but with the same proportions of the other OPs (''reduced'' ray). In both adult and preweanling rats, the analyses revealed significant greater-than-additive (synergistic) responses for blood and brain ChE inhibition, motor activity, gait alterations, and tail–pinch response (pups only). In general, the deviation from additivity in pups was greater than or equal to that seen in the adults. Comparing the full and reduced rays showed that malathion interacts with the other OPs for some endpoints; however, the deviation from additivity cannot fully be attributed to the malathion in the mixture. Thus, greater-than-additive responses were observed for most endpoints, with more pronounced effects in the young rats. Keywords: Organophosphate, Mixture, Behavior. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

58

EXPOSURE SEQUENCE INFLUENCES CHOLINERGIC TOXICITY IN NEONATAL RATS EXPOSED TO TWO ORGANOPHOSPHORUS INSECTICIDES. Carey N Pope, *Department of Physiological Sciences, Center for Veterinary Health Sciences, Oklahoma Sate University, Stillwater, OK, USA*

We previously reported that sequence of exposure to chlorpyrifos and parathion in adult rats can markedly influence toxic outcome, possibly based on relative contributions of A-esterase- and carboxylesterase-mediated detoxification. Neonatal rats are more sensitive than adults to acute toxicity of both chlorpyrifos and parathion, and lesser A-esterase- and carboxlyesterase-mediated detoxification is proposed to play a role. We evaluated the interactive toxicity of chlorpyrifos (8 mg/kg, po) and parathion (0.5 mg/kg, po) in neonatal (7 days old) rats exposed either concurrently or sequentially (separated by 4 h). Animals were sacrificed at 4, 8 and 24 h after the first exposure for biochemical measurements (cholinesterase and carboxylesterase activities). Concurrently exposed rats showed more cumulative lethality than either of the sequential dosing groups. Rats treated initially with chlorpyrifos (CPF-first) exhibited significantly higher lethality compared to those treated with parathion first (PS-first). At 4 h, concurrently dosed rats showed higher brain cholinesterase (ChE) inhibition compared to both sequential dosing groups. At 8 h, brain ChE inhibition was higher in CPF-first compared to PS-first group. Plasma and liver carboxylesterase inhibition was relatively similar among treatment groups across time points. In liver homogenates from untreated rats, A-esterases efficiently detoxified chlorpyrifos oxon in vitro (carboxylesterases were less effective) whereas A-esterases had little apparent effect on paraoxon. In homogenates from parathion pre-treated rats A-esterases effectively detoxified chlorpyrifos oxon, while homogenates from chlorpyrifos pre-treated rats had essentially no effect on paraoxon (via A-esterases or carboxylesterases). While it is generally thought that limited oxon detoxification in neonates is a major factor in their higher sensitivity, relative detoxification of chlorpyrifos oxon and paraoxon appears important in the observed differences in toxicity based on sequence of exposure. Keywords: Acetylcholinesterase, Cumulative, Interactive. *(Supported by STAR grant R825811 from US EPA and OSU Board of Regents.)*

59

EFFECTS OF BINARY OR TERNARY MIXTURES OF ORGANOPHOSPHATES ON ESTERASES IN VITRO. Janice E Chambers, *Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA*

Cholinesterase inhibition by mixtures of the anticholinesterase organophosphates must be determined in order to effectively predict pesticide risk in the cumulative risk assessments mandated by the Food Quality Protection Act. These experiments were designed to determine whether target (brain) and non-target (serum) cholinesterase inhibition by mixtures of organophosphates in vitro was additive, and whether predictive mathematical models for the inhibition could be developed. Cholinesterase of rat brain homogenates or serum was exposed to binary or ternary mixtures of organophosphates, specifically the oxon metabolites of several organophosphorus insecticides, to determine the level of inhibition and to assess additivity. With binary mixtures exposures were either simultaneous or sequential, and with ternary mixtures exposures were simultaneous only. Relatively low concentrations were used, with a maximal inhibition of 30% from individual compounds. Cholinesterase inhibition from these mixtures was additive and was predictable with mass action models using ordinary differential equations. Inhibition of serum cholinesterase from similar experimental paradigms was not additive, was not predictable by the similar mass action models, and was influenced greatly by the likelihood of the compounds being stoichiometrically detoxified by the serum carboxylesterases. Keywords: Organophosphate, Mixtures, Esterases. *Supported by American Chemistry Council grant CRAM2a-99.*

60

IN VITRO MODELS FOR ASSESSING NEUROTOXICITY OF MIXTURES. Evelyn Tiffany-Castiglioni, Yongchang Qian, K.C. Donnelly, *Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA*

Rapid, inexpensive methods are needed to investigate the interactions of complex mixtures. Presented here are results of three studies from our lab investigating the toxicity of binary mixtures in in SY5Y human neuroblastoma cells. Chemicals tested for cytotoxicity included benzo-a-pyrene (BaP), chrysene, anthracene, pentachlorophenol (PCP), vinyl chloride monomer (VC), trichloroethylene (TCE), and dichloroethylene (DCE). In addition, effects of a binary mixture of metals, Cu and Pb on cell homeostasis endpoints were carried out. The most sensitive measure of PAH cytotoxicity in neural cells was amino acid incorporation into proteins. Single PAHs were capable of producing damage to neural cells only at concentrations near their solubility limits, though BaP, the most toxic of all PAHs tested, induced a toxic response at concentration levels of 3 and 30 μM after metabolic activation (0.25% S9). With mixtures of PAHs the majority of samples induced additive responses. The minimum concentration required to induce a significant response was reduced for the mixture of chrysene and BaP when compared to BaP alone. In addition, PCP appeared to increase the inhibition of acetylcholinesterase by mipafox. VC was inactive, while both TCE and DCE induced a cytotoxic response. The viability of cells exposed to a mixture of TCE and VC induced a response that was significantly lower than both the control and from TCE alone. These results suggest general insensitivity of cytotoxicity assays to PAH and HAH binary mixtures. In rat astrocyte primary cultures exposed to both Pb (10 μM) and Cu (10 μM), the generation of reactive oxygen species (ROS) was significantly higher than after treatment with either metal alone. Cu accumulation and induction of glucose regulated protein 78 and 94 (GRP78 and GRP94) were also increased more by both metals than either metal alone. Preliminary stoichiometric data showed that the heavy metal binding region of the copper efflux pump ATP7a (Menkes protein), bounds nearly twice as many Pb as Cu molecules. This observation, coupled with previous results that Pb enhances Cu accumulation in glia, suggests the additive effects observed resulted from blockage of the Cu pump by Pb. Keywords: In vitro, Chemical mixtures, Neurotoxicity

61

DIETARY COPPER SUPPLEMENTATION ENHANCES THE PERIPHERAL MYELINOPATHY PRODUCED BY DITHIOCARBAMATES IN RATS. William M Valentine, *Center for Molecular Toxicology and Center for Molecular Neuroscience, Vanderbilt University Medical Center, Nashville, TN, USA*

Some dithiocarbamates have been demonstrated to elevate copper levels within the peripheral and central nervous systems and produce a peripheral myelinopathy. Dithiocarbamate-mediated accumulation of copper and myelin injury may be mediated through the ability of dithiocarbamates to form lipophilic copper complexes and promote lipid peroxidation, respectfully. To assess whether tissue copper levels are correlated with the severity of injury produced in peripheral nerve by dithiocarbamates, rats were administered either parenteral *N*,*N*-diethyldithiocarbamate for 8 weeks or oral pyrrolidine dithiocarbamate chronically and maintained on a diet containing 13 ppm copper or 200 ppm copper. Controls for each diet were also examined. At the end of the exposure periods the levels of tissue copper and severity of lesions in sciatic nerve were determined. To assess the contribution of lipophilicity to dithiocarbamate mediated accumulation of copper in the nervous system a series of dithiocarbamates differing in the polarity of their nitrogen substituents were subchronically administered to rats by abdominal osmotic pumps and the levels of tissue copper in brain determined. Both the level of copper within the nervous system and the severity of lesions in peripheral nerve were augmented by dietary copper supplementation in conjunction with administration of both

dithiocarbamates; whereas increased dietary copper alone produced no significant differences relative to controls. Administration of dithiocarbamates predicted to generate lipophilic copper complexes produced significant elevations in brain copper levels; whereas those forming more hydrophilic complexes were not significantly different from controls. These results suggest a role for copper accumulation in dithiocarbamate mediated myelinopathy and that the potential of a particular dithiocarbamate to elevate nervous system copper and produce neurotoxicity may be related to the polarity of its nitrogen substituents. Keywords: Dithiocarbamate, Copper, Myelin

62

COPPER-REGULATED APP EXPRESSION IN HUMAN ASTROCYTOMA CELLS. Yongchang Qian, Ying Zheng, Evelyn Tiffany-Castiglioni, *Texas A&M University, College Station, TX, USA*

Copper (Cu) is an essential trace element in human health. The disturbance of Cu homeostasis in the nervous system has been implicated in lead (Pb) neurotoxicity and in neurodegenerative diseases, including Alzheimer's disease (AD). Amyloid precursor protein (APP) is a Cu-binding protein that is cleaved to generate $A\beta_{40–42}$ in AD. We hypothesize that the elevation of Cu levels in the nervous system alters APP expression and augments Aβ production in neural cells. In this study, we used human CCF-STTG1 astrocytoma and SY5Y neuroblastoma cells as glial and neuronal models, respectively, to study the regulation of APP expression by Cu elevation in the medium. Both cell lines were treated with 0, 50, and 150 μM $CuSO_4$ for 1 or 3 days. Their cytotoxicity, *APP*, *BACE1* (beta-site APP cleaving enzyme 1) and *GRP78* gene expression, and XBP-1 mRNA splicing were measured in parallel. On day 1, Cu-induced cytotoxicity was observed in astrocytoma cells treated with 150 μM but not 50 μM Cu, as indicated by 39 and 38% declines of cell proliferation and MTT reduction activity, respectively, compared to control. Levels of APP770 mRNA, a predominant isoform in astrocytes, were 1.6- and 1.9-fold of control in 50 and 150 μM Cu groups, respectively. BACE1 mRNA, a key enzyme in the process of APP to generate $A\beta_{40–42}$, was 1.8- and 3.8-fold of control in 50 and 150 μM Cu groups. The level of GRP78 mRNA, a molecular chaperone that is involved in $A\beta_{40–42}$ clearance, was 6.7-fold that of control in the 150 μM Cu group. In agreement with the change in GRP78, a 25 and a 90% increase of spliced and unspliced XBP-1 mRNA levels, respectively, compared to control, were observed in 150 μM Cu group. Interestingly, no change in the above parameters measured was observed in neuroblastoma cells. On day 3, greater reduction of cell proliferation (a 34% reduction in 50 μM Cu and a 67% reduction in 150 μM Cu) and MTT reduction activity (a 15% reduction in 50 μM Cu and 44% reduction in 150 μM Cu) were observed in astrocytoma cells. APP770 and BACE1 mRNA levels were elevated in astrocytoma cells treated with 150 μM Cu and were 2.4- and 2.5-fold, respectively, of control. However, GRP78 levels were reduced and XBP-1 levels returned to the control level. In neuroblastoma cells, changes observed with Cu increase were a slight reduction of cell proliferation and significantly decreased APP770 and APP751 mRNA levels. These preliminary data suggest that Cu induced APP expression in astrocytoma cells, and astrocytoma and neuroblastoma cells had different responses to Cu treatment. Furthermore, upregulation of BACE1 and GRP78 implied the increase of $A\beta_{40–42}$ production as a result of Cu neurotoxicity in Cu-treated astrocytoma cells. Keywords: Amyloid precursor protein, Copper, Astrocytes

Symposium – continued

SESSION IX-A: **CONTEMPORARY HEALTH ISSUES ASSOCIATED WITH OVER EXPOSURE TO MANGANESE**

Co-Chairs: Tomás Guilarte, Ph.D.
Anumantha Kanthasamy, Ph.D.

This session is sponsored by the Manganese Health Research Program (MHRP).

63

Mn^{2+} INTERFERENCE WITH Ca^{2+} ACTIVATION OF ATP PRODUCTION BY MITOCHONDRIA: A NOVEL HYPOTHESIS OF Mn NEUROTOXICITY. Thomas E. Gunter[1], Karlene K. Gunter[1], Michael Aschner[2], *[1]Department of Biochem. and Biophys.; Univ. of Rochester Medical School; Rochester, NY; [2]Department of Pediatrics; Vanderbilt University Medical School; Nashville, TN, USA*

Using XANES spectroscopy, which distinguishes Mn^{2+} and Mn^{3+} complexes, we have shown that there is no evidence for formation or accumulation of Mn^{3+} complexes from Mn^{2+} in brain mitochondria, neuron-like cells or astrocytes even under pro-oxidizing conditions. This suggests that damage to cells in Mn neurotoxicity is caused by transport of Mn^{3+} into brain cells or damage by the Mn^{2+} oxidation state. Mn^{2+} is sequestered by neurons and astrocytes, and once in the cell, is rapidly taken up by mitochondria via the Ca^{2+} uniporter. Intramito-chondrial Ca^{2+} controls the rate of ATP production by oxidative phosphorylation and can increase this rate several fold. Mn^{2+} readily binds to Ca^{2+} binding sites, usually with a higher affinity than Ca^{2+} itself, and often inhibits the functions of Ca^{2+}. We hypothesize that Mn^{2+} interference with Ca^{2+}'s role in increasing the rate of ATP production in metabolically active cells like those of the globus pallidus or striatum could be a cause of Mn neurotoxicity. The best known sites at which Ca^{2+} activates metabolic reactions leading to accelerated ATP production are αketoglutarate dehydrogenase (αKGDH), isocitrate dehydrogenase, and pyru-vate dehydrogenase. We have begun tests on Mn^{2+} inhibition of Ca^{2+} activation of NADH production by αKGDH as the first step in testing the above hypothesis of

damage by Mn^{2+}. While the results are incomplete, they show significant Mn^{2+} inhibition of Ca^{2+} activation of NADH production at αKGDH.

64

THE ROLE OF PRION PROTEINS IN MANGANESE NEUROTOXICITY. Anumantha Kanthasamy, *College of Veterinary Medicine, Iowa State University, Ames, IA, USA*

Prion diseases are fatal neurodegenerative disorders that affect humans (Creutzfeldt-Jacob disease), cattle (bovine spongiform encephalopathy), deer (chronic wasting disease), and sheep (scrapie). Normal prion proteins (PrP^c) are abundantly expressed in the CNS but the effect of environmental agents on the physiological function of prion proteins remains to be characterized. Because PrP^c have high affinity for certain divalent cations, we characterized in the present study the effect of manganese on prion-expressing neural cells (PrP^c) and prion-knockout (KO) cells. Manganese was toxic to both cell types, but PrP^c-expressing cells were less susceptible to manganese toxicity than prion-KO cells, suggesting that endogenous prion protein may protect against manganese toxicity. Depletion of cellular glutathione (GSH) levels was observed in a concentration dependent manner in both cell types, but the depletion was significantly attenuated in PrP^c-expressing cells. Treatment with manganese also activated the apoptotic cascade, including caspase-9 and caspase-3 in a time-dependent manner in both prion and prion-KO cells. Likewise, manganese-treated cells showed a dose-dependent increase in DNA fragmentation, which was blocked by the caspase inhibitors ZDEVD-fmk and ZVAD-fmk. Trace element analysis through induction coupled plasma mass spectrometry (ICP-MS) revealed that prion cells have higher basal manganese levels as compared to prion KO cells. We also tested another divalent metal cation, copper, in our cell models to determine the specificity of the prion protein effect on manganese neurotoxicity. Interestingly, we found copper treatment did not cause any toxic effects, but rather enhanced the antioxidant status of PrPc cells. Together, these results demonstrate that the cellular prion protein interacts with manganese and alters the manganese-induced neurotoxicity. This novel observation may have some functional implications in the pathogenesis and/or progression of prion diseases. Keywords: Prion diseases, Manganese, Neurotoxicity

65

MANGANESE-INDUCED DOPAMINE NEURODEGENERATION IN *C. ELEGANS*: PHARMACOGENETIC ANALYSIS IN A NOVEL MODEL OF MANGANISM. M Fullard, K Fallen, JM Andresen, CR McManus, M Marvanova, R Nass, *Departments of Anesthesiology and Pharmacology, and Center for Molecular Neuroscience, Vanderbilt University Medical Center, Nashville, TN, USA*

Manganese (Mn^{2+}) neurotoxicity resembles a number of aspects of the dopamine (DA) neuron degenerating disorder Parkinson's disease (PD). Both PD and Mn^{2+} toxicity are characterized by motor deficits and damage to substantia nigra and other basal ganglia nuclei, and dopamine or its metabolites are believed to contribute to the disorders[1]. Furthermore, expression of the pre-synaptic protein α-synuclein, and the oxidative stress-induced protein parkin have been proposed to contribute to the pathogenesis of both disorders, and occupational exposure to Mn^{2+} has been invoked to predispose individuals to PD. Despite the initial characterization of the disorder over 150 years ago, and intensive research within the past several decades, the origin of the pathogenesis and the molecular determinants involved in Mn^{2+} neurotoxicity have yet to be fully elucidated. A significant hindrance in dissecting the molecular components of Mn^{2+}-induced neurotoxicity is the high complexity of the vertebrate brain and lack of facile in vivo genetic models to determine and explore the mechanisms involved in the cell death. We have developed a novel pharmacogenetic model using the genetically tractable nematode *C. elegans* to dissect and characterize the molecular components involved in DA neuron degeneration (see Nass et al., PNAS, 2002; Nass and Blakely, Ann. Rev. Toxicol. Pharmacol., 2003). At the molecular level, the *C. elegans* nervous system is highly conserved both genetically and functionally with mammals, and all the genes responsible for DA biosynthesis, packaging, and reuptake are present and functional in the worm. We now show that the DA neurons are sensitive to brief exposures to Mn^{2+}, and Mn^{2+} amplifies the 6-OHDA-induced as well as α-synculein-induced DA neuron cell death. We have also identified several molecular transporters that play roles in the DA neuron degeneration. This system will allow us a facile test to examine the role that Mn^{2+} and xenobiotics play in the degeneration of DA neurons. Keywords: Manganese, Neurodegeneratoin, *C. elegans*.

66

A STUDY OF THE NERVOUS SYSTEM IN WELDERS. Dag G Ellingsen[1], Ludmila Merkurjeva[2], Valery Chaschin[2], Rita Bast-Pettersen[1], Maxime Chaschin[2], Yngvar Thomassen[1], *[1]National Institute of Occupational Health, Oslo, Norway; [2]Northwest Public Health Research Center, St. Petersburg, Russia*

Ninety-six male current welders (mean age 36.3 years, range 20–65; mean education 12.7 years, range 8–17) were recruited from one plant producing heavy machinery and from one shipyard. They were compared with 96 turners/fitters (mean age 36.1 years, range 18–66; mean education 12.3 years, range 8–19 years) in a cross-sectional study. The referents were matched for age 1:1 in pairs with the exposed subjects with a maximum age difference of ±2 years within each pair. In addition 27 patients, all former welders, who have become the diagnosis manganism were examined. These cases have been officially recognized as an occupational disease in the Russian insurance system. The average duration of welding in the current welders was 13.5 years (range 1–0) and their current

geometric mean (GM) exposure to manganese (Mn) in the welding aerosol was 121 $\mu g/m^3$ (range 6.5–322) as the mean of samples collected on two successive days. The patients had on average received the diagnosis 5.8 years prior to the examinations (range 4–7), at a mean age of 44.9 years (range 34–51). They had welded for 23 years on average (range 15–30). All subjects were examined with the Tremor 7.0, postural sway and maximum frequency tests from the DPD (Danish Product Development) test system. Other neurobehavioral tests that were used were Grooved Pegboard, Foot tapping, Finger tapping, Dynamometer, Static Steadiness test, Digit span and Digit symbol. Blood samples and urine samples for the determination of trace elements were collected. The concentration of B-Mn was significantly higher in the current welders than in the referents (GM 8.6 $\mu g/l$ versus$\mu g/l$; $p < 0.001$). B-Mn was significantly associated with the current concentration of Mn in the welding aerosol, in particular in air samples collected the day before the blood samples were collected (Pearson's $r = 0.33$; $p < 0.01$). Also the patients had somewhat higher concentrations of B-Mn. In addition to data on exposure, neurobehavioral test scores of the current welders, referents and the patients with will be presented. Keywords: Manganese, Welding, Neurobehavioral tests

Platform Session

SESSION X: **ENVIRONMENTAL TOXICANTS AND DISEASES**

Co- Chairs: Toshio Narahashi, Ph.D.
Ram Ramabhadran, Ph.D.

Theme: *Environmental agents not only could cause direct toxic effects on humans but also are suspected to be related to various diseases. These direct and indirect effects are in most cases the result of interactions with specific target receptors or molecules. This session deals with a few examples of such studies ranging from pesticides/heavy metals to Alzheimer's disease/peripheral myelinopathy.*

67

STUDIES OF AUTOIMMUNE AND NEUROLOGICAL DISEASES IN COMMUNITIES CONCERNED ABOUT ENVIRONMENTAL EXPOSURES. DM Williamson, *Division of Health Studies, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA*

Approximately 22 million Americans have been diagnosed with an autoimmune or neurological disease. However, these diseases have not been a priority health condition for most public health agencies. There is a lack of basic epidemiologic data concerning these diseases, particularly current background prevalence estimates for many geographic regions and ethnic groups in the United States. Numerous communities around the country located near hazardous waste sites have expressed concern about the potential exposure to environmental toxicants and the number of individuals residing near these sites that have an autoimmune or neurological disease. This presentation will (1) provide information about the role of environmental exposures and the onset of autoimmune and neurological diseases and (2) describe activities being conducted by the Agency for Toxic Substances and Disease Registry (ATSDR) to address this issue. These activities include a cluster investigation of multiple sclerosis (MS) in an elementary school cohort in Texas, a pilot study to estimate the prevalence of MS, a case-control study to examine the joint role of select environmental exposures and genetic susceptibility as potential risk factors of MS, and additional surveillance studies of MS and amyotrophic lateral sclerosis (ALS). In conclusion, a proposal for future surveillance activities of MS and other autoimmune and neurological diseases will be discussed.

68

ROLE OF NEURORECEPTORS IN SELECTIVE TOXICITY OF INSECTICIDES IN INSECTS AND MAMMALS. Toshio Narahashi, *Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL, USA*

Most insecticides are much more toxic to insects than mammals. A variety of mechanisms that underlie this phenomenon are conceivable including detoxication and target site sensitivity. Recently, some of the insect-specific target sites are receiving much attention as they are not present in mammals. We will present a few of our recent studies dealing with the mechanisms of selective toxicity. Fipronil is a relatively new insecticide with a high degree of selective toxicity in insects, and is known to block GABA receptors. Cockroach neuron's GABA receptors are 50 times more sensitive to fipronil block than rat $GABA_A$ receptors. In addition, fipronil at 10 nM potently blocks cockroach glutamate-activated chloride channels (GluCls) which are not present in rats. Therefore, these two factors can account for the highly selective fipronil toxicity against insects compared with mammals. On the contrary, GABA receptor sensitivity to dieldrin is not much different between cockroaches and rats, and its blocking potency for GluCls is even less than that for GABA receptors. Thus, dieldrin's selective toxicity is due to factors other than the target site sensitivity. Pyrethroids represent another example in which the target sodium channels are approximately 1000-fold more sensitive in cockroaches than in rats. Developing new compounds that have a high affinity for a target site unique for insects is deemed an excellent strategy. Keywords: Insecticide, Selective toxicity, Glutamate-activated chloride channel

69

DEVELOPMENTAL PESTICIDE EXPOSURE ALTERS THE DOPAMINERGIC SYSTEM AND INCREASES MPTP TOXICITY. Jason R Richardson[1,2], W. Michael Caudle[2], Minzheng Wang[2], Kurt D. Pennell[3], Gary W. Miller[2], *[1]Environmental and Occupational Health Sciences Institute, and Department of Environmental and Occupational Medicine, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, [2]Department of Environmental and Occupational Health, Rollins School of Public Health, Emory University, [3]Department of Civil and Environmental Engineering, Georgia Institute of Technology*

The dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) are phenotypic markers of dopamine neurons and are sensitive indicators of the dopaminergic neurodegeneration observed in Parkinson's disease (PD). Previously, we have observed that acute exposure of adult mice to organochlorine pesticides increases DAT levels while inhibiting VMAT2 activity, which may increase vulnerability to dopaminergic neurotoxicants. In this study, we sought to determine whether perinatal exposure to low levels of dieldrin (0.3, 1, or 3 mg/kg every 3 days) or heptachlor (3 mg/kg) to female mice would alter dopaminergic neurochemistry in their male offspring and exacerbate MPTP toxicity. At 12 weeks of age, striatal DAT levels were increased in male offspring by perinatal dieldrin exposure in a dose-related manner (30, 41, and 52%). VMAT2 levels were increased by 16, 16, and 27% in these same animals. Striatal DOPAC levels were increased in the offspring of dieldrin treated animals (34, 42, and 57%), indicating the ability of these dosages to inhibit VMAT2 activity. MPTP exposure (2×10 mg/kg s.c.) at 12 weeks of age resulted in a greater reduction of striatal dopamine in dieldrin exposed mice (73, 76, and 74%) compared to controls (62%). Additionally, dieldrin treatment potentiated the increases in GFAP (26% in controls and 54, 60, and 73% in dieldrin treated offspring) and α-synuclein levels (27% in controls and 47, 43, and 46% in dieldrin treated offspring), indicating MPTP caused more damage in the dieldrin treated offspring. Similar results were observed with heptachlor treated offspring. The increased toxicity of MPTP in the pesticide exposed offspring was associated with an increase in DAT:VMAT2 ratio. These data suggest that the developing dopaminergic system is particularly sensitive to dieldrin and heptachlor exposure during the perinatal period and that developmental pesticide exposure may result in increased risk of dopaminergic damage and to PD. Keywords: Parkinson's disease, Developmental neurotoxicity, Organochlorine pesticide. *Supported by NIH R21ES012315 and F32ES013457.*

70

EFFECTS OF PERIPHERAL INFLAMMATION ON THE DOPAMINERGIC TOXICITY OF THE FUNGICIDE MANEB IN TWO STRAINS OF MICE. NM Filipov, AB Norwood, SC Sistrunk, A Coban, *Center for Environmental Health Sciences, Department of Basic Sciences, College of Vet. Medicine, Mississippi State University, Mississippi State, MS, USA*

Adult male C57BL/6 mice exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the most widely used animal model of Parkinson's disease (PD), but other strains of mice, i.e., BALB/c, are relatively resistant. Brain inflammation and several pesticides, including the fungicide maneb, have been implicated in the etiology of PD. Excessive exposure to manganese which is a part of the maneb structure also causes PD-like symptomatology. Because (i) role of peripheral inflammation in PD etiology has not been studied before and (ii) mouse strains with differential sensitivity to MPTP differ substantially in their immune responsiveness, the objective of this study was to determine whether (i) peripheral lipopolysaccharide (LPS) administration will modulate maneb's dopaminergic toxicity and (ii) this modulation will be strain-specific. Male C57BL/6 and BALB/c mice were exposed for 14 days to a dose range of maneb. On day 12, half of the mice were challenged with a single dose of LPS and all mice were sacrificed on day 15 or 22. From the data analyzed so far, basal striatal levels of dopamine (DA), serotonin (5-HT) and their metabolites DOPAC and 5-HIAA were greater in the C57BL/6 strain; the C57BL/6 mice were also more active than the BALB/c mice in all behavioral tests. LPS increased DA, 5-HIAA, and HVA (a DA metabolite) levels of in both strains. Maneb exposure by itself did not produce any appreciable alterations in striatal neurochemistry. However, mice from both strains exposed to maneb that were also challenged with LPS, spent more time than control mice in the corner in the locomotor activity test performed 5 days after the LPS challenge. Interestingly, strain-specific neurochemical alterations were observed in the maneb-exposed mice that were also challenged with LPS. Notably, striatal DA levels were decreased dose-dependently only in the C57BL/6 mice on day 15. This decrease was accompanied with a decrease in HVA levels, an indication of either decreased DA synthesis or loss of DA neurons in the substantia nigra. The fact that peripheral inflammation enhances the dopaminergic toxicity of maneb preferentially in a mouse strain with already demonstrated sensitivity to the prototypical dopaminergic toxicant MPTP, suggests possible genotype-dependent selectivity in the contribution of peripherally induced inflammation to the etiology of PD. *Supported by NIEHS ES11654.*

71

GAMMA-INTERFERON (IFNγ) CAUSES DENDRITIC RETRACTION IN SYMPATHETIC NEURONS IN VIVO. P.J. Lein[1], E. Gonsiorkek[2], M. Garred[1], D. Higgins[2], *[1]Center for Research in Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR, USA; [2]Department of Pharmacology and Toxicology, SUNYAB, Buffalo, NY, USA*

There is evidence that the pro-inflammatory cytokine IFNγ is elevated in children with autism, schizophrenia, and a variety of other neurological disease states in which viral infections or immune dysregulation are presenting features. We hypothesize that elevated IFNγ contributes to the neuropathology associated with inflammation by inducing dendrite retraction and synapse loss. We have previously reported that IFNγ causes dendritic retraction and synapse loss in cultured sympathetic neurons and hippocampal neurons derived from embryonic rats, but whether IFNγ elicits the same response in vivo is not known. The goal of these studies, therefore, was to determine if these effects are recapitulated in vivo following systemic administration of IFNγ. Six-week-old male rats were injected with IFNγ (50, 100 or 500 U/g/day, i.p.) or an equal volume of vehicle daily for 7 days. Immunocytochemical analyses indicated that IFNγ caused a dose-dependent increase in MHC-I expression in SCG in the absence of effects on body weight. Subsequent studies, therefore, used the highest IFNγ dose. Dendritic arbors of individual neurons within the SCG were labeled in their entirety by Diolistics, a method in which tungsten particles (1 μM) coated with the fluorescent lipophilic dye DiI are fired into fixed ganglia using a gene gun. Morphometric analyses of DiI labeled neurons indicated that IFNγ treatment (500 U/g/day) did not significantly alter the number of primary dendrites; however, it did significantly decrease the number of secondary and tertiary dendrites, the number of dendritic branch points, lengths of primary, secondary and tertiary dendrites, total dendritic length and overall dendritic area. In contrast, IFNγ treatment did not alter the diameter of neuronal somata, the volume of the SCG or the volume of the submandibular salivary gland, which is a principal SCG target tissue. IFNγ did cause a subtle, but significant increase in the innervation of the submandibular gland by tyrosine hydroxylase-positive fibers. These data are consistent with previous in vitro observations and suggest that IFNγ acts directly on neurons in vivo to cause retraction of dendritic arbors. This work has significant implications with respect to the potential of IFNγ to alter the morphogenesis of CNS neurons in autistic, schizophrenic and other children with neuropathological conditions associated with inflammation. Moreover, since the sympathetic nervous system provides the principal neural regulation of the immune system, this work may also be of significance for explaining immune deficits in autistic children. Keywords: Interferon-gamma, Dendrite, Inflammation. *This work was supported by NINDS (R01 NS46649 to P.J.L).*

72

ALZHEIMER'S DRUG MODULATION OF NICOTINIC RECEPTORS AND NMDA RECEPTORS: BASIS FOR THERAPEUTIC EFFECTS. Toshio Narahashi, *Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL, USA*

It is well established that accumulation of β-amyloid in the brain is a hallmark of Alzheimer's disease. However, no strategies for curing the disease have been developed yet as we do not know the exact cause of the disease. Since Alzheimer's disease is associated with down-regulation of the nicotinic acetylcholine receptors (nAChRs), several anticholinesterases have been developed into clinical use. However, their effectiveness is limited because of side effects and short periods of effects. Meanwhile, NMDA receptors have received much attention these days. First, NMDA receptors are also down-regulated in Alzheimer's disease patients; second, we have recently found that some of these drugs (nefiractam, galantamine, and donepezil) stimulate NMDA receptors; and third, the newly approved memantine blocks NMDA receptors in a very complex manner. Our hypotheses are that reactivating nAChRs and NMDA receptors to their normal levels contributes to therapeutic effects in improving the memory, learning, and cognition of Alzheimer's patients and that memantine caused therapeutic effects by blocking voltage dependently extrasynaptic NMDA receptors which would be activated by glutamate released from ischemic tissues leading to cell death while sparing normal activity of synaptic NMDA receptors. In summary, we have to consider multiple mechanisms of action of Alzheimer's drugs which are woven in a complicated manner. Keywords: Alzheimer, Nicotinic acetylcholine receptor, NMDA receptor

Poster Session

SESSION VII: **GENERAL POSTER SESSION**

See pages 26–44 for abstracts presented from Poster. Poster abstracts are numbered from P-73 to P-134.

The poster session is a highlight of this conference series. It has proven to be an effective venue for informal, in-depth discussion and collaboration building – as well as an important networking opportunity for all participants. Papers on any aspect of neuroscience, toxicology, children's environmental health, public health and policy are welcome! Judging and selection of Student Awardees will be made during the poster session.

Student award competition

The Student Award Competition is divided into four groups: 1 for post-doctoral competition and 3 for pre-doctoral competition by general topic (i.e., metals, pesticides and PCBs, other). There is approximately the same number of posters in each group.

A winner will be chosen from each group for a total of four awards. Competing students are expected to give an overview of their work in 2–3 min to the judges followed by a brief set of questions and answers. Originality, significance, hypothesis, presentation material and style, as well as knowledge of the subject, will be considered in selecting the winners. All papers presented for the Student Awards must be presented from poster.

Group 1: Post-Doctoral Competition

Group 1: Post-Doctoral Student Award Committee

1. Kenneth Reuhl, Ph.D. ~ *Chair*
2. Stephanie Padilla, Ph.D.
3. Isaac Pessah, Ph.D.

Group 1: Post-Doctoral Students (9)

Ambuja Bale	*Mentor*: Timothy J. Shafer, Ph.D.
Wendy Donlin	*Mentor:* M. Christopher Newland, Ph.D.
Anne Dreiem	*Mentor*: Richard Seegal, Ph.D.
Julia Gohlke	*Mentor:* Christopher J Portier, Ph.D.
Ruth Jameson	*Mentor:* Ted Slotkin, Ph.D.
Kennita Johnson	*Mentor:* Robert Maronpot, Ph.D.
Tal Kenet	*Mentor:* Michael Merzenich, Ph.D.
Elizabeth Roberts	*Mentor:* David Dorman, D.V.M., Ph.D.
Marcelo J. Wolansky	*Mentor:* Kevin Crofton, Ph.D.

Group 2: Neurotoxicity of Metals

Group 2: Pre-Doctoral Student Award Committee

1. Michael Aschner, Ph.D. ~ *Chair*
2. David Dorman, D.V.M., Ph.D.
3. Timothy J. Shafer, Ph.D.

Group 2: Pre-Doctoral Students (11)

Christopher Choi	*Mentor:* Anumantha G. Kanthasamy, Ph.D.
Joel F. Cooper	*Mentor:* Alexander Kusnecov, Ph.D.
Jeremy J. Day	*Mentor:* M. Christopher Newland, Ph.D.
John C. Heath	*Mentor:* M. Christopher Newland, Ph.D.
Christina J. Herden	*Mentor:* William D. Atchison, Ph.D.
Jayme D. Mancini	*Mentor:* William D. Atchison, Ph.D.
Erin F. Pesek	*Mentor:* M. Christopher Newland, Ph.D.
Miranda Reed	*Mentor:* M. Christopher Newland, Ph.D.
M.A. Polunas	*Mentor:* Kenneth Reuhl, Ph.D.
Feng-Chiao Su	*Mentor:* Pau-Chung Chen, M.D., Ph.D.
Blair C. Weig	*Mentor:* Kenneth Reuhl, Ph.D.

Group 3: Neurotoxicity of Pesticides and PCBs

Group 3: Pre-Doctoral Student Award Committee

1. Toshio Narahashi, Ph.D. ~ *Chair*
2. Anumantha G. Kanthasamy, Ph.D.
3. Virginia (Ginger) Moser, Ph.D.
4. Bob Sonawane, Ph.D.

Group 3: Pre-Doctoral Students (10)

Cary Coburn	*Mentor:* Margarita C. Curras-Collazo, Ph.D.
Lisa M. Domica	*Mentor:* Keith R. Cooper, Ph.D., Gail Zeevalk, Ph.D. (co-advisor)
Josh A. Harrill	*Mentor:* Kevin Crofton, Ph.D.
Chia-Jung Hsieh	*Mentor:* Pau-Chung Chen, M.D., Ph.D.
Zhenquan Jia	*Mentor:* Hara Misra, B.V.Sc., M.S., Ph.D.
Todd A. Jusko	*Mentor:* Irva Hertz-Picciotto, M.D.
Edward C. Meek	*Mentor:* Janice E. Chambers, Ph.D.
David S. Sharlin	*Mentor:* R. Thomas Zoeller, Ph.D.
Tram-Anh N. Ta	*Mentor:* Isaac N. Pessah, Ph.D.
Jennifer Watkins	*Mentor:* Timothy J. Shafer, Ph.D.

Group 4: Other Compounds/General Neurotoxicology

Group 4: Pre-Doctoral Student Award Committee

1. M. Christopher Newland, Ph.D. ~ *Chair*
2. Edward Levin, Ph.D.
3. Eva Polston, Ph.D.

Group 4: Pre-Doctoral Students (8)

Michele A. Cheh	*Mentor:* Margarita C. Curras-Collazo, Ph.D.
Robert Giddings	*Mentor:* Timothy J. Shafer, Ph.D.
Elizabeth Gribble	*Mentor:* Kevin Crofton, Ph.D.
Lynn Parsons Heibrun	*Mentor:* Claudia S. Miller, M.D., M.S.
Jinghong Kou	*Mentor:* Jeffrey R. Bloomquist, Ph.D.
Sharon Oxendine	*Mentor:* Stephanie Padilla, Ph.D.
Faneng Sun	*Mentor:* Anumantha G. Kanthasamy, Ph.D.
Daniella Urbach	*Mentor:* Alexander Kusnecov, Ph.D.

P-73

AUTISM AND ENVIRONMENTAL GENOMICS.

MR Herbert*, JP Russo, S Yang, J Roohi, M Blaxill, SG Kahler, L McCoy, DA Ziegler, E Hatchwell, *CMA & Pediatric Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA*

Autism spectrum disorders (ASD) are behaviorally defined syndromes with no clearly established biomarkers whose heterogeneous biological causes may include environmental as well as genetic factors. Candidate genes have generally been chosen from genes directly related to the central nervous system, but the apparent increase in autism incidence supports examination of environmental factors including those that may not primarily target the brain. The NIEHS has implemented the Environmental Genome Project (EGP) to study genetic susceptibility to environmental disorders and has identified a set of ''environmental response genes that may include previously uninvestigated candidate genes for autism''. *Methods:* This study utilizes bioinformatics methodologies to identify genes with functional SNPs from three environmentally relevant genome databases (NIEHS Environmental Genome Project, SeattleSNPS (inflammatory related), and Toxicogenomics) that are in linkage regions identified in published autism genome scans. We will also report the results of genotyping SNPs of TNFalpha, a gene that is found both in an autism linkage region

and in all three databases. *Results:* Sixty-seven genes with functional SNPs were identified that had not previously been studied in autism. We will present this candidate gene list, selected pathway analyses oriented toward discerning relationships with metabolic and neurological abnormalities in autism, and the results of genotyping. *Conclusion:* Finding multiple overlaps between autism and environmental genomics suggests that ASD brain abnormalities could be downstream from metabolic or regulatory changes that are more widespread, or that originate in other systems (e.g. immune) and could be modulated by environmental factors. This deserves systematic investigation. Keywords: Autism, Environmental genomics, Inflammation. *Funding: Cure Autism Now, Cody Autism Center.*

P-74

A NEW DEVELOPMENTAL NEUROTOXICITY STUDY FOCUSING ON THE FETAL BRAIN: EVALUATION OF A RAT AUTISM MODEL INDUCED BY VALPROATE AND THALIDOMIDE. T. Ogawa[1], M. Kuwagata[2], S. Shioda[1], *[1]Department of Anatomy, Showa University School of Medicine, Tokyo, Japan; [2]Hatano Research Institute, FDSC, Kanagawa, Japan*

Prenatal exposures to chemicals such as alcohol, lead, PCBs and valproate (VPA) are well known to induce developmental abnormalities in the central nervous system of children. In the developmental neurotoxicology to protect the pediatric brain from those chemical-induced tragedies, the majority of studies focus on postnatal subjects rather than the fetuses. We have been investigating the usefulness of histological observation of the fetal brain in the evaluation of chemicals on the development of the central nervous system. Since prenatal exposure to VPA or thalidomide has been reported to increase children with autistic features, these chemicals are often used to produce animal models of autism. In this study, we exposed pregnant rats to thalidomide (500 mg/kg) or valproate (800 mg/kg) orally on gestation day 9, and observed the fetal brain at embryonic day 14. To avoid selection bias and evaluate more justly, we selected the fetuses located at specific positions in each mother's uteri. We observed these brains very carefully using serial coronal and sagital sections after Nissl staining, and/or 5-HT immunohistorical staining. The exposure to thalidomoide did not induce any alternations even if there are reports suggesting this chemical affects the development of 5-HT neurons. The fetal brains exposed to VPA showed an altered structure in the thalamus, which was rounder with an enlarged third ventricle. Not all VPA-exposed brains were affected, but the incidence was almost one of three. The alternation seemed not to be a developmental delay, since the size of the brain (maximum lateral diameter and maximal longitudinal diameter) was not smaller compared to the controls. Some fetal brains affected by VPA showed the normal development of 5-HT neurons (5-HT fibers arrived at the thalamus, and 5-HT cell bodies appeared in the pons and medulla oblongata as observed in the controls). Other affected brains showed diminished development of the 5-HT neurons. The relationship between the altered fetal thalamus and abnormal function observed in children with autism will be investigated in the future. This study suggests that examination of fetal brains after chemical exposure can be a good new endpoint to support postnatal data from current developmental neurotoxicity studies. Keywords: Fetal brain, Valproate, Developmental neurotoxicity. *(Supported partly by a grant of Long-range Research Initiative (LRI) by Japan Chemical Industry Association (JCIA).)*

P-75

CULTURED LYMPHOCYTES FROM AUTISTIC PATIENTS AND NON-AUTISITC SIBLINGS UPREGULATE HEAT SHOCK PROTEIN RNA IN RESPONSE TO THIMEROSAL CHALLENGE. SJ Walker, *Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC, USA*

There are reports suggesting that many autistic children exhibit an abnormal response when exposed to heavy metals, and this correlation, coupled with the similarity in clinical presentations of autism and some heavy metal toxicities, has led to the suggestion that susceptibility to heavy metal poisoning might play a role in the etiology of autism in some individuals. Thimerosal, an antimicrobial preservative previously added routinely to some childhood multi-dose vaccines, is composed of 49.6% ethyl mercury and, based on the levels of this compound that children receive in the first years of life, has been postulated as a potential triggering mechanism contributing to autism in some individuals. To investigate this hypothesis, cultured lymphocytes from autistic children and non-autistic siblings (obtained from the Autism Genetic Research Exchange; AGRE) were challenged for 6 h either with: (1) Hg = 10 μM thimerosal; (2) Zn = 150 mM zinc; or (3) control = fresh media only. Following the challenge, total RNA was extracted from the cells and used to query ''whole genome'' microarrays (Affymetrix HG U133A; 22,000 genes and expressed sequence tags). Cultured lymphocytes challenged with zinc responded with an impressive upregulation of metallothionenin (MT) transcripts (at least seven different MTs were overexpressed) while cells challenged with thimerosal responded by upregulating numerous heat shock protein transcripts, but not MTs. Although there were no apparent differences between autistic and non-autistic sibling response in this very small sampling, the differences in expression profiles between those cells treated with zinc versus treatment with thimerosal were dramatic. Determining cellular response, at the level of gene expression, has important implications for the understanding and treatment of conditions that result from exposure to neurotoxic compounds. Keywords: Autism, Lymphocytes, Thimerosal. *This work was supported in part by funds from SafeMinds and The Wallace Foundation.*

P-76 ***Post-Doctoral Student (Group 1)***

A DIRECT COMPARISON OF ALGORITHM-BASED AND LITERATURE-BASED SYSTEMS BIOLOGY APPROACHES: APPLICATIONS IN NEURODEVELOPMENT. Julia M. Gohlke, Fredrick M. Parham, Christopher J. Portier, *Environmental Systems Biology Group, Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, RTP, NC, USA*

Computational models can help to elucidate dynamic interactions of biological systems that cannot be discovered by studying individual parts of that system. In the field of systems biology, there has been much discussion in regards to ''algorithm-based'' versus ''literature-based'' approaches. In particular, algorithm-based approaches have been criticized for utilizing data only at the genomic level, while excluding data generated using traditional molecular biology approaches. However, literature-based approaches may miss important novel discoveries and therefore not fully realize the potential of incorporating broad genomic scans. Here, we directly compare algorithm-based and literature-based methodologies by developing two gene regulatory networks (GRN). First, we build a GRN from compilation of the current literature on regulation of forebrain development using diverse experimental approaches, including transgenics, in-situ hybridization, and utilization of specific agonists and antagonists to specify interactions within the overall network. We then develop another GRN using a Bayesian algorithm to discover the optimal GRN utilizing only microarray data from brain tissues of several transgenic mouse strains. Finally, we quantify each network using Bayesian statistical approaches in order to directly compare these two methodologies. By quantifying and directly comparing these two networks, we are able to show that both methodologies have distinct benefits. We conclude that the ''literature-based'' approach is particularly useful in testing current hypotheses of gene regulation and refining the network structure, whereas the ''algorithm-based'' approach is particularly suited for developing hypotheses of potential novel linkages thereby directing future experimental research. We suggest iterations between these two approaches results in a consensus GRN that simultaneously incorporates diverse datasets of information and prioritizes hypothetical linkages for future research. Keywords: Computational, Microarray and forebrain

P-77 ***Post-Doctoral Student (Group 1)***

EVALUATING THE NMDA-GLUTAMATE RECEPTOR AS A SITE OF ACTION FOR TOLUENE USING PATTERN ELICITED VISUAL EVOKED POTENTIALS. AS Bale[1], QT Krantz[2], PJ Bushnell[1], TJ Shafer[1], WK Boyes[1], *[1]Neurotoxicology Division and [2]Experimental Toxicology Division, US Environmental Protection Agency. Research Triangle Park, NC*

In vitro studies have demonstrated that toluene disrupts the function of NMDA-glutamate receptors, as well as other channels. This has led to the hypothesis that effects on NMDA receptor function may contribute to toluene neurotoxicity, CNS depression, and altered visual evoked potentials observed in animals and humans. However, this hypothesis has not been tested in vivo. It has been shown previously that toluene exposure alters visual evoked potentials (VEPs) in rats, and there is a significant population of NMDA-glutamate receptors in the visual system. The present experiment examined the NMDA-glutamate receptor as a potential toluene target in Long-Evans rats by measuring VEPs during toluene exposure and challenging with the agonist NMDA, hypothesized to counteract toluene's action at the NMDA receptor. Awake, restrained rats were presented with an onset/offset pattern and baseline VEPs were recorded. Rats were injected with either saline or NMDA (2.5 or 10 mg/kg, i.p.), and 10 min later were exposed to air or toluene (2000 ppm). VEP amplitudes were calculated for 2× stimulus frequency (F2). In the toluene-exposed groups, toluene/saline ($n = 11$) decreased F2 by 60%, toluene/NMDA (10 mg/kg) ($n = 11$) decreased F2 amplitude by 50%, and toluene/NMDA (2.5 mg/kg) decreased F2 amplitude by 60% ($n = 13$). These results indicate that NMDA did not counteract the F2 amplitude decrease caused by toluene. Therefore, the acute actions of toluene on visual function do not appear to be mediated through blockage of NMDA receptors. Keywords: Visual evoked potential, Toluene, NMDA-glutamate receptor. *(This abstract does not reflect EPA policy.)*

P-78

Rx FOR PREVENTION: PEDIATRIC ENVIRONMENTAL HEALTH TOOLKIT PILOT STUDY FINDINGS. Kathleen Schuler, *MHP, Environmental Scientist, Institute for Agriculture and Trade Policy, 2105 First Av. S, Minneapolis, MN, USA*

Children have heightened vulnerability to toxicants in their environment. In national educational programs on toxic threats to child development, pediatricians and other health care providers have consistently expressed a need for practical clinical tools to enable them to incorporate environmental health guidance into practice. In response pediatricians in Boston and San Francisco developed a Pediatric Environmental Health Toolkit. The Toolkit's peer-reviewed materials include reference and anticipatory guidance components for providers as well as ''Rx for Prevention'' slips and magnets for patients, designed for use during well-child visits. Thirty-four pediatric and family practitioners at 17 sites have pilot tested the Toolkit for 6 months in California and Massachusetts. The measurement phase of the study included baseline and 6 month written surveys, monthly telephone contacts, and in-depth telephone interviews with a random sample of participants. This session will report the evaluation results, including measurement of: (a) use and perceived utility of Toolkit materials; (b) frequency of advising patients about 21 environmental health issues; (c) barriers and facilitators to using Toolkit materials; (d) perceptions of confidence,

knowledge, and enthusiasm for discussing environmental health topics with patients; and (e) familiarity with and use of resources at regional Pediatric Environmental Health Specialty Units (PEHSUs). We will also report on results from focus groups with patients' families to measure behavioral changes among a sample of caregivers. Strategies for more widely disseminating and implementing the Toolkit will also be discussed. The Toolkit, and the related information, was developed for pediatric health care providers but is relevant for policymakers, child care providers and administrators, psychologists, and others that are charged with promoting the health and well-being of young children. Keywords: Pediatric practice, Prevention, Educational tools

P-79 ***Post-Doctoral Student (Group 1)***

APPLICATION OF MAGNETIC RESONANCE IMAGING IN DEVELOPMENTAL NEUROTOXICITY TESTING: A PILOT STUDY. K. Johnson[1], L. Ryan[2], J. Davis[3], A. Elmore[3], B. Guenther[2], J. Marcus[1], R. Maronpot[1], *[1]Laboratory of Experimental Pathology, National Institute of Environmental Health Sciences/NIH/DHHS, RTP, NC, USA; [2]MRPath, Inc., Durham, NC, USA; [3]Integrated Laboratory Systems, Inc., RTP, NC, USA*

In a rat developmental neurotoxicity study, three-dimensional magnetic resonance (MR) images were used to compliment traditional light microscopic evaluation of histologic sections. Sprague–Dawley dams were treated with 0, 1.25, 4.0 or 7.5 mg/kg methylazoxymethanol acetate (MAM) during gestation days 13–15. At postnatal days 23 and 60, brains from representative male and female rats from 2 dams in each dose group were fixed with 10% neutral buffered formalin by transcardial perfusion for in situ MR imaging. A 7T small animal magnet system was used to obtain three-dimensional isotropic images with a 125 μm resolution. Data from a rapid screening method based on midpoint MR slices of whole brain, cerebrum, cerebellum, hippocampus, thalamus and corpus callosum showed a dose-related decreased volume of whole brain, cerebrum and hippocampus. Subsequent volumetric measurements using the Cavalieri method confirmed these findings and showed decreased volume of whole brain, cerebrum and hippocampus. The brains were subsequently removed and processed for conventional histologic examination of hematoxylin and eosin-stained sections. It is concluded that MR imaging in rat developmental neurotoxicity studies offers the advantages of in situ volumetric measurements of brain structures while preserving the samples for conventional optical microscopy. Keywords: MR imaging, Stereology, Methylazoxymethanol acetate

P-80 ***Post-Doctoral Student (Group 1)***

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON CRITICAL PERIOD PLASTICITY IN RATS AUDITORY CORTEX—IMPLICATIONS FOR DEVELOPMENTAL DISORDERS? Tal Kenet[1], Isaac Pessah[2], Michael Merzenich[1], *[1]University of California, San Francisco, CA, USA; [2]University of California, Davis, CA, USA*

The recent increase in the number of children diagnosed with autism suggests that non-genetic factors may be etiologically important. We hypothesize that genetic polymorphisms confer increased susceptibility to neurotoxic agents, which alter brain maturational processes in a catastrophic manner, with autism being one possible outcome. To test that model, we studied the impact of PCB95 on the development of auditory cortex (A1) in rats. Early exposure to PCBs is already known to impair functioning in rodents in ways that are reminiscent of human autism. Using multiunit electrophysiological recording, we found that the development of A1 in rats exposed to PCB95 was highly abnormal. Specifically, we observed large-scale differences in the cortical areas with A1 response characteristics, degraded and disrupted A1 tonotopic gradients, and grossly abnormal receptive field properties. Such abnormalities in the human model would almost certainly result in a disturbance in aural language development, which is a prominent trait of autism. In spite of these powerful cortically expressed effects, recorded auditory brainstem responses were normal. A likely marker of autism arising from inherited factors is a deficit in inhibition leading to increased cortical noise. To simulate this inherited expression of the at-risk child, we added moderate-level pulsed noise/tone to our developing rats' environment. Rats exposed to both PCBs and external pulsed noise – i.e., with an inherently noisy cortex through the critical period – had highly amplified pathologies. This study suggests the possibility that environmental factors combine synergistically with genetic predisposition to contribute to the increased incidence of autism. Keywords: PCBs, Auditory cortex, Rats

P-81

A STUDY OF LEAD LEVELS IN BREAST FED INFANTS AND THEIR MOTHERS. M.M. Ahmed, D.A. Salem*, Zeinab, M. Mohie-El-Din, Asmaa, S.G. Mohamed, **Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt (diefysalem57@yahoo.com); Department of Pediatric, Assiut University Hospital, Faculty of Medicine, Assiut University, Assiut, Egypt*

To study the condition of infants exposure to lead and the relation to maternal exposure and their breast milk levels, the present study included exclusively breast fed 56 infants and their mothers who were admitted to the Pediatric Department, Assiut University Hospital, Egypt. All the studied infants were subjected to full history taking, complete clinical examination, complete blood picture besides the estimation of lead levels in their whole blood, their mothers blood and maternal breast milk. The concentrations of lead were measured by using GF- AAS (SIMAA 6000 PE Simultaneous Multielement Atomic Absorption Spectrophotometer fitted with graphite furnace, combined with PE AS 72 autosampler) and a Zeeman background

correction. The mean blood lead levels of the studied mothers, their infants and in breast milk were 180.19 ± 5.89, 83.72 ± 4.93 and 19.51 ± 1.52 μg/L, respectively. In the studied candidates, we found that 10.7% of the mothers had high lead level in their whole blood (greater than 25 μg/dL): primiparous mothers represented 23.2% while multiparous ones were 76.8%. While 67.9% were coming from rural areas, 32.1% were from urban localities. Mothers of fair socioeconomic status were 23.2% while those with low socioeconomic level were 76.8%. Overt malnutrition was detected in 50% of the infants of all mothers. It was found that lead levels exceeded the recommended permissible daily intake in three breast milk samples (5.36%). A significant positive correlation was found between the maternal whole blood and breast milk lead levels ($r = 0.47$, $P < 0.0001$). On the other hand, a significant negative correlation was found between infants' whole blood lead levels and their hemoglobin values ($r = -0.45$, $P < 0.0001$). It could be concluded that infants are at risk of exposure to lead toxicity, despite the fact that breast milk is the ideal nutrient for the baby; the environmental pollution to which the mothers are exposed is directly affecting their babies besides the baby's own exposure to lead. Older maternal age, primiparity, rural residence and low socio-economic condition as well as infant malnutrition and delayed mental development were associated with elevated lead levels in blood and breast milk in the studied infants and mothers. Anemia in infancy can exaggerate the existence of/or may be due to the increased lead level in infants' blood. Keywords: Lead; Whole mother blood; Child blood; Breast milk; Economic status

P-82 ***Post-Doctoral Student (Group 1)***

EFFECTS OF METHYLMERCURY ON MITOCHONDRIAL FUNCTION, REACTIVE OXYGEN SPECIES FORMATION AND CYTOSOLIC CALCIUM LEVELS IN STRIATAL SYNAPTOSOMES FROM RAT. A. Dreiem[1], R.F. Seegal[1,2], *[1]New York State Department of Health, Wadsworth Center, Albany, NY, USA; [2]School of Public Health, University at Albany, Albany, NY, USA*

Methylmercury (MeHg) is an environmental neurotoxicant that is especially harmful to animals and humans during development. The purpose of this study was to examine the relationship between mitochondrial function, reactive oxygen species (ROS) formation, and calcium (Ca^{2+}) levels in isolated nerve terminals (synaptosomes) after in vitro MeHg exposure. MeHg, at concentrations ≥ 0.5 μM, reduced mitochondrial membrane potential ($\Delta\Psi_m$) measured by the fluorescent indicator JC-1. The ability of MeHg to cause irreversible cell damage was investigated by measuring the conversion of MTT to formazan. MeHg dose-dependently reduced formazan formation in exposed synaptosomes at concentrations ≥ 5 μM. MeHg also caused a dose-dependent increase in ROS levels after exposure to 5–15 μM (assessed by measuring oxidation of 2′,7′-dichlorofluorescin). To assess whether ROS formation could be the cause of mitochondrial dysfunction, we repeated the experiments in the presence of Trolox, a synthetic antioxidant. Trolox completely prevented the increase in ROS levels induced by MeHg; however, it had no protective effect against reductions in $\Delta\Psi_m$ or mitochondrial function, indicating that ROS formation is not the cause of mitochondrial damage, but rather could be an effect of mitochondrial disruption. MeHg is also known to increase cytosolic Ca^{2+} ($[Ca^{2+}]_i$), which can cause increased uptake of Ca^{2+} into mitochondria and mitochondrial uncoupling with subsequent ROS formation. Therefore, we investigated the effects of MeHg on $[Ca^{2+}]_i$ levels using the Ca^{2+} indicator Fura-2. We found that MeHg increased $[Ca^{2+}]_i$ levels after exposure to MeHg concentrations similar to those that reduced $\Delta\Psi_m$, indicating that loss of $\Delta\Psi_m$ and increase in $[Ca^{2+}]_i$ are key events in the mechanism of MeHg-induced toxicity in striatal synaptosomes. Further studies are currently being performed to elucidate the relationship between the effects of MeHg on Ca^{2+}, mitochondrial function, and ROS formation. Keywords: Methylmercury, Mechanisms, Neurotoxicology. *Supported by NIEHS grant 1P01ES11263 and EPA grant R829390 to RFS.*

P-83

BENCHMARK CONCENTRATIONS FOR METHYL MERCURY OBTAINED FROM THE 9-YEAR FOLLOW-UP OF THE SEYCHELLES CHILD DEVELOPMENT STUDY. E van Wijngaarden[1], C Beck[2], PW Davidson[3], GJ Myers[3,4], *[1]Department of Community and Preventive Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, United States; [2]Department of Biostatistics and Computational Biology, University of Rochester School of Medicine and Dentistry, Rochester, NY, United States; [3]Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, NY, United States; [4]Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY, United States*

Methyl mercury (MeHg) is highly toxic to the developing nervous system. Human exposure is mainly from fish consumption since small amounts are present in all fish. Findings of developmental neurotoxicity following high-level prenatal exposure to MeHg raised the question of whether children whose mothers consumed fish contaminated with background levels during pregnancy are at an increased risk of impaired neurological function. Benchmark doses determined from studies in New Zealand, and the Faroese and Seychelles Islands indicate that a level of 10–20 parts per million (ppm) measured in maternal hair may carry a risk to the infant. However, there are numerous sources of uncertainty that could affect the derivation of benchmark doses, and it is crucial to continue to investigate the most appropriate derivation of safe consumption levels. Earlier, we published the findings from benchmark analyses applied to the data collected on the Seychelles cohort at the 66-month follow-up period. Here, we expand on these analyses by determining the benchmark doses of MeHg level in maternal

hair based on a cohort of 643 Seychellois children for whom 26 different neurobehavioral endpoints were measured at 9 years of age. Dose–response models applied to these continuous endpoints incorporated a variety of covariates and included the *k*-power model, the Weibull model, and the logistic model. Evaluation of the Child Behavior Checklist, and full scale and verbal IQ yielded benchmark levels of 20–22 ppm in maternal hair; results for all 26 endpoints will be presented. The Seychelles Child Development Study continues to provide a solid basis for the derivation of safe levels of MeHg consumption. Keywords: Mercury, Neurodevelopment, Benchmark analysis

P-84 ***Post-Doctoral Student (Group 1)***

INCREASED SENSITIVITY TO PENTOBARBITAL ON THE BEHAVIOR OF RATS EXPOSED TO METHYLMERCURY AND SELENIUM. W.D. Donlin, Ph.D.*, M.C. Newland, Ph.D.†, *Department of Psychiatry & Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD, USA; †Department of Psychology, Auburn University, Auburn, AL, USA*

Behavioral studies of postnatal methylmercury exposure have revealed motor deficits. Operant schedules that engender high rates of lever pressing are sensitive to methylmercury exposure, elucidating these deficits. Sixty-four female rats were separated into six groups. Methylmercury (0, 0.5, OR 5 PPM) was added to drinking water. Rats consumed a diet either marginal or enriched with selenium, creating a 2×3 design of diet and mercury exposure. Lever-pressing behavior was placed on a multiple percentile 10:0.5 differential reinforcement of high rates (DRH) 6:3 schedule of food reinforcement. The percentile schedule reinforces responses, under a variable interval 30 s schedule, if their interresponse times (IRTs) were shorter than 50% of the last 10 IRTs, which maintains high rates of behavior while adjusting reinforcement criterion to the subject's own performance. The DRH schedule delivers a reinforcer if the lever is pressed six times in 3 s. Response rates were not significantly different between the schedules. Overall response rates under both schedules were lower in animals exposed to methylmercury, especially if they were maintained on a diet containing marginal selenium. A drug challenge of pentobarbital (1, 3, 5.6, 10 and 17 mg/kg) revealed an interaction, with animals exposed to methylmercury and low levels of selenium being the most sensitive the rate-decreasing effects of pentobarbital. Keywords: Methylmercury, Selenium, High-rate behavior. *Supported by ES10865.*

P-85

A NEW DEVELOPMENTAL NEUROTOXICITY STUDY FOCUSING ON THE FETAL BRAIN: EVALUATION OF A RAT AUTISM MODEL INDUCED BY VALPROATE AND THALIDOMIDE. T. Ogawa[1], M. Kuwagata[2], S. Shioda[1], *[1]Department of Anatomy, Showa University School of Medicine, Tokyo, Japan; [2]Hatano Research Institute, FDSC, Kanagawa, Japan*

Prenatal exposures to chemicals such as alcohol, lead, PCBs and valproate (VPA) are well known to induce developmental abnormalities in the central nervous system of children. In the developmental neurotoxicology to protect the pediatric brain from those chemical-induced tragedies, the majority of studies focus on postnatal subjects rather than the fetuses. We have been investigating the usefulness of histological observation of the fetal brain in the evaluation of chemicals on the development of the central nervous system. Since prenatal exposure to VPA or thalidomide has been reported to increase children with autistic features, these chemicals are often used to produce animal models of autism. In this study, we exposed pregnant rats to thalidomide (500 mg/kg) or valproate (800 mg/kg) orally on gestation day 9, and observed the fetal brain at embryonic day 14. To avoid selection bias and evaluate more justly, we selected the fetuses located at specific positions in each mother's uteri. We observed these brains very carefully using serial coronal and sagital sections after Nissl staining, and/or 5-HT immunohistorical staining. The exposure to thalidomoide did not induce any alternations even if there are reports suggesting this chemical affects the development of 5-HT neurons. The fetal brains exposed to VPA showed an altered structure in the thalamus, which was rounder with an enlarged third ventricle. Not all VPA-exposed brains were affected, but the incidence was almost one of three. The alternation seemed not to be a developmental delay, since the size of the brain (maximum lateral diameter and maximal longitudinal diameter) was not smaller compared to the controls. Some fetal brains affected by VPA showed the normal development of 5-HT neurons (5-HT fibers arrived at the thalamus, and 5-HT cell bodies appeared in the pons and medulla oblongata as observed in the controls). Other affected brains showed diminished development of the 5-HT neurons. The relationship between the altered fetal thalamus and abnormal function observed in children with autism will be investigated in the future. This study suggests that examination of fetal brains after chemical exposure can be a good new endpoint to support postnatal data from current developmental neurotoxicity studies. Keywords: Fetal brain, Valproate, Developmental neurotoxicity. *(Supported partly by a grant of Long-range Research Initiative (LRI) by Japan Chemical Industry Association (JCIA).)*

P-86 ***Post-Doctoral Student (Group 1)***

CHLORPYRIFOS AFFECTS NEURONAL CELL REPLICATION AND PHENOTYPIC OUTCOMES. RR Jameson, FJ Seidler, D Qiao, TA Slotkin, *Dept. of Pharmacology & Cancer Biology, Integrated Toxicology Program, Duke Univ. Med. Ctr., Durham, NC, USA*

The widely used organophosphate insecticide, chlorpyrifos (CPF), has adverse effects on brain development that are mediated through multiple mechanisms. To determine if CPF has a direct effect on neuronal cell replication and phenotypic fate, we assessed its effects on PC12 cells during active mitosis and during differentiation induced by nerve growth factor (NGF). Upon addition of NGF, PC12 cells develop neuritic projections and differentiate into two distinct phenotypes, catecholaminergic and cholinergic. In undifferentiated cells, CPF reduced the rate of DNA synthesis as monitored by [^{3}H]thymidine incorporation, an effect that was sustained over a period of several days and that occurred in the absence of cytotoxicity as determined by trypan blue staining. CPF also increased the expression of tyrosine hydroxylase (TH), the enzyme responsible for catecholamine biosynthesis, but had no effect on choline acetyltransferase (ChAT), the corresponding enzyme for formation of acetylcholine. Upon addition of NGF, the expression of TH and ChAT increased several-fold in conjunction with differentiation into the two phenotypes. CPF again promoted the expression of TH while having little or no effect on ChAT. The advancement of the catecholaminergic phenotype by CPF in PC12 cells suggests strongly that CPF has a direct effect on the differentiation of neuronal precursors and can thus influence their ultimate phenotypic fate. Keywords: Chlorpyrifos, Neuronal differentiation, PC12 cells. *Support: NIH ES10356 and ES07031.*

P-87

USE OF ANIMAL TOXICITY DATA TO PREDICT ACUTE EFFECTS OF ORGANIC SOLVENTS ON PUBLIC HEALTH. PJ Bushnell, VA Benignus, WK Boyes, TJ Shafer, AS Bale, *Neurotoxicology Division, NHEERL, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA*

Most published information about the acute neurotoxicity of organic solvents is aimed at identifying the hazard of exposure. Synthesizing this information into predictive relationships between exposure and effect in humans is difficult because (1) data are usually derived from experimental animals whose sensitivity to the chemical relative to humans is unknown; (2) the effects measured in the laboratory infrequently translate into effects of concern in humans; and (3) the mode of action of the chemical is rarely understood. To utilize existing information to estimate the hazard and cost of exposure to organic solvents, we focused on the acute effects of toluene on visual and cognitive functions. A meta-analysis of published data suggested that a 10% change in rat avoidance behavior occurs at a blood concentration 25 times higher than the concentration at which a 10% change in human choice reaction time occurs. In contrast, in vitro studies comparing human- and rat-derived nicotinic acetylcholine receptor proteins indicated that the receptors do not differ in sensitivity to toluene. Analysis of other dose–response relationships suggests that the apparent difference between rats and humans may be driven by the endpoints measured in the two species rather than by inherent differences in sensitivity to toluene. Dose–equivalence relationships may also be used to compare the societal costs of two chemicals. For example, ethanol, for which societal costs are known, may be used as a benchmark chemical for estimating the monetary benefits of controlling exposure to organic solvents. Keywords: Organic solvent, Animal–human extrapolation, Dose–equivalence

P-88 ***Post-Doctoral Student (Group 1)***

CUMULATIVE RISK OF PYRETHROIDS: RELATIVE POTENCIES FOR ACUTE EFFECTS ON MOTOR FUNCTION IN RATS. M.J. Wolansky[1], C. Gennings[2], K.M. Crofton[3], *[1]National Research Council, Research Triangle Park (RTP), NC; [2]Department of Biostatistics, Virginia Commonwealth University, Richmond, VA, USA; [3]Neurotoxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, RTP, NC, USA*

The prevalence of pyrethroids in insecticide formulations has increased in the last decade. A common mode-of-action has been proposed for pyrethroids based on in vitro studies, which includes alterations in sodium channel dynamics in nervous system tissues, consequent disturbance of membrane polarization, and abnormal discharge in targeted neurons. The objective of this work was to characterize individual dose–response curves for in vivo motor function and calculate relative potencies for eleven commonly used pyrethroids. Acute oral dose–response functions were determined in adult male Long Evans rats for five Type I (bifenthrin, *S*-bioallethrin, permethrin, resmethrin, tefluthrin), five Type II (β-cyfluthrin, λ-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate), and one mixed Type I/II (fenpropathrin) pyrethroids [n = 8–18 per dose; 6–11 dose levels per chemical, vehicle = corn oil, at 1 ml/kg]. Motor function was measured using Fig. 8 mazes. Animals were tested for 1 h during the period of peak effects. All pyrethroids, regardless of structural class, produced dose-dependent decreases in motor activity. Relative potencies were calculated based on the computed ED30s. Deltamethrin, with an ED30 of 2.51 mg/kg, was chosen as the index chemical. Relative potency ratios ranged from 0.009 (resmethin) to 2.092 (esfenvalerate). These data suggest a common final toxicity pathway for pyrethroids that includes general motor function in rats. Additional work with environmentally based mixtures

is needed to test the hypothesis of dose-additivity of pyrethroids. Keywords: Pyrethroid, Neurotoxicity, Risk.

P-89 ***Post-Doctoral Student (Group 1)***

HYDROGEN SULFIDE EXPOSURE REDUCES THE INTRACELLULAR BUFFERING CAPACITY OF RAT NASAL OLFACTORY EPITHELIAL CELLS. E.S. Roberts, V.A. Wong, B.E. McManus, D.C. Dorman, *CIIT Centers for Health Research, Research Triangle Park, NC, USA*

Hydrogen sulfide (H_2S) is a naturally occurring gas that is also associated with several industries. The potential for widespread human inhalation exposure to this toxic gas is recognized as a public health concern. Human epidemiological investigations and experimental laboratory animal studies show that the nasal olfactory epithelium is selectively damaged by exposure to H_2S. To further understand the mechanism by which H_2S damages the nasal epithelium, respiratory and olfactory epithelial explants were isolated from naïve rats; loaded with the pH-sensitive dye, SNARF-1; and exposed to air or 10, 80, 200, or 400 ppm H_2S for 90 min. Exposures to vinyl acetate and potassium cyanide (KCN) were used as controls. The average pH for air exposed explants was 7.25 ± 0.05. Vinyl acetate (pH 6.99 ± 0.06) significantly decreased intracellular pH ($P < 0.05$), while KCN did not affect pH (pH 7.22 ± 0.02). Incremental decreases in ΔpH occurred with increasing H_2S dose exposure. A significant decrease in ΔpH occurred with 400 ppm H_2S exposure ($P < 0.05$) in both respiratory and olfactory explants. While the dose–response pattern was similar between respiratory and olfactory epithelial tissue, a greater decrease in pH occurred in the olfactory epithelium ($P < 0.03$). We used the intracellular chromofluor SNARF-1 with confocal microscopy to show that rat nasal olfactory epithelium may have a decreased capability to buffer pH compared to the respiratory epithelium, further validating the selectivity of H_2S-induced olfactory toxicity. Keywords: Hydrogen sulfide, Olfactory epithelium, pH

P-90

DEVELOPMENTAL NEUROTOXICITY OF METHYLMERCURY AND METHYLAZOXYMETHANOL: BODY WEIGHT, MOTOR ACTIVITY AND BRAIN DAMAGE COMBINED. *Didima de Groot[1], Marja Moerkens[1], Renate Janskin[1], Marlies Otto[1], Linda van de Horst[1], Marga Bos-Kuijpers[1], Ine Waalkens[1], James O'Callaghan[2], Hans-Jorgen Gundersen[3], Wolfgang Kaufmann[4], Jan Lammers[1], Bente Pakkenberg[5], *[1]TNO Quality of Life, Zeist, NL; [2]NIOSH, Morgantown, USA; [3]University of Aarhus, DK; [4]BASF, Ludwigshafen, FRG; [5]Research Laboratory for Stereology & Neuroscience, Copenhagen, DK*

Motor activity was studied in rats, prenatally exposed to methyl mercury (MeHg) or methylazoxy methanol (MAM) (five dose levels each) on PN 13, 17, 21 and 62, as required by the EPA guideline for developmental neurotoxicity (DNT) testing (OPPTS 870.6300). Neuropathology was studied on PN 22 and PN 62. Both neurotoxicants are known to affect brain morphology during development. MeHg, however, primarily causes systemic toxicity. The results of the motor activity profiles of both compounds were mutually compared and considered in view of body weight changes and brain damage.

Statistically significant effects on motor activity per test age were not observed either for MAM or MeHg, males or females. However, in the top dose MAM group, the time pattern of motor activity differed from controls: hypo-activity on PN 13 and hyper-activity particularly on PN 21. In MeHg-exposed F1-rats, the time pattern of motor activity also differed from that in the top-dose MAM group and showed higher activity than controls on PN17 and lower activity on PN 21.

Brain morphology showed considerable effects of MAM in the forebrain including also a loss of hippocampal CA1 neurons. Effects on cerebellum were not found for MAM. Body weight changes were the same for MAM and MeHg and did not explain the differences in the time pattern of motor activity between the two model compounds. Relating the motor activity results with the neuropathology findings it was concluded that the hyperactivity on PN 21 (MAM) suggested a loss of spatial memory due to observed neuron loss in the hippocampal CA1 region, rather than motor impairment. Neuron loss in the cerebellum and changes found in volume of regions in cerebellum and brain stem (MeHg) most likely account for motor impairment.

The results of the present study illustrate the relevance of combining functional and morphological data when considering their impact with regard to developmental neurotoxicity. Keywords: Developmental neurotoxicity, Methylmercury and methylazoxy methanol, Motor activity and brain damage

Acknowledgements: This project is financially supported by the American Chemistry Council (ACC contract no. 1847). The views given here are not necessarily the views of the sponsor but are solely the responsibility of the authors.

P-91 ***Pre-Doctoral Student (Group 2)***

DEVELOPMENTAL EXPOSURE TO METHYLMERCURY AND *N*-3 FATTY ACIDS: PERFORMANCE ON SPATIAL AND VISUAL DISCRIMINATION REVERSAL TASKS IN ADULT AND AGED RATS. JJ Day[1], EM Paletz[2], MC Craig-Schmidt[3], MC Newland[4], *[1]Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Department of Psychiatry, University of Wisconsin at Madison, Madison, WI, USA; [3]Nutrition and Food Science, Auburn University, Auburn, AL, USA; [4]Deparment of Psychology, Auburn University, Auburn, AL, USA*

Methylmercury (MeHg) is a pernicious neurotoxicant that produces both motor and learning deficits. Developmental MeHg exposure retards the ability of organisms to readjust their behavior following reinforcement contingency changes in

concurrent choice experiments. This effect may suggest either a disruption in the spatial discrimination between two response alternatives or a reduction in a reinforcer's ability to strengthen behavior. In the present study, female rats received in utero exposure to 0, 0.5, or 5 ppm MeHg via maternal drinking water, approximating exposures of 0, 40, and 400 μg/kg/day. These rats also received life-long exposure to a diet containing either fish oil or coconut oil. The fish oil diet was rich in docosahexaenoic acid (DHA), an *N*-3 fatty acid thought to prevent or attenuate the neurotoxic effects of MeHg, while the coconut oil diet contained no DHA. When middle-aged, rats were tested on a spatial discrimination reversal task (SDR). Upon completion of the SDR, the rats were tested on a non-spatial, visual discrimination reversal task (VDR). There was a profound effect of MeHg exposure on number of errors to criterion during the initial reversal condition of both the SDR and VDR tasks, but diet did not mediate this effect. After aging, the rats were re-exposed to the previously learned SDR task. This time, MeHg exposure did not alter discrimination as before. In the elderly rats, however, there was an effect of diet not observed in younger animals. These findings suggest that MeHg weakens the capacity to track reward sources during environmental transitions. With respect to spatial discrimination, this deficit may be overcome as experience with the task increases, even after aging. The fish oil did not weaken MeHg's effect, but it did improve performance among aged rats. Keywords: Methymercury, Developmental exposure, Docosahexaenoic acid. *(Supported by ES10865 from NIEHS.)*

P-92 ***Pre-DoctoralStudent (Group 2)***

INVOLVEMENT OF THE $GABA_A$ RECEPTOR IN METHYLMERCURY-INDUCED DISRUPTION OF Ca^{2+} HOMEOSTASIS IN CEREBELLAR SLICES. Jayme D. Mancini, William D. Atchison, *Department of Pharm/Tox and Neurosci. Prgrm. Mich. State Univ., Coll. Osteopathic Med., E. Lansing, MI, USA*

Perinatal exposure to methylmercury (MeHg) impairs development of cerebellar granule cells causing motor dysfunction. The mechanism by which this occurs is unknown. Acute cerebellar slice preparations were used to investigate changes in intracellular calcium ($[Ca^{2+}]_i$) during development in response to the $GABA_A$ receptor ($GABA_AR$) agonist, muscimol, and/or MeHg. Sagittal cerebellar slices (200 μm thick) of 9–11 day old rats were loaded with calcium indicator dyes Fluo4-AM or Fluo5-AM. Calcium images of the external germinal cell layer (EGL), molecular layer (ML), and internal granule cell layer (IGL) were simultaneously recorded within one visual field by confocal laser microscopy. Recordings through the depth of the slice were obtained using *z*-series stacked. MeHg caused a time- and concentration-dependent increase in $[Ca^{2+}]_i$ in granule cells of all stages of development. Immature granule cells in the EGL showed an increased $[Ca^{2+}]_i$ in response to muscimol pulses. Within the EGL, application of a 100 μM muscimol pulse in the presence of 20 μM MeHg increased the average $[Ca^{2+}]_i$ by 154% relative to controls. This is significantly greater than that caused by application of muscimol in the absence of MeHg. Application of a 50 μM muscimol pulse in the presence of 10 μM MeHg in the EGL increased $[Ca^{2+}]_i$ moreso than that induced by 10 μM MeHg alone. The $[Ca^{2+}]_i$ at subsequent pulses of muscimol in the presence of MeHg was not as high as that in the presence of MeHg alone. Postmigratory granule cells in the IGL are presumed to generate inhibitory responses to $GABA_AR$ activation. Within the IGL, pulses of 50 or 100 μM muscimol alone did not increase $[Ca^{2+}]_i$. The MeHg-induced increase in $[Ca^{2+}]_i$ was dampened by muscimol. The $[Ca^{2+}]_i$ continues to increase suggesting that MeHg may stimulate, then block the mature $GABA_AR$ as described by other studies. Thus, effects of MeHg on the $GABA_AR$ at different stages of development may be responsible for the differential changes in $[Ca^{2+}]_i$. Keywords: Cerebellum, Methylmercury, Calcium. *Supported in part by John Hopkins CAAT.*

P-93

HPLC-BASED METHOD FOR MEASUREMENT OF COPPER IN BIOLOGICAL SAMPLES. V. Amarnath, Kalyani Amarnath, Holly Valentine, William Valentine, *Department of Pathology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*

An elevated level of copper has been implicated in some neurodegenerative diseases necessitating the measurement of copper in biological samples. The most commonly used ICPMS method involves the use of expensive instrumentation and requires 50–100 mg samples. A cost-effective method that uses acid-precipitation of proteins from body fluids or microwave digestion of tissue samples with nitric acid followed by chelation of Cu^{2+} with 3-hydroxypyrrolidne dithiocarbamate (HPDC) is presented. The resulting copper complex exhibiting a characteristic absorption maximum at 431 nm is extracted for measurement by HPLC. Both the reagent HPDC, and the copper chelate ($Cu[HPDC]_2$) are stable and give highly repeatable results. The limit of detection of copper chelate is 10 pmol in the injected solution and, assuming a 4 ppm level of copper in tissues, analysis could be performed with as little as 4 mg of samples. We performed the analysis on 10 mg (dry weight) samples of rat brain to show that subcutaneous exposure to disulfiram in silastic tubes resulted in an elevated copper level of 46.3 ppm compared to 5.11 ppm for controls. Keywords: Copper determination, Dithiocarbamates, HPLC.

P-94 ***Pre-Doctoral Student (Group 2)***

SPATIAL DISCRIMINATION IN RATS CONTINUALLY EXPOSED TO SELENIUM AND GESTATIONALLY EXPOSED TO METHYLMERCURY. Erin F. Pesek, Miranda Reed, M.C. Newland Ph.D., *Department of Psychology, Auburn University, AL, USA*

Previous studies of choice suggest methylmercury (MeHg), retards adjustment when sources of reinforcement change in relative value. A spatial discrimination task using two levers was completed using rats continually exposed to selenium and gestationally exposed to MeHg. Acquisition was completed on the left lever. Rats that were exposed to high selenium required more sessions to complete the first reversal. High selenium, control animals drastically decreased the number of sessions needed to reach 85% accuracy from first reversal to subsequent reversals. Rats exposed to .50 ppm of MeHg made more errors than the other rats on the first reversal. The time it took the rats to respond was also recorded for both the back center and front levers. Measurements were taken from first response and last response. For the first responses: low selenium animals took longer to respond to back center lever than high selenium on the first reversal. High mercury animals responded faster than controls in the choice phase on the first reversal. Controls require longer time to respond in the choice phase than higher levels of mercury on the first reversal to the left lever. For the last responses: controls require longer time to respond on the back center lever than higher mercury rats, on the first reversal. Controls require longer time to respond on the back center lever than higher levels of MeHg on the second reversal to the left lever. Blood and brain samples from littermates of the rats in this study showed blood and brain levels of selenium were significantly lower in the high mercury animals. Keywords: Selenium, Methymercury, Discrimination. *Supported by NIH ES 10865.*

P-95 ***Pre-Doctoral Student (Group 2)***

EFFECTS OF METHYLMERCURY ON THE CRITICAL FUSION FREQUENCY OF RATS. John C. Heath MS, M.C., Newland Ph.D., *Department of Psychology, Auburn University, AL, USA*

Chronic methylmercury (MeHg) exposure causes constriction of the visual field and changes in contrast sensitivity in primates. The Critical Fusion Frequency (CFF, the frequency at which a flickering stimulus appears steady) is sensitive to both these phenomenon. A rodent model of MeHg-induced changes in CFF is being developed to chart the course of visual deficits arising from chronic methylmercury exposure. Four Long-Evans rats were trained to discriminate between a flickering and a steady light. The CFFs were determined using three different psychophysical techniques. In addition, running wheel activity, gait, hind-limb cross, and food consumption were monitored. Rats were exposed to 25 ppm of mercury (as MeHg) in drinking water using a multiple baseline experimental design, i.e., exposure onset occurred in a staggered fashion. Exposure ceased when running wheel activity dropped below baseline levels. During baseline the CFF was consistent across psychophysical method and subject, ranging from 16.3 to 19.2 Hz across rats. Approximately 6–7 weeks of chronic exposure to methylmercury produced an elevated CFF as well as decreased response rate and running, Disrupted gait and hindlimb cross were also observed. Coincident with impairment was an unexpected but substantial increase in food intake required to maintain the rats' body weight. In at least one rat, response rates and CFF returned to baseline levels after MeHg exposure stopped. Keywords: Vision, Methymercury, Rats. *(Supported by NIH ES 10865.)*

P-96

TOXIC EFFECTS OF METHYLMERCURY IN YOUNG DROSOPHILA ARE AMELIORATED BY THE EXPRESSION OF ALZHEIMER'S BETA-AMYLOID PEPTIDES. T Gangi[1], A Halladay[2], K Reuhl[2], M Konsolaki[1], *Rutgers, The State University of New Jersey, Departments of [1]Genetics and [2]Pharmacology & Toxicology, Piscataway, NJ, USA*

Alzheimer's disease (AD) is a neurodegenerative disorder that results in loss of cognitive function. Although the disease pathways are not completely understood, it is widely accepted that the formation of neurofibrillary tangles and beta-amyloid (Aβ) neuritic plaques participate in neuronal cell death and ultimately in the manifestation of disease symptoms. It has been shown that Aβ binds heavy metals such as copper, iron and zinc with high affinity. A suggested physiological role of the Aβ peptide is to aid in the clearance of metals from the extracellular fluid of the brain. We have examined the effects of methylmercury (MeHg), a heavy metal known for its neurotoxic properties, in *Drosophila melanogaster.* We also tested the effect of Aβ in flies treated with MeHg. Exposure of wild type flies to low doses of MeHg caused an early death phenotype. The severity of this phenotype correlated directly with the concentration of MeHg. To examine whether Aβ expression in flies might interfere with the MeHg phenotype, we exposed a strain of Aβ transgenic flies to MeHg. Flies expressing Aβ show dose- and age-dependent neurodegeneration, manifested as a rough eye phenotype or a shorter lifespan, at around 30 days of age. Interestingly, when young Aβ-expressing flies were exposed to MeHg, their viability was improved relative to MeHg-exposed wild type flies. We conclude from this observation that at a young age, before the effects of Aβ are manifested, Aβ is ameliorating the neurotoxicity of MeHg in flies. Keywords: Methyl-mercury, Drosophila, Alzheimer's disease

P-97 ***Pre-Doctoral Student (Group 2)***

MOTOR FUNCTION AND TISSUE LEVELS IN DAMS CHRONICALLY EXPOSED TO METHYLMERCURY AND SELENIUM. Miranda N. Reed, M.C. Newland, *Auburn University, Behavioral Toxicology Lab., Auburn, AL, USA*

It has been suggested that selenium (Se) may prevent or ameliorate methylmercury's neurotoxicity. To examine interactions between Se and methylmercury (MeHg) exposure, 114 Long-Evans rats were exposed to a diet containing either 0.05 or 0.5 ppm Se at 18 weeks of age. Continuous methylmercury exposure via drinking water began approximately 3 weeks later at 21 weeks of age. Water included

methylmercury concentrations of 0, 0.5, and 5.0 ppm, creating estimated intakes of about 0, 40, and 400 μg/kg/day across exposure groups, and a 2×3 design of Se by MeHg. Neurological testing of motor functions was performed on 59 of these rats using several dependent measures taken repeatedly throughout the lifespan. These measures included: run wheel revolutions, forearm grip strength, tail pressure tests, latency on sticky dot tests, and presence of hindlimb cross, flexion, or gait abnormalities. For all repeated measures, there was detrimental effect of the 5.0 ppm MeHg. For some of the measures, there was protection of Se in the high MeHg group but not enough to reach statistical significance, partially due to the euthanasia of animals exhibiting severe MeHg-related deficits in the low Se, high MeHg group. This created an artificial improvement in performance on measures taken later in life for this group relative to the high Se, high MeHg group, thus, masking Se protection. Twenty-eight rats not used in neurological tests were euthanized immediately after breeding in order to access tissue levels. Se blood levels were higher for the high Se group, and both low MeHg groups exhibited higher Se blood levels relative to their control and high MeHg groups. Se brain levels were approximately the same for all groups with the exception of the high Se, high MeHg, which had approximately two times as much Se in the brain. MeHg blood levels increased as a function of MeHg exposure, but for the low MeHg, high Se group, there was more MeHg relative to the low MeHg, low Se group. MeHg brain levels increased as a function of MeHg exposure, regardless of diet. Keywords: Methylmercury, Selenium, Behavior. *Research supported by NIH ES 10865*.

P-98 ***Pre-Doctoral Student (Group 2)***

BEHAVIORAL EFFECTS OF COCAINE AND DESIPRAMINE FOR RATS GESTATIONALLY EXPOSED TO METHYLMERCURY AND SELENIUM. Miranda N. Reed, M.C. Newland, *Auburn University, Behavioral Toxicology Lab., Auburn, AL, USA*

Both the in vitro and in vivo literature suggests alterations of catecholamine functioning following gestational methylmercury (MeHg) exposure. The present experiment was designed to examine the interaction of MeHg and selenium on behavior under stimulus control following cocaine and desipramine administration. Female Long-Evans rats were exposed to 0, 0.5, or 5 ppm MeHg via maternal drinking water during gestation as well as a diet either high or low in selenium that continued throughout their lifespan, creating a 2×3 factorial design. Upon reaching adulthood, forty-four female offspring were placed on a MULT FI_{120} $FIClock_{120}$. The $FIClock_{120}$ component was divided into five 24 s bins. Each of the five bins was associated with a different auditory stimulus, thus providing the rats with a ''clock''. After 42 sessions, the rats were exposed to acute doses of cocaine (1–30 mg/kg) followed by desipramine (1–17 mg/kg). Increased sensitivity to cocaine on measures of low-rate behavior was found in low selenium animals, regardless of MeHg exposure. Mercury-exposed animals exhibited increased sensitivity on measures of high-rate behavior following cocaine administration. Differences between the FI_{120} and $FIClock_{120}$ components following cocaine administration were only seen on measures of low-rate behavior for all groups. There were no differences between selenium exposed animals on any measures following desipramine administration. Mercury-exposed animals displayed increased sensitivity to desipramine on measures of high-rate behavior. Differences between the FI_{120} and $FIClock_{120}$ components following desipramine administration were seen on all measures of behavior for all groups. These findings suggest alteration of dopamine functioning for low selenium animals engaging in low-rate behavior, whereas mercury exposure disrupts high-rate behavior following catecholinergic agonists. Keywords: Methylmercury, Selenium, Behavior. *Research supported by NIH ES 10865*.

P-99

USE OF MAGNETIC RESONANCE IMAGING (MRI) TO DETERMINE BRAIN MANGANESE DEPOSITION IN MALE SPRAGUE–DAWLEY RATS. VA Fitsanakis[1], N Zhang[2], KM Erikson[3], JC Gore[2], M Aschner[1,4], *[1]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN, USA; [2]Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Nashville, TN, USA; [3]Department of Nutrition, University of North Carolina, Greensboro, NC, USA; [4]Department of Pharmacology and the Kennedy Center, Vanderbilt University Medical Center, Nashville, TN, USA*

Manganese (Mn) is an essential metal found in food and the environment. Despite its ubiquity, it is toxic at high levels. While Mn toxicity is usually associated with specialized activities, such as Mn mining, recent animal data suggests that iron (Fe) deficiency and/or anemia may lead to increased brain Mn deposition. Mn is a paramagnetic metal, a property that permits it to be visualized on T_1-weighted magnetic resonance images (MRIs). In this study, two groups of adult male Sprague–Dawley rats received normal rodent chow for a total of 14 weeks (10 ppm Mn). However, the treated group was also intravenously injected weekly with 5 mg Mn/kg intravenously during the course of the study. Animals were weighed weekly, as a measure of general health, and imaged every 2 weeks to determine the temporal pattern of brain Mn deposition. At the conclusion of the study, blood was collected, brains were removed and dissected into various regions (cerebellum, brain stem, midbrain, hippocampus, striatum or cortex) and the amount of Mn and Fe determined. Treated animals weighed significantly less than controls beginning at week 2 and continuing throughout the course of the study. Preliminary MRI data indicate that treated animals have a

significant accumulation of Mn in the olfactory bulb, hippocampus, periventricular zone and cerebellum. These data will be useful as baselines in determining whether Fe deficiency changes the deposition pattern of Mn, and what effect Fe repletion may have on the ability to reverse brain Mn accumulation. Keywords: Manganese neurotoxicity, Magnetic resonance imaging, Rat. *Supported by DoD, Manganese Research Health Project (04149002) and NIEHS ES 10563.*

P-100 ***Pre-Doctoral Student (Group 2)***

ANTIOXIDANT PROTECTION AGAINST MeHg-INDUCED NEUROTOXICITY IN VIVO AND IN VITRO. M. Polunas[1,2], A.K. Halladay[1,2], G.C. Wagner[1,3], K.R. Reuhl[1,2], *[1]Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ, USA; [2]Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, USA; [3]Department of Psychology, Rutgers University, Piscataway, NJ, USA*

We have previously reported an elevation in reactive oxygen species (ROS) production following exposure to methylmercury (MeHg). A marker for ROS, DCF fluorescence, was elevated in both differentiated neurons and neonatal mouse pup brain following administration of MeHg. The MeHg-induced increase in ROS, as well as pertubation of mitochondrial membrane potential in differentiated neurons, was attenuated by co-administration with the water soluble vitamin E derivative Trolox®. In order to determine if behavioral effects of MeHg following early postnatal exposure were altered by antioxidants, male BALB/c mice were treated with MeHg 4 mg/kg on alternate days P3–15 of life. Trolox® at 2.5 or 1.0 mg/kg was administered daily and 1 h before each MeHg injection, and mice were examined for neurobehavioral development daily through PND 33. MeHg administration at 4 mg/kg on alternate postnatal days 3–15 resulted in a delay in the mid-air righting response when tested on postnatal days 14–18, and a significant impairment in spatial and working memory using the water maze when examined on postnatal days 26–33. These impairments were not attributed to swimming ability. Pretreatment with Trolox® at 2.5, but not 1.0 mg/kg, protected against these cerebellar and hippocampal-mediated behavioral deficits. In addition, Trolox® at 2.5 mg/kg prevented the increase in aggressive behaviors and decrease in social interaction on PND 90 consequent to MeHg exposure. Together, these results obtained from both in vivo and in vitro models demonstrate that ROS production contributes to neurodevelopmental deficits. Keywords: Mercury, Oxidative stress, Behavior. *Supported by ES05022, ES07148 and ES11256.*

P-101 ***Pre-Doctoral Student (Group 2)***

EFFECT OF PRION PROTEINS ON MANGANESE-INDUCED OXIDATIVE INSULT AND MITOCHONDRIAL DYSFUNCTION. Christopher Choi, Vellareddy Anantharam, Arthi Kanthasamy, Anumantha Kanthasamy, *Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, USA*

Normal prion protein is highly expressed in the central nervous system and binds to divalent metal cations at the octapeptide repeat regions of the protein. Manganese is one of these divalent cations purported to interact with the prion protein. Manganese is an essential trace element required for various cell functions; yet, excessive exposure to chronic manganese causes neurological deficits of varying degree. Recently, we noted that the prion protein-expressing mouse neural cells (PrP^{c}) were less susceptible to neurotoxic effects of manganese as compared to the knock-out mouse neural cells (PrP^{ko}), indicating that cellular prion protein may have a neuroprotective role against metal toxicity. In the present study, we further examined the cellular mechanisms underlying the protective effect of prion protein against manganese neurotoxicity. Manganese-induced ROS generation and mitochondrial membrane depolarization were significantly attenuated in PrP^{c} cells as compared to PrP^{ko} cells. Measurement of antioxidant status revealed no significant difference in the basal levels of cellular reduced glutathione (GSH) and superoxide dismutase (SOD) activity between PrP^{c} cells and PrP^{ko} cells. However, manganese treatment caused greater depletion of GSH in PrP^{ko} cells as compared to PrP^{c} cells, indicating that prion protein may alter antioxidant utilization in metal toxicity. We also determined whether manganese exposure alters cellular prion protein levels by quantifying the prion protein levels in cells as well as in the extracellular medium. Western blot analysis revealed increased expression of cellular prion protein and shedding into the supernatant over time following manganese treatment. Collectively, these data suggest that prion protein interacts with divalent manganese and protects against metal-induced neurotoxicity by attenuating oxidative stress and mitochondrial function. Keywords: Prion, Manganese, Neurotoxicity

P-102

METHYL MERCURY (MeHg) EXPOSURE ALTERS NEUROGENESIS SELECTIVELY IN THE NEONATAL RAT HIPPOCAMPUS. A Falluel-Morel[1], X Zhou[1], A Litterman[1], KR Reuhl[2], E DiCicco-Bloom[1], *[1]Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School – UMDNJ, Piscataway, NJ, USA; [2]Department of Toxicology and Pharmacology, Rutgers, Piscataway, NJ, USA*

Environmental toxicants, like MeHg, may contribute to disorders of developing brain. Toxicants may act at multiple times, eliciting acute stage-dependent effects on neurogenesis that influence later ontogeny. Indeed, our previous work

indicates that MeHg exposure decreases DNA synthesis acutely in hippocampus, leading to diminished DNA content 2 weeks later (Burke et al., 2004). While high MeHg exposure causes gross defects, retardation and seizures, effects of lower levels unassociated with deformities are less well defined, especially regarding precursor proliferation. Seven-day-old rats were injected subcutaneously with MeHg (2.5–10 μg/gbw). Two hours before sacrifice we injected [3H]thymidine to examine effects on DNA synthesis, or BrdU to label cells in S phase of the cell cycle. After 24 h, DNA synthesis was reduced 6% at 2.5 μg (NS), 24% at 5 μg and 35% at 10 μg/gbw in the hippocampus ($P < 0.02$). The dose of 5 μg/gbw reduced DNA synthesis 14% at 6 h (NS), 29% at 8 h, 26% at 10 h and 24% at 24 h ($P < 0.03$) indicating rapid effects on cell cycle progression, raising the possibility of a G1-S block. In contrast, MeHg did not effect cerebellum, suggesting differential vulnerability, since blood flow and mercury content were similar to hippocampus. This reduction in DNA synthesis could be attributed to several processes including a block in the G1/S transition, initiation of cell death or energy failure. To examine the G1/S transition, we used BrdU labelling to quantify cells in S phase in the hilus of the hippocampal dentate gyrus. Following MeHg exposure, the number of BrdU positive cells decreased by 35% at 8 h and 37% at 24 h, indicating that MeHg affects DNA synthesis by reducing S phase entry. In light of continuing concern about the pharmaceutical preservative thimerosal (49.6% mercury), which exhibits different transport and metabolism kinetics, we performed dose–response comparison. At 24 h thimerosal was four times more potent that MeHg in inhibiting DNA synthesis in the hippocampus. These studies suggest that mercury compounds rapidly and directly alter brain development through modulation of regional neurogenesis. Keywords: Mercury, Neurogenesis, Hippocampus. *Support Contributed By: NIEHS 11256, EPA R82939101, ES 05022.*

P-103 ***Pre-Doctoral Student (Group 2)***

TIME COURSE OF METHYLMERCURY BLOCK OF $GABA_A$ RECEPTOR CURRENTS IS NOT CHANGED BY FLUMAZENIL IN RAT CORTICAL CELLS IN CULTURE. C. Herden, Y. Yuan, W.D. Atchison, *Neuroscience Program and Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI, USA*

$GABA_A$ receptor-mediated synaptic transmission is extremely sensitive to the effects of methylmercury (MeHg), as it is blocked more quickly by MeHg than is excitatory synaptic transmission. However, the site at which MeHg interacts with the $GABA_A$ receptor has not yet been determined. Previous studies in our lab using cerebral cortical cells in primary culture have found that block of $GABA_A$ receptor-mediated currents (I_{GABA}) by MeHg is voltage-independent, suggesting that MeHg acts at a site outside of the channel pore. Furthermore, time to block of I_{GABA} by MeHg was independent of [GABA] (10 μM–1 mM) in these cells, suggesting that MeHg does not compete with GABA for its binding site. However, in addition to GABA, the $GABA_A$ receptor has modulatory binding sites for agents such as barbiturates and benzodiazepines. To test if MeHg interacts with the benzodiazepine binding site of the receptor, flumazenil (10 μM), a benzodiazepine antagonist, was applied to cerebral cortical cells in primary culture in the absence and presence of MeHg (1 μM). I_{GABA} were obtained by means of the whole-cell voltage patch-clamp technique under symmetrical chloride conditions. In the absence of flumazenil, the time-course of I_{GABA} block by MeHg (1 μM) was approximately linear, in that 40% of I_{GABA} block occurred at approximately 40% of the time needed to cause complete block. At 1 μM MeHg, time to 20% I_{GABA} block by MeHg occurred at an average of 456 s and complete I_{GABA} block by MeHg occurred at about 2146 s. Similarly, in the presence of flumazenil (10 μM), time to 20% block and time to complete block of I_{GABA} by MeHg (1 μM) occurred at approximately 546 and 2337.5 s, respectively, and did not differ significantly from those obtained by MeHg alone. Thus, co-application of MeHg (1 μM) and flumazenil (10 μM) did not appear to affect the time-course of effects of MeHg on I_{GABA} in cortical cells. These results indicate that MeHg maintains its effects on the $GABA_A$ receptor in the presence of flumazenil, a benzodiazepine antagonist, suggesting that MeHg acts at a site independent of that used by benzodiazepines. Keywords: Methylmercury, GABA(A), Benzodiazepines. *Supported by: NIH grant ES11662.*

P-104 ***Pre-Doctoral Student (Group 2)***

CELLULAR REPOPULATION OF THE MURINE HIPPOCAMPUS FOLLOWING TRIMETHYLTIN INJURY. BC Weig, HE Lowndes, KR Reuhl, *Department of Pharmacology and Toxicology, and Joint Graduate Program in Toxicology, Rutgers University and University of Medicine and Dentistry of New Jersey, USA*

Following the postnatal decline of cell proliferation in the mammalian central nervous system, the adult brain retains a low rate of active neurogenesis in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampus. The mechanisms regulating this neurogenesis are unclear and the functional role of new neurons in the adult animal is unknown. One role may be to compensate for cell loss due to injury or aging. The response of stem cells and their progeny to injury was studied in 2-month-old male C3H mice treated with the selective limbic system neurotoxicant trimethyltin (TMT). Using bromodeoxyuridine (BrdU) as a proliferation marker, TMT exposure (2.8 mg/kg; i.p.) resulted in the appearance of a significant number of BrdU-labeled cells in the dentate gyrus granular and subgranular zones, and in the molecular layer of the hippocampus. The number of SVZ cells incorporating BrdU

was not significantly different following trimethyltin treatment. BrdU+ cells in the hippocampus were classified as neurons based on NeuN co-labeling. Onset of hippocampal neurogenesis occurred 48 h after TMT exposure and continued at an accelerated rate for several days. To determine whether migrating progenitor cells from the SVZ contributed new cells to the injured hippocampus, mice were injected icv with the carbocyanine dye DiI prior to TMT exposure. Twenty-eight days after TMT injection, DiI+ cells were detected in the hilus and granule cell layer of the dentate gyrus. Though most DiI-labeled cells in the dentate gyrus did not appear to express NeuN or the astrocytic marker GFAP, a small proportion of labeled cells did present with these lineage-specific markers. These studies indicate that while most cells repopulating the chemically damaged hippocampus are derived from proliferating precursors resident in the hippocampus, some SVZ cells are also capable of migrating to the site of injury. The functional role of these cells is presently unknown. Keywords: Trimethyltin, Stem cells, Hippocampus.*(Supported by ES011256 and ES07148.)*

P-105

MICGROGLIA ACTIVATION AND FATE FOLLOWING TMT-INDUCED NEURODEGERATION IN THE MOUSE HIPPOCAMPUS. C.A. McPherson, R.N. Wine, C.L. d'Hellencourt, G.J. Harry, *NIEHS, NIH, DHHS, Laboratory of Neurobiology, Research Triangle Park, NC, USA*

Activation of microglial cells and production of inflammatory factors contribute to neuronal damage in various forms of brain injury. Previous studies associated regulation of microglial activation with proliferative and apoptotic processes. This study examined the temporal and spatial response of microglia within the framework of hippocampal neuronal death or survival. In young male CD-1 mice, selective damage of dentate gyrus (DG) granule neurons was produced by an acute injection of trimethyltin (TMT; 2 mg/kg, i.p.). Pyramidal CA neurons were not damaged. QT-PCR of RNA from DG or CA cells, as collected by laser capture microdissection, showed early elevations of TNFα and the TNFα receptors p55 and p75. The temporal pattern of increased TNFα and TNFα receptor mRNA levels coincide with morphological responses of activated microglia in the DG and reactive microglia in the CA layer. Proliferation, as determined by immunostaining for BrdU and Ki-67, was predominantly localized to neuronal cells rather than in microglia. TUNEL and active caspase 3 immunohistochemistry for apoptotic cell death was evident in DG neurons. Data from this study suggest hippocampal microglia activation via differentiation from a resting to phagocytic phenotype, rather than via cell proliferation. Morphological evidence suggested a down regulation of activated microglia to a resting phenotype rather than a gross microglia cell death. Keywords: Neurodegeneration, Microglia, Mouse

P-106 ***Pre-Doctoral Student (Group 2)***

METHYLMERCURIC CHLORIDE AND PSYCHOGENIC STRESSORS DIFFERENTIALLY ACTIVATE c-FOS EXPRESSION IN THE MURINE BRAIN. Joel F. Cooper, Alexander W. Kusnecov, *Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ, USA*

Organic mercury compounds are well known environmental neurotoxicants that can directly lead to mitotic arrest, protein dysfunction, faulty neuronal migration and targeting, as well as excitotoxicity; all capable of leading to neuropathologic states. While mercury exposure, in and of itself, may be conceptualized as a chemical stressor, little evidence exists on its ability to influence stress circuits in the brain responsible for neural adaptation to psychogenic stressors. Measurement of early gene activation of stress-mediated transcription factors such as cFos, cJun, MEK, ERK in response to a toxic insult are commonly used to assess the response of a biologic system to chemical and psychogenic stressors. In addition, proinflammatory cytokines (e.g., tumor necrosis factor, interleukin-1 [IL-1], and IL-6) are known to be generated by glial cells and associated with neurodegenerative diseases (e.g., Parkinson's disease), although little is known regarding their role in organic mercury neurotoxicity. As an initial step in addressing these gaps in the literature, we have determined the impact of an acute exposure to methylmercuric chloride (8 mg/kg), with and without coincidental exposure to a psychogenic stressor (open field, OF), on c-Fos immunoreactivity in the brain. Male C57BL/6J mice given intraperitoneal (i.p.) injections of methylmercuric chloride (CH_3HgCl) in the presence of the open field stressor were found to exhibit high numbers of cFos-positive cells in the bed nucleus of the stria terminalis (BST), paraventricular thalamic nucleus (PV), preoptic nuclei (PO), locus coeruleus (LC), solitary tract (sol), and dorsal motor nucleus of vagus (10N). Exposure to CH_3HgCl in the absence of the OF stress displayed similarly located, but less robust, presence of cFos positive cells. CH_3HgCl-dosed mice exhibited a markedly reduced number of stereotypic, thigmotactic line crossings in the open field, when compared to mice dosed with saline. Mice given an i.p. injection of saline and placed into the open field exhibited a broad distribution of cFos-positive cells within the lateral septum (LS) and BST with more focal aggregates in the PV; all of which were less robust than both CH_3HgCl-treated groups. However, saline/OF mice had more robust presence cFos-positive cells in the septal and cortical regions. Mice given i.p. saline injection with no OF stressor had minimal amounts of PV, BST, and cortical cFos protein. Studies are currently in progress to further define these changes as a function of methylmercury dose, as well as the ability of methylmercury to induce other immediate early genes and proinflammatory cytokine expression. Keywords: Neurotoxicology, Immunology, Pathology

P-107 ***Pre-Doctoral Student (Group 2)***

BACKGROUND LEVELS OF HEAVY METALS ON FETAL GROWTH AND NEONATAL NEURODEVELOPMENT. HC Wu[1], YH Hwang[1], SF Jeng[2], WS Hsieh[3], HF Liao[2], YN Su[3], FC Su[1], PC Chen[1], *[1]National Taiwan University College of Public Health; [2]National Taiwan University College of Medicine; [3]National Taiwan University Hospital, Taipei, Taiwan*

The objective in this study was to estimate the relation between background levels of heavy metals in the general population and fetal growth and neonatal neurodevelopment. A total of 393 pregnant women and their neonates were recruited in the pilot of Taiwan Birth Cohort Study between April 2004 and January 2005. We interviewed them by a structured questionnaire before delivery, collected umbilical cord blood at birth, and performed the neonatal neurobehavioral examination (NNE) after birth. We measured fetal umbilical cord blood As, Cd, Hg, Mn, and Pb concentrations by using the ICP-MS. The geometric mean concentrations of As, Cd, Hg, Mn, and Pb in cord blood were 4.1, 0.6, 11.8, 50.0, and 11.0 μg/L, respectively. There were significant dose–response relations between cord blood As, Hg, and Pb concentrations and low NNE scores. Compared with lowest quartile concentration groups, the highest quartile As (>5.47 μg/L), Hg (>24.95 μg/L), and Pb (>22.35 μg/L) concentration groups had higher risks of having low NNE scores (As: OR = 4.45, 95% CI = 1.28–15.48; Hg: OR = 2.98, 95% CI = 0.72–12.40; and Pb: 5.71, 95% CI = 1.14–28.58). In summary, we concluded that background levels of metals still affected neonatal neurodevelopment at birth. Keywords: Heavy metals, Fetal growth, Neurodevelopment

P-108 ***Pre-Doctoral Student (Group 3)***

EXPLORING THE RISK OF CHLORPYRIFOS ON FETAL GROWTH AND NEONATAL NEURODEVELOPMENT. CJ Hsieh[1], HP Li[2], WS Hsieh[3], SF Jeng[4], HF Liao[4], YN Su[3], SN Yu[1], PC Chen[1], *[1]National Taiwan University College of Public Health; [2]Taiwan Agricultural Chemicals and Toxic Substances Research Institute; [3]National Taiwan University Hospital; [4]National Taiwan University College of Medicine, Taiwan*

The aim of the study was to explore the risk of chlorpyrifos exposure on fetal growth and neurodevelopment in the general population. This study was a cross-sectional in design. The study population was 81 mothers who were 18–40 years, had no smoking history, and gave births in the pilot of Taiwan Birth Cohort Study between August 2004 and January 2005 and their neonates. We interviewed them by a structured questionnaire before delivery, collected umbilical cord blood at birth, and performed the neonatal neurobehavioral examination (NNE) after birth. The plasma chlorpyrifos concentration was analyzed by using gas chromatography coupled to tandem mass spectrometry. The median chlorpyrifos concentration was 1.29 ppb. There were negative relations between cord plasma chlorpyrifos and short birth length (OR = 5.86, 95% CI = 1.68–20.43) and small for gestational age (OR = 13.22, 95% CI = 1.3–134.4). However, we did not find a significant result on the NNE. Therefore, chlorpyrifos exposure in the general population may be related to fetal growth. We're going to follow-up these newborns to investigate any potentially prenatal exposure delayed effect. Keywords: Chlorpyrifos, Fetal growth, Neurodevelopment

P-109 ***Pre-Doctoral Student (Group 3)***

RYANODINE RECEPTOR TYPE 1 (RYR1) POSSESSING MALIGNANT HYPERTHERMIA MUTATION R615C EXHIBITS HEIGHTENED SENSITIVITY TO DYSREGULATION BY NONCOPLANAR PCB 95. Tram-Anh N. Ta, Isaac N. Pessah, *VM: Molecular Biosciences and UC Davis Center for Children's Environmental Health and Disease Prevention, University of California, Davis, CA*

Malignant hyperthermia susceptibility (MHS) is an inherited pharmacogenetic disorder of skeletal muscle in human and animals possessing any one of ~60 missense mutations within ryanodine receptor type 1 ($_{MH}$RyR1). RyR1, a highly regulated microsomal Ca^{2+} channel that orchestrates the release of Ca^{2+} from ER/SR stores and entry of Ca^{2+} across the plasma membrane, is widely expressed in human tissues. Since patients susceptible to MH do not always have a fulminant MH episode when exposed to triggering agents, and there are different degrees of severities in patients possessing the same point mutation, we are examining the possible influence of environmental modifiers that may augment MH susceptibility. In the present study we tested the potency and efficacy of non-coplanar polychlorinated biphenyl PCB 95 toward sensitizing activation of $_{Wt}$RyR1 and $_{MH}$RyR1 isolated from skeletal muscle junctional microsomes obtained from normal Pietrain pigs and those homozygous for MHS mutation R615C. No significant difference in the level of $_{Wt}$RyR1 and $_{MH}$RyR1 expression was found ($p < 0.85$). Using [^{3}H]ryanodine-binding analysis to measure specific aspects of channel function, we discovered that $_{MH}$RyR1 is significantly more sensitive to channel activation by Ca^{2+}, and less sensitive to channel inhibition by Ca^{2+} and Mg^{2+}, compared to $_{Wt}$RyR1. At a level of 100 nM Ca^{2+} that is typically present in the cytosol of the typical mammalian cell at rest, PCB 95 (50–250 nM) enhanced the activity of $_{MH}$RyR1, whereas it has no detectable effect on $_{Wt}$RyR1. Furthermore, PCB 95 (2 μM) decreases channel inhibition by Mg^{2+} to a greater extent in $_{MH}$RyR1 (IC_{50} increased nine-fold) compared to $_{Wt}$RyR1 (IC_{50} increased by 2.5-fold). PCB95 also reduced inhibition by Ca^{2+} two-fold more with $_{MH}$RyR1 than $_{Wt}$RyR1. Collectively our data suggest that non-coplanar PCBs have a more profound effect on cation regulation of $_{MH}$RyR1 channels than $_{Wt}$RyR1 channels. Considering the important roles of Ca^{2+} and Mg^{2+} in regulating Ca^{2+} signals involving RyR channels, these data provided the first mechanistic

evidence of a genetic susceptibility to the exposure of non-coplanar PCBs may synergize MHS. Keywords: PCBs, RyR1, Malignant Hyperthermia. *This work was supported by Grant P01 ES11269 and T32 ES07059.*

P-110 ***Pre-Doctoral Student (Group 3)***

AROCLOR 1254 MAY INDUCE LONG-TERM ALTERATIONS IN CENTRAL VASOPRESSIN RELEASE BY INHIBITING NITRIC OXIDE SYNTHESIS WITHIN THE SUPRAOPTIC NUCLEUS. C.G. Coburn[1], B. Hou, L. Lin, C. Cheetham, E.R. Gillard, O. Loson, D. Prodon, M.C. Curras-Collazo[2], *[1]Environmental Toxicology Program and [2]Department of Cell Biology & Neuroscience, University of California at Riverside, Riverside, CA, USA*

Our research aims at understanding the relationship between exposure to polychlorinated biphenyls (PCBs) and mechanisms underlying subtle neuroendocrine toxicity. Our work explores the effects of PCBs on osmoregulation and in particular on magnocellular neuroendocrine cells (MNCs) of the supraoptic nucleus (SON). MNCs release vasopressin (VP) within the SON, a phenomenon contributing to autoregulation of systemic VP release. Recently, we have shown that oral exposure to PCBs suppresses the dehydration-induced intranuclear release of VP in adult male rats (Coburn et al., 2005). Because several PCB congeners contained in Aroclor 1254 reduce nitric oxide synthase (NOS) activity in the rat hypothalamus (Sharma and Kodavanti, 2002) and NO production is required for the efficient neuroendocrine activity of MNCs (Kadekaro, 2004), it is possible that PCBs might compromise the MNC system by decreasing compensatory NO production within the SON in response to dehydration in vivo. To quantify the contribution of NO to SON VP release during dehydration we have measured NO release from SON tissue punches using an enzymatic NO assay (Oxford Biomedical). Pilot data show that PCB (Aroclor 1254/30 μM); 10 min exposure in vitro decreases NO release from the SON of dehydrated adult male rats by 62% compared to its release from the SON of normosmotic animals. This decrease yields values that are very similar to normosmotic animals. In another set of studies we quantified changes in NOS activity using NADPH-d diaphorase histochemistry. Densitometry analysis revealed that in utero exposure to PCBs caused a dramatic inhibition of NOS function in aged (15 month) dehydrated male rats. Pilot work has shown that NOS activity in SON was diminished by 53% compared to dehydrated controls and moreover, these values were reduced to levels similar to NOS activity in normosmotic animals. Taken together, these data are consistent with our previous findings that Aroclor 1254 treatment of SON punches in vitro completely abolishes dehydration-induced central VP release and support our hypothesis that NO synthesis may be compromised by exposure to PCBs in utero, and furthermore, that the deleterious effects of these toxicants on neuroendocrine systems may have lifelong consequences. Keywords: Polychlorinated biphenyls, Supraoptic nucleus, Nitric oxide

P-111

SEXUALLY DIMORPHIC GENE EXPRESSION PATTERNS IN THE DEVELOPING MOUSE EMBRYONIC BRAINS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN. Y Kagami[1], T Mitsui[2], S Maeda[2], *[1]Ecogenomics, Inc., Fukuoka, Japan; [2]Department of Biochemistry, University of Yamanashi, Yamanashi, Japan*

Perinatal developmental exposure of dioxin and dioxin-like compounds are known to cause reproductive and nueroendocrinological disruptions in human and laboratory animals, and thus far majority of environmental endocrine disruptor studies were to understand the mechanisms of reproductive endocrine disruption. However, a number of studies of dioxin- and dioxin-like compound-exposure studies have indicated aberrations in non-reproductive sex-linked behavioral responses of laboratory animals. This suggested us that the causes of alterations in non-reproductive behaviors should be studied further at gene expression level. We exposed pregnant C57BL/6 mice to 5 μg/kg-body weight of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by single gavage at gestational day (GD) 12.5, and then gene expression profiles of the whole embryonic brain at GD18.5 were analyzed by CodeLink™ UniSet Mouse I Bioarrays (10,012 gene probes, Amersham Biosciences). The data analysis by ANOVA statistics revealed that 30.1% (3011/10,012) of the genes were significantly ($p < 0.05$) altered their expressions by in utero TCDD-exposure. Among these TCDD-responsive genes, 23.9% (719/3011) were up-regulated in both sexes, and 10.3% were down-regulated in both sexes (310/3011). Sexually dimorphic gene responses were very interesting, and they showed that 62.9% (1894/3011) were up-regulated in male but down-regulated in female, whereas only 2.9% (88/3011) were up-regulated in female but down-regulated in male. Gene ontology classification study showed striking differences between the sexes in the expression profiles of the genes with catalytic, signal transduction, and transcriptional regulation activities, suggesting some functional links of these TCDD-affected genes to the disorders that occur later in non-reproductive sex-linked behavioral responses. Keywords: DNA microarray, Sexual dimorphism, Dioxin-responsive genes.

P-112 ***Pre-Doctoral Student (Group 3)***

MANCOZEB AND MANEB NEUROTOXICITY IN MESENCEPHALIC CELLS: POSSIBLE RISK FACTOR FOR PARKINSONISM. LM Domico[1], GD Zeevalk[2], B Buckley[3], B Winnik[3], MJ Thiruchelvam[1], KR Cooper[1], *[1]Joint Graduate Program in Toxicology, Rutgers, The State University of New Jersey, Piscataway, NJ, USA; [2]Neurology Department, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA; [3]Environmental and Occupational Health Sciences Institute, Rutgers/UMDNJ, Piscataway, NJ, USA*

Recent studies suggest that exposure to agrochemicals may contribute to the development of idiopathic Parkinsonism.

The Mn-ethylene-bis-dithiocarbamate (EBDC) fungicide, maneb (MB), inhibits complex III of the electron transport chain and has been implicated in selective dopaminergic neurodegeneration. We examined the potential neurotoxicity of mancozeb (MZ), a Mn-Zn-EBDC fungicide that is structurally similar to MB, as compared to MB using a rat primary mesencephalic cell culture. Previous studies in our laboratory have shown that MZ and MB produce equipotent toxic effects in both DA and GABA neurons and perturb mitochondrial respiration. At low doses, MZ and MB act as uncouplers, whereas at higher toxic doses they both uncouple resting respiration and inhibit active respiration. To further investigate the mechanism of neurotoxicity and the role of ROS, peroxide generation was measured in mesencephalic cells exposed to low doses of MZ, MB, or nabam (NB), a Na-EBDC, alone or in combination with $MnCl_2$ (NB/$MnCl_2$). Exposure to MZ or MB resulted in a 100-fold increase of peroxides as compared to control, while NB or $MnCl_2$ alone resulted in only a two-fold increase. Interestingly, exposure to NB/$MnCl_2$ constituted 24 h prior to use produced peroxide levels similar to those of MZ and MB. In addition, pre-treatment with the NADPH oxidase inhibitor DPI partially decreased peroxide levels, suggesting a role for NADPH oxidase in EBDC toxicity. Pre-treatment with NS 398 (COX2 inhibitor) or allopurinol (XO inhibitor) were without effect. Analysis of MZ, MB, NB, and NB/$MnCl_2$ via mass spectrophotometry revealed that MZ, MB, and 24 h old NB/$MnCl_2$ form similar high molecular weight (HMW) polymers when put into solution. It is not likely that such HMW polymers gain entry into the cell, thereby supporting the hypothesis that NADPH oxidase, which resides on the cell membrane, may play a role in the toxic action of these compounds. Keywords: Pesticides, Reactive oxygen species, Parkinson's disease. *(Supported by ES07148 and a Cook College NJAES grant.)*

P-113 ***Pre-Doctoral Student (Group 3)***

CONCENTRATION DEPENDENT ACCUMULATION OF [^{3}H]-DELTAMETHRIN IN *XENOPUS LAEVIS* OOCYTES. J.A. Watkins[1], C.A. Meacham[2], A.S. Bale[2], K.M. Crofton[2], T.J. Shafer[2], *[1]North Carolina State University, Raleigh, NC, USA; [2]Neurotoxicology Div., NHEERL, ORD, U.S. EPA, Res. Tri. Park, NC, USA*

Pyrethroid insecticides such as deltamethrin have been demonstrated to target and disrupt voltage-sensitive sodium channels (VSSCs). VSSCs were expressed in *Xenopus laevis* oocytes and used to study the effects of deltamethrin on VSSCs. This study evaluated the amount of deltamethrin (DLT) absorption by VSSC-expressing oocytes. Previous research concerning the uptake of DLT in non-injected oocytes was performed by Harrill et al. (2005). Na_v 1.2 is the primary VSSC in the adult rat brain and was selected to model mammalian VSSCs in this experiment. Since deltamethrin disrupts sodium channels, it was hypothesized that oocytes expressing the Na_v 1.2/β_1 would have a higher uptake of [^{3}H]-deltamethrin (DLT) than non-injected oocytes. Oocyte expression of Na_v 1.2/β_1 channels was assessed by two-electrode voltage-clamp. The expression rate was 98% in all experiments (n = 5); deltamethrin increased tail currents for all the tested oocytes. Tetrodotoxin (TTX) was used to test for specificity of the channel, and blocked both peak and tail currents. Accumulation of DLT (0.1 and 1.0 μM) after a 1 h exposure, in both injected and non-injected oocytes, was determined using liquid scintillation counting. There was no significant difference in DLT uptake between VSSC-injected oocytes and non-injected controls, although the transfected oocytes consistently had less accumulation. In 0.1 μM incubation solution, non-injected oocytes accumulated 0.162 ± 0.076 μg [^{3}H]-DLT, water injected oocytes accumulated 0.110 ± 0.062 μg, and transfected oocytes accumulated 0.088 ± 0.044 μg. In 1.0 μM incubation solution, non-injected oocytes accumulated 1.04 ± 0.409 μg, water injected oocytes accumulated 0.914 ± 0.447 μg, and transfected oocytes accumulated 0.804 ± 0.287 μg. The results suggest that deltamethrin disrupts VSSCs and demonstrate that the presence of the channel did not cause greater accumulation. In fact, less accumulation may take place. Keywords: Sodium channel, Deltamethrin, *Xenopus* oocyte. *This abstract does not reflect EPA policy.*

P-114 ***Pre-Doctoral Student (Group 3)***

DELTAMETHRIN INDUCED ALTERATIONS IN THE TRANSCRIPTION OF CALCIUM RESPONSIVE AND IMMEDIATE EARLY GENES IN VIVO. JA Harrill[1], KM Crofton[2], *[1]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC, USA; [2] Neurotoxicology Division, NHEERL, ORD, USEPA, RTP, NC, USA*

Pyrethroid insecticides disrupt nervous system function by prolonging sodium current through voltage-sensitive sodium channels (VSSCs) following nerve depolarization. Pyrethroid effects on nervous system tissue have been investigated in terms of in vitro molecular and cellular electrophysiology and on a whole-organism level through various behavioral tests. However, a critical data gap exists concerning transcriptional and translational alterations that occur in an intact nervous system following pyrethroid exposure in vivo. A set of transcriptional markers was identified for a representative pyrethroid, deltamethrin (DLT), through the use of high-density oligonucleotide microarrays. Three doses corresponding to one-third below a NAOEL (0.3 mg/kg), a NAOEL (1.0 mg/kg), and an ED_{30} (3.0 mg/kg) for motor activity depression were administered by oral gavage and tissue was collected at 6 h. cDNA generated from frontal cortex was hydridized to Affymetrix 230 2.0 GeneChip® arrays. The set of candidate markers identified includes transcripts that code for calcium-responsive proteins such as calcium-calmodulin dependent protein kinase 1γ (CamkIγ), calcium-regulated heat-stable protein 1 (Carhsp1)

and protein tyrosine kinase 2B (Ptk2b) as well as immediate early genes such as activity regulated cytoskeletal-associated protein (Arc), FBJ murine osteosarcoma viral oncogene homolog (c-fos) and immediate early gene transcription factor NGFI-B (Nr4a1). qRT-PCR analysis of a replicate set of animals confirms that Camk1γ transcript increases linearly as a function of administered dose of DLT ($p = 0.02$). Future studies include examination of candidate markers at the translational and post-translational level as well as expansion of validated markers to other members of the pyrethroid insecticide class. Keywords: Pyrethroids, Dose–response, Microarray

P-115

ADULT AND JUVENILLE RAT SODIUM CHANNEL (NAV1.2 AND NAV1.3) SENSITIVITY TO THE PYRETHROID INSECTICIDE DELTAMETHRIN. C.A. Meacham[1], P.D. Brodfuehrer[2], A.S. Bale[1], J. Watkins[3], K.M. Crofton[1], T.J. Shafer[1], *[1]Neurotoxicology Div., NHEERL, ORD, U.S. EPA, Res. Tri. Park, NC, USA; [2]Biol. Dept., Bryn Mawr Col., Bryn Mawr, PA, USA; [3]North Carolina State University, Raleigh, NC, USA*

Adult rats are less sensitive than juveniles to the acute neurotoxicity of the Type II pyrethroid insecticide deltamethrin (DLT). Voltage-sensitive sodium channels (VSSCs) are the primary target of DLT and are differentially expressed during development, with expression of Nav1.2 predominating in the adult CNS, and Nav1.3 predominating in the embryo and juvenile animal. To investigate the hypothesis that pharmacodynamic differences contribute to the differential sensitivity of young animals to DLT, effects of DLT on Nav1.2 and Nav1.3 were compared following expression of these channels in *Xenopus laevis* oocytes. Two electrode voltage clamp recordings demonstrated expression of VSSCs in oocytes injected with either Nav1.2 or Nav1.3 mRNA in the absence or presence of β_1 subunit mRNA. Tetrodotoxin (1 μM) demonstrated channel specificity by blocking peak and tail current through both channels. DLT increased tail currents in both Nav1.2 and Nav1.3-injected oocytes in a concentration-dependent manner, with 0.1 and 1.0 μM DLT significantly increasing tail current amplitude after 2 min of exposure. DLT (0.1 and 1 μM) increased tail current by 51.21 ± 5.82 and 134.15 ± 14.09 ($n = 18$) percent over control respectively in oocytes expressing Nav1.2/β_1; by 76.38 ± 11.81 ($n = 19$) and 106.36 ± 8.99 ($n = 15$) in Nav1.3/β_1; and by 42.07 ± 13.87 ($n = 6$) and 71.79 ± 11.47 ($n = 6$) in Nav1.3/Noβ_1 (without β_1). These results demonstrate that both Nav1.2 and Nav1.3 are sensitive to DLT; and VSSCs may be less sensitive to pyrethroids without co-expression with β subunits. These results may partially account for the differential sensitivity of juvenile and adult animals to the acute neurotoxicity of DLT. *(This work was supported by the U.S. EPA and does not necessarily reflect EPA policy.)*

P-116 ***Pre-Doctoral Student (Group 3)***

POLYCHLORINATED BIPHENYLS EXERT SELECTIVE EFFECTS ON WHITE MATTER COMPOSITION IN A MANNER INCONSISTENT WITH HYPOTHYROIDISM. David S. Sharlin, R. Thomas Zoeller, *Morrill Science Center/Biology Department, University of Massachussetts at Amherst, USA*

Recent evidence in children suggests that PCB neurotoxicity is associated with changes in white matter development. We have tested the hypothesis that PCB exposure affects the development of white matter tracts by interfering with thyroid hormone signaling. Pregnant Sprague–Dawley rats were exposed to the PCB mixture, Aroclor 1254 (5 mg/kg) with or without co-treatment of a combination of methimazole and perchlorate from gestational day-7 until postnatal day-15. Treatment effects on white matter development were determined by measuring the cellular density and relative proportion of mature oligodendrocytes (myelin-associated glycoprotein-positive) and astrocytes (glial acidic fibrillary protein-positive) in the genu of the corpus callosum (CC) and in the anterior commissure (AC). As expected, both goitrogen treatment and PCB exposure significantly reduced circulating levels of TH. Hypothyroidism induced an overall reduction in cellular density with a disproportionate decrease in the cellular density of oligodendrocytes and a simultaneous increase in the density of astrocytes. In contrast, PCB exposure significantly reduced total cell density, but did not disproportionately alter oligodendrocyte density, or change the ratio of oligodendrocytes to astrocytes. These data indicate that PCB exposure selectively reduces the size and cellular density of the anterior commissure and genu of the corpus callosum during development, but that these effects are not completely attributable to PCB-induced TH insufficiency. Keywords: Polychlorinated biphenyls, Hypothyroidism, White matter. *This work was supported in part by a STAR EPA Graduate Fellowship FP916424 (to D.S.S), a National Institutes of Health Grant ES10026 (to R.T.Z), and an EPA STAR Grant RD-3213701-0 (to R.T.Z.).*

P-117 ***Pre-Doctoral Student (Group 3)***

AGE-RELATED DIFFERENCES OF ACETYLCHOLINESTER-ASE INHIBITION FROM TWELVE ORGANOPHOSPHATE INSECTICIDES. Edward C. Meek, Howard Chambers, Alper Coban, Benjamin E. Hurley, Jay Pittman, Kristin R. White, Janice E. Chambers, *Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State, MS, USA*

The organophosphate insecticides which result in acetylcholinesterase (AChE) inhibition display a wide range of acute toxicity levels. The greater sensitivity of young animals to the acute toxicity of many organophosphate insecticides is most likely due to the diversity of their chemistries yielding differences in both anticholinesterase potency and metabolism. The organophosphates chosen for this study were the active

metabolites (oxons) of commercial insecticides or model compounds. The OPs were either diethyl or dimethyl phosphates and had a variety of ''leaving groups''. The ability of these compounds to inhibit AChE was investigated using rat tissues at ages 70 (adult), 12 (juvenile), and 1 (neonate) days of age, using a spectrophotometric assay. The tissues studied included: brain, lung, heart, skeletal muscle, and serum. The inhibitory concentration 50 (IC50) was determined for each of the 12 compounds to compare the relative potencies. Four dimethyl and eight diethyl compounds were used. The IC50s of the 12 compounds ranged from 2 nM to 1.5 μM and varied amongst tissues. The differences in the potencies of the 12 OP compounds studied varied with differences in the chemistries of the OPs with respect to age. Keywords: Organophosphate, Acetylcholinesterase, Pesticide. *(Supported by NIH ES R01 ES 11287.)*

P-118 ***Pre-Doctoral Student (Group 3)***

MATERNAL DDT CONCENTRATIONS AND SEX RATIO OF OFFSPRING. TA Jusko[1], PA Shaw[2], TA Greenfield[3], MJ Charles[4], I Hertz-Picciotto[3], *[1]Department of Epidemiology, University of Washington, Seattle, WA, USA; [2]Department of Biostatistics, University of Washington, Seattle, WA, USA; [3]Division of Epidemiology, University of California-Davis, Davis, CA, USA; [4]Department of Environmental Toxicology, University of California-Davis, Davis, CA, USA*

Alterations in the sex ratio of offspring have been noted experimentally in relation to several endocrine-disrupting chemicals, where treatment with estrogenic and anti-androgenic compounds increases the ratio of female to male births. However, few studies have investigated this possible association in humans, particularly in relation to DDT exposure. Furthermore, results from human studies are mixed with regard to an excess of female births. To determine whether in utero DDT exposure alters the sex ratio of offspring, we analyzed data from mothers and children who participated in the Child Health and Development Study (CHDS). CHDS was a cohort study of 20,754 women and their pregnancies conducted in the San Francisco Bay area during the 1960s when DDT use peaked in the United States. From the CHDS, we sampled 399 women who gave birth between 1964 and 1967, had an adequate serum sample from their second or third trimester of pregnancy, and met additional eligibility criteria. We determined *p*,*p*′-DDE, *o*,*p*′-DDT, and *p*,*p*′-DDT concentrations from stored sera; all organochlorine levels were adjusted for maternal lipid concentrations. To quantify the association between maternal DDT concentrations and sex ratio of offspring, we modeled quartiles of *p*,*p*′-DDE, *o*,*p*′-DDT, and *p*,*p*′-DDT concentrations in relation to male birth. Overall, estimates were imprecise and did not exhibit clear dose–response relations. For example, the odds of male birth decreased across quartiles of *p*,*p*′-DDE concentration, but not consistently (ORs = 1.0, 0.5, 0.8, 0.6; trend $p = 0.37$) and all confidence intervals included 1.0. Despite relatively high maternal exposures to DDT in the CHDS sample, DDT compounds did not appear to affect the sex ratio of offspring. Keywords: Endocrine disruptor, Sex ratio, Organochlorine

P-119 ***Pre-Doctoral Student (Group 3)***

EXPOSURE TO MIXTURES OF ENDOSULFAN AND ZINEB INDUCES APOPTOTIC CELL DEATH IN NEURONAL CELLS (SH-SY5Y), IN VITRO. Z Jia[1], HP Misra[1,2], *[1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA; [2]Edward Via Virginia College of Osteopathic Medicine, Blacksburg, VA, USA*

A number of epidemiological studies have demonstrated a strong association between the incidence of Parkinson's disease and factors that increase the likelihood of exposure to pesticides. Our earlier data indicated that exposure to endosulfan and zineb could result in neurodegeneration in vivo. We hypothesized that these pesticides cause neurotoxicity, at least in part, by enhancing apoptotic cell death. SH-SY5Y human neuroblastoma cells were exposed to endosulfan, zineb or mixtures of these chemicals, in vitro. Cytotoxicity was evaluated using lactate dehydrogenase release. A dose-dependent response was obtained by exposing to individual pesticides. Exposure to mixture of pesticides (endosulfan 150 μM + zineb150 μM) exhibited significantly ($p \leq 0.05$) higher toxicity, more than additive effect, in this assay. A flow cytometric (7-aminoactinomycin D) assay was used to distinguish live, early apoptotic and late apoptotic/necrotic populations. Both pesticides were found to cause apoptotic cell death that was both concentration (50–400 μM) and time (4–16 h) dependent. For mixture studies, concentrations of pesticides (100 μM each) were chosen based on LC_{25} (lethal concentration) that would result in minimum cell death. Exposure to mixtures of pesticides enhanced both the early apoptosis and late apoptosis/necrosis compared to either chemical alone. Visual evaluation using DNA ladder assay and fluorescence Annexin V/PI assay confirmed the contribution of both apoptotic and necrotic processes. These findings suggest that the neurotoxicity of endosulfan and zineb, both individually and in mixtures, is associated with the occurrence of early and late apoptotic/necrotic processes. Keywords: Pesticides, Apoptosis, Neurotoxicity

P-120

ALTERED GENE EXPRESSION AND GROWTH RESTRICTION IN FETAL BRAIN FOLLOWING EXPOSURE TO THE WATER DISINFECTANT BYPRODUCT (DBP); CHLOROACETONITRILE (CAN). A E Ahmed, S Jacob, T Wood, H Fouad, *Dept of Pathology and Molecular Genomics Facility, University of Texas Medical Branch, Galveston, TX, USA*

Disruption of fetal development results in reduced cellular proliferation and differentiation leading to various forms of APO. Reports indicate an association between DBP ingestion and increased susceptibility to APO; skeletal defects, intra

uterine growth retardation and CNS anomalies. Correlation between spontaneous abortion and consumption of more than five glasses of chlorinated tap water has been demonstrated. Our objective is to examine the mechanism by which DBP-induce APO, using altered brain development as a predisposing factor. Pregnant mice were treated at gestation day 6 (GD6) until GD18 with a daily oral dose of the DBP; CAN (25 mg/kg). Animals were then anesthetized and fetuses were collected and weighed. Fetal brains were extracted, weighed and total RNA was isolated. cDNA synthesis was performed and biotin labeled target cRNA was prepared. Target cRNA for each mouse sample was hybridized on a mouse genome chip. Hybridized biotin labeled cRNA was quantitated using Gene Array Scanner and Affymetrix Analysis software. Genes with two-fold change (up or down-regulated) in hybridized target intensity were identified. The results indicated that CAN treatment caused a significant decrease in total fetal weight (78% of control) and in fetal brain weight (80% of control). Concordance analysis identified 39 genes with unidirectional (increase/decrease) change. Five genes demonstrated an increased RNA expression and 34 genes showed decreased level of expression as compared to control. Thirteen of the down-regulated genes belonged to the crystallin gene family. Four-fold decrease was indicated in parathyroid hormone related peptide (PTHrP) and cartilage matrix protein genes. RT-PCR analysis indicated that mRNA levels of PTHrP were significantly lower in treated mouse brain. In conclusion, as PTHrP regulates fetal development, down regulation of PTHrP and matrilin genes may be a factor in the observed decrease in fetal weight. Future studies will explore, in detail, the molecular cascades of DBP-induced APO. Keywords: Gene expression, Fetal brain development, Environmental insults

P-121 ***Pre-Doctoral Student (Group 4)***

DEVELOPMENTAL EFFECTS OF ETHANOL IN THE JAPANESE MEDAKA FISH (*Oryzias latipes*): WINDOWS OF VULNERABILITY. S. Oxendine[1,2], D.E. Hinton[3], J. Cowden[1], S. Padilla[1], *[1]Neurotox. Div.,U.S. EPA, RTP, NC, USA; [2]Curr. in Toxicol., UNC-CH, Chapel Hill, and [3]Nicholas School of the Environ., Duke Univ., Durham, NC, USA*

Developmental EtOH is a well-known mammalian toxicant that produces a range of phenotypes including craniofacial abnormalities and growth retardation. While the toxic potential of EtOH exposure is well characterized clinically, questions regarding factors that influence toxicity, such as the timing of exposure, remain unanswered. In this study, fish embryos were exposed to EtOH during specific stages of development to assess temporal variations in EtOH toxicity. Fertilized medaka eggs were collected at the 64-cell stage and incubated during early, middle or late gestation (e.g., 0–3, 3–6 or 6–9 days post fertilization) with various, sublethal concentrations of EtOH (0.1, 0.5 or 1%) ($n = 20$ per dose group). [Because long term (8 days) treatment with 2% EtOH produced significant lethality and malformations, 1% EtOH was chosen as the highest dose in the above studies.] Viable embryos were photographed on the day of hatching (control embryos normally hatch by day 10) and linear measures of outer eye distance and total body length were used to investigate the effects of developmental EtOH. EtOH decreased eye width and total body length at all concentrations; however, the window of exposure only affected hatching and body length. Dose-related hatching delays and growth inhibition were consistently observed in treated embryos (e.g., approximately 45% of controls hatched out by day 9, whereas only 17% of embryos treated with 1% EtOH hatched by that time). Delays in hatching were most pronounced when exposures occurred early in development. The incidence of EtOH-induced growth inhibition, however, appeared to be most pronounced when exposures occurred late in development (e.g., 1% EtOH only decreased total body length by 8.8% if exposure occurred on days 0–3, but that decrement doubled to 17.6% when exposure occurred on days 6–9). The observed temporal variations in EtOH-induced growth inhibition may be due to stage-specific pharmacokinetic differences in EtOH uptake, as the EtOH target dose was significantly higher in embryos treated late in development. These data suggest that critical periods for heightened sensitivity to developmental EtOH may vary according to the endpoint used to assess toxicity. *This is an abstract of a proposed presentation and does not imply Agency policy.*

P-122 ***Pre-Doctoral Student (Group 4)***

INDUCTION OF C-FOS AND BEHAVIORAL ASSESSMENT IN C57BL/6J AFTER TREATMENT WITH CUPRIZONE. Urbach D., Kusnecov A.W., *Joint Graduate Program in Toxicology, Rutgers University and University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA*

Demyelinating neuropathology is associated with a number of diseases, most commonly multiple sclerosis (MS), a suspected autoimmune disease that in various stages of severity can impair motor, cognitive and emotional functions. Relapses in MS have been associated with exposure to stressful life events (Ackerman et al., Psychosom Med. 2002;64(6):916–20), but the degree to which a demyelinated neuropathological state influences differential neuroanatomical engagement and behavioral reactivity to psychogenic stressors has not been adequately addressed. In the present study, the copper-chelating agent, cuprizone, was used to produce CNS demyelination in male C57BL/6J mice. Subsequently, mice were exposed to a psychogenic stressor (open field [OF] exploration, together with investigation of a novel object). Animals were monitored for various behavioral parameters indicative of altered emotionality, and following the behavioral test were sacrificed for CNS quantification of the immediate early gene *c-fos*. Exposure of non-cuprizone treated animals to the OF significantly elevated the number of c-Fos immunoreactive cells in the hippocampus, thalamus, hypothalamus and amygdala. Similarly, cuprizone-treated animals displayed significant elevations in c-Fos,

although in some regions (e.g., PVN of the hypothalamus, and dentate gyrus of the hippocampus), there appeared to be a blunted response. Assessment of behavior in the OF revealed no major impairment in motor activity due to cuprizone treatment, although cuprizone-treated animals spent less time in the center of the OF, a possible indication of heightened anxiety. These results reveal that after exposure to a demyelinating neurotoxicant, excitation of specific brain regions involved in learning and stress-reactivity may be blunted. Moreover, demyelination may increase the anxiety response to a novel environment. Keywords: Neurotoxicology, Immunology, Behavior. *Support Contributed By: Grants MH60706, DA141186, NIEHS P30 ES05022 and NIEHS Graduate Training grant.*

P-123

BEHAVIORAL EFFECTS OF DIRECT EXPOSURE OF CNS TO HYPER-IL-6 IN THE PERINATAL CD-1 MOUSE. S.H. Brunssen[1*], S.S. Moy[1], G.J. Harry[2], *[1]University of North Carolina, Chapel Hill, NC, USA; [2]NIEHS, NIH, DHHS, RTP, NC, USA*

Early inflammatory insults to the human fetal/premature brain were modeled by direct sub-cranial injection of a biologically active form of interleukin-6 into CD-1 mice at post-natal day (P)4. Randomly reconstituted litters ($n = 12$) of 10 male mice were exposed to 5 ng or 10 ng/μL hyper-IL-6 or saline to verify the emergence of a previously identified hyper-reactive behavioral phenotype during development and to test for its persistence in adults. One animal per group per litter was tested at P8, 12, and 16 with a modified Fox Screen. Additionally, one animal was screened either between P21 and 24 or in adulthood on a battery of neurophysiologic, sensory, motor, cognitive, and emotional performance tests. The hyper-reactive phenotype was identifiable by P8, replicating effects seen in prior experiments. Neither physical development nor sensori-motor functions were altered by treatment. In contrast, mature adults showed no systematic differences in sensory, motor, activity, balance, anxiety, spatial navigation, or cognitive measures. Behavioral results support the previous finding that early postnatal inflammatory exposure may alter responses to stimulation and attention processes in young males, but suggest that these subtle behavioral alterations are modifiable during adulthood. Keywords: Neuroinflammation, Development, Behavior

P-124 ***Pre-Doctoral Student (Group 4)***

POTENTIATING EFFECT OF THE K_{ATP}^+ CHANNEL BLOCKER GLIBENCLAMIDE ON THE NEUROTOXICITY OF MITOCHONDRIAL COMPLEX I INHIBITORS. J Kou, JR Bloomquist, *Neurotoxicology Laboratory, Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA*

Previous studies have demonstrated a deficiency in mitochondrial function in Parkinson's disease (PD), particularly in the activity of complex I. To determine the effect of mitochondrial inhibitors on dopamine depletion, we measured their ability to release preloaded dopamine from murine striatal synaptosomes. Mitochondrial inhibitors of complexes I (rotenone, MPP^+, and HPP^+), II (hydramethylnon), IV (Na cyanide), and an uncoupler (dinoseb) were tested. All tested compounds were potent dopamine releasers, with EC_{50} values in the micromolar or nanomolar range, and this effect was calcium-dependent. The striatum also contains a significant density of ATP-dependent potassium channels, which are thought to play a protective role during ATP decline. We found that blockage of these channels with glibenclamide only potentiated the dopamine release by complex I inhibitors, but not inhibitors acting on the other sites. The selective potentiating effect of glibenclamide on the toxicity of MPTP was also observed in vivo using C57 mice. Western blots of striatal dopamine transporter (DAT) and tyrosine hydroxylase (TH) proteins demonstrated that 30 mg/kg of glibenclamide alone did not affect the expression of DAT and TH after 2 weeks of daily treatments, but it significantly enhanced the reduction of DAT and TH expression caused by a single dose of 20 mg/kg of MPTP. Treatment of mice with hydramethylnon or dinoseb alone, or in conjunction with glibenclamide did not alter the expression of DAT and TH. The mechanism underlying the selectivity of glibenclamide is uncertain. Analysis of ATP titers in treated synaptosomes did not support a correlation between mitochondrial inhibition and K_{ATP}^+ channel activation. However, greater amounts reactive oxygen species (ROS) generated by complex I inhibitors might be a contributory factor. After incubating striatal synaptosomes with mitochondrial blockers, malondialdehyde (MDA) level was measured using a TBARS assay as an indicator of lipid peroxidation. Both rotenone and MPP^+ enhanced MDA production by 20% compared to control, but this effect was not seen in tissue treated with either hydromethylnon or dinoseb. Overall, these findings suggest that K_{ATP}^+ channels are activated by ROS, and that co-exposure to mitochondrial complex I inhibitors and sulfonylureas such as glibenclamide or a genetic defect in K_{ATP}^+ channel function, may increase neurotoxicity and play a role in the development of Parkinson's disease. Keywords: Parkinson's disease, Dopamine transporter, Tyrosine hydroxylase

P-125

THE NEW APPROACH FOR THE EFFECTS OF THE TOXIC CHEMICAL EXPOSURE ON THE PROLIFERATION OF EMBRYONIC STEM CELLS IN THE DEVELOPMENTAL NEUROTOXICITY STUDY. M Kuwagata, T Ogawa, S. Shioda, *Department of Anatomy I, Showa University School of Medicine, Tokyo, Japan*

While the current developmental neurotoxicity guideline focuses on the postnatal behavior, some problems still remain in respect of the reliability and reproducibility, so we are investigating a new endpoint focusing on the fetal brain. The proliferation of the neural stem cells can be one of the targets for toxic agents initially. To evaluate the proliferation of cells, 5-bromo-2′-deoxyuridine (BrdU) has been widely used in the neuroscience field. However, we have previously reported that

BrdU induced apoptosis in the fetal brain. Here, we examined the expression of phosphorylated H3 protein, which phosphorylated in the response to mitogenic stimulus, as a new proliferation marker for the neural stem cells. We exposed C57BL pregnant mice to BrdU once, and confirmed the BrdU-induced toxicity in the fetal brain 24 h after exposure. The number of H3 positive cells on the ventricular surface of several brain areas was counted and their distribution analyzed morphometrically. In the control embryos, the distribution of H3 positive cells showed a variety of patterns in the fetal brain areas, suggesting that the embryonic neural stem cell mitosis does not occur uniformly. BrdU significantly decreased the number of H3 positive cells in the neocortex, and induced apoptosis severely, compared to those of the controls. In the cerebellum, where apoptosis was not induced, the H3 immunoreactivity was similar to that of the controls, indicating that BrdU inhibited embryonic stem cell mitosis. These results suggest that evaluation of neural stem cell proliferation by H3 immuno-histological observation can be useful to detect the inhibition of neural stem cells in developmental neurotoxicity studies. Keywords: Developmentalneurotoxicity study (DNT), Phosphorylated histone 3 ICH, Neural stem cell. *(Supported partly by a grant of Long-range Research Initiative (LRI) by Japan Chemical Industry Association (JCIA).)*

P-126 ***Pre-Doctoral Student (Group 4)***

HUMAN ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES ARE INHIBITED BY TRICHLOROETHYLENE (TCE). R Giddings[1], CA Meacham[2], AS Bale[2], PJ Bushnell[2], TJ Shafer[2], *[1]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC, USA; [2]Neurotoxicology Division, NHEERL, ORD, US EPA, RTP, NC, USA*

Trichloroethylene (TCE) is a volatile organic solvent (VOC) that is used as a metal degreasing agent and in paints and glue. In addition to being a commonly abused inhalant, run-off from hazardous waste sites contain enough TCE and other VOCs to contaminate ground water and nearby water supplies. As with other VOCs, TCE produces neurobehavioral effects such as euphoria, motor incoordination, visual deficits, and memory loss. Studies with VOCs such as alkylbenzenes and 1,1,1-trichloroethane have correlated these neurobehavioral changes to modifications in brain receptor function. Specifically, the α-7 nicotinic acetylcholine receptor (nAChR), which is linked to cognition and memory, has been shown to be inhibited by two other VOCs, toluene (TOL) and perchloroethylene (PERC). Based on the similar properties of these compounds to TCE, it was hypothesized that TCE would act similarly. *Xenopus laevis* oocytes were injected with human α-7 subunits and the current across the membrane was measured using two electrode voltage clamp. Human α-7 nAChR inhibition by TCE was rapid, reversible, and concentration-dependent. After a 30 s pretreatment, at 0.25 mM [TCE], α-7 nAChRs were inhibited by 25.7% ± 6.2% (n = 5) and at 5 mM, by 77.1% ± 3.29% (n = 3), and after a 2 min washout, receptor function returned to normal. This initial data shows that TCE inhibits the α-7 nAChR, and, like TOL and PERC, acts on the central nervous system to inhibit normal neurological function and potentially damage memory and cognition functions by directly modulating nAChRs. This abstract does not reflect EPA policy. Keywords: Nicotinic acetylcholine receptor, Trichloroethylene, *Xenopus* oocyte

P-127

CYTOKINE RECEPTOR EXPRESSION AND GLIAL CONTACT FOLLOWING ACUTE HIPPOCAMPAL INJURY. Robert N. Wine[1], Christian Lefebvre d'Hellencourt[2], Christopher A. McPherson[1], G. Jean Harry[1], *[1]Laboratory of Neurobiology, NIEHS, NIH, DHHS, Research Triangle Park, NC, USA; [2]Universite de La Reunion, Reunion-France-DOM*

Two factors influencing neuronal fate following acute brain injury are the cellular response to a local inflammatory environment and contact with glial cells. In this study we induced selective apoptotic cell death in hippocampal dentate granule cells by administering a single dose of trimethyl tin (TMT) to young, male, CD-1 mice. Using widefield epifluorescence microscopy the relationship between neuronal cell death (active caspase 3) and survival was evaluated in the context of cytokine expression and glial contact. Neurons were identified using a fluorescent Nissl stain which redistributes in injured or regenerating neurons, providing an additional marker of the physiological state of neurons. The expression of tumor necrosis factor receptors p55 (TNFR1) and p75 (TNFR2), and the glial markers GFAP (astrocytes) and isolectin B4 (microglia) were evaluated by immunofluorescence. In normal tissue the cellular localization of TNFR1 appeared as punctate foci distributed in both the perinuclear cytoplasm and nucleus. Six hours after dosing with TMT dying neurons showed a normal cell morphology but were positive for both active caspase 3 and TNFR1. At this time neuronal TNFR1 staining became predominantly nuclear with microglia occasionally found in close proximity, yet direct contact was not evident. As neuronal degeneration in the dentate proceeded to its peak at 48 h after dosing, the distribution of TNFR1 in dying neurons became primarily cytoplasmic and these cells were often in direct contact with microglia. Seventy-two hours into the TMT lesion TNFR1 (+) neurons possessed condensed chromatin, often appeared in clusters, and were being phagocytized by microglia. In contrast, a distinctly different pattern was seen in neurons expressing TNFR2. These neurons showed a lack of co-localization with active caspase 3, maintained contact with astrocytes, and had no microglia present. Our observations demonstrate a temporal correlation between the expression of TNFR1, TNFR2, the presence of microglia, and cell death in dentate granule cell neurons. Quantitation of TNFR1 protein expression, together with imaging of direct glial contacts, should further our understanding of the interaction between these factors in

determining neuronal fate following exposure to TMT. Keywords: Glial contact, Cytokines, Neurodegeneration

P-128 ***Pre-Doctoral Student (Group 4)***

ANIMAL MODEL OF AUTISM USING En2$^{-/-}$ MICE. MA Cheh[1], JH Millonig[2], E Jacobsen[3], X Ming[4], GC Wagner[1,3*], *[1]Department of Neuroscience, Rutgers University, New Brunswick, NJ, USA; [2]Center for Advanced Biotechnology and Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA; [3]Department of Psychology, Rutgers University, New Brunswick, NJ, USA; [4]Department of Neurosciences, UMDNJ-New Jersey Medical School, Newark, NJ, USA*

Autism spectrum disorder (ASD) is a prevalent psychiatric condition that has a neurodevelopmental and genetic basis. Previous family based linkage disequilibrium methods have demonstrated that the homeobox transcription factor, *ENGRAILED 2* (*EN2*), is significantly associated with ASD, indicating that *EN2* may be involved in increased genetic susceptibility to ASD. To investigate whether *En2* knockout (*En2*$^{-/-}$) mice displayed autistic-like behavior, a battery of tests was performed that our lab has developed to detect ASD-associated deficits. Results revealed deficits in motor, learning, and social development of *En2*$^{-/-}$ mice compared to wild-type mice. Specifically, *En2*$^{-/-}$ mice were impaired in mid-air righting and hanging wire grip strength and were hyperactive in the open-field in early postnatal life. They displayed a spatial memory impairment in acquisition and retention of the Morris water maze in early life and in the modified open-field with objects as adults. *En2*$^{-/-}$ mice also displayed marked deficits in play behavior, social sniffing and social grooming. Additionally, these mice were administered pentelyenetetrazole (PTZ), a seizure-inducing agent, to determine whether *En2*$^{-/-}$ mice would display altered sensitivity to toxicant exposure compared to controls. Results revealed a shorter latency to seizure onset and a longer duration of seizure in *En2*$^{-/-}$ mice, with knockout males being more sensitive than females. Finally, neurochemical analysis was performed and revealed significant region-specific changes in monoamines that could account for some of the behavioral changes observed in these mice. We conclude that *En2*$^{-/-}$ mice represent a unique animal model of ASD that reflects the genetic and behavioral changes associated with the disorder, and may be useful in identifying toxicants that interact with this susceptibility gene to exacerbate behavioral disruption. Keywords: Animal model, Autism, Engrailed-2

Acknowledgements: NS43981, ES05022, ES11256, EPAR829391, NJ Governor's Council on Autism, Johnson & Johnson, Busch Biomedical Research Grant, National Alliance for Autism Research, Cure Autism Now.

P-129

THE EFFECTS OF LIPOPOLYSACHARIDE INJECTION ON BAX AND BCL2, REGULATORS OF APOPTOSIS, IN NEURAL TISSUE OF NEWBORN MICE. David F Sorrentino, MD[1], Alexander Kusnecov, Ph.D.[2], *[1]UMDNJ, New Brunswick, NJ 08901, United States; [2]Psychology, Rutgers University, Piscataway, NJ, United States*

Newborn sepsis is associated with poor neurologic outcomes and increased rates of periventricular leukomalacia. The inflammatory mediators present in sepsis have been shown to cause spontaneous neurodegeneration. This may occur through intrinsic apoptotic pathways that are regulated by the Bcl-2 family of proteins. Two regulators in this family of proteins are Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) which inhibits Bax activity. The present study tests the hypothesis that exposure to LPS alters the mRNA and protein content of Bax and Bcl-2 in neural tissue in newborn mice. Five-day-old mice were injected intraperitoneally with LPS, to simulate sepsis, or saline. 6 h later they were sacrificed and brain tissue was harvested for protein and RNA isolation. Bax and Bcl-2 mRNA was quantified using Q-RT-PCR. Protein content of Bax and Bcl-2 was quantified by western blot analysis. There was no statistically significant difference in mRNA content after LPS treatment vs. control for Bax (mean: 3.34 versus 4.59, $p = 0.063$) or Bcl-2 (mean: 4.18 versus 4.21, $p = 0.75$). There was a significantly altered ratio of Bax to Bcl-2 mRNA in LPS treated mice vs. control (mean: 1.25 versus 0.77, $p = 0.048$). The alteration was likely due to increased Bax mRNA as this approached significance while Bcl-2 production was essentially unchanged. We conclude that, 6 h after LPS exposure, there are altered ratios of mRNA for Bax and Bcl-2, leading to a predominance of anti-apoptotic mechanisms but no alterations in existing protein in neural tissue in newborn mice. We speculate that this is an early response to prevent apoptosis in neural tissue of newborns after infectious insult. Keywords: Sepsis, Apoptosis, Lipopolysaccharide

P-130 ***Pre-Doctoral Student (Group 4)***

PROTEASOMAL INHIBITOR MG-132 INDUCES DOPAMINERGIC DEGENERATION IN CELL CULTURE AND ANIMAL MODELS. Faneng Sun, Calivarathan Latchoumycandane, Danhui Zhang, Vellareddy Anantharam, Arthi Kanthasamy, Anumantha Kanthasamy, *Parkinson's Disorder Research Laboratory, Dept. of Biomedical Sciences, Iowa State University, USA*

Dysfunction of the Ubiquitin-Proteasome System (UPS) is implicated in the pathogenesis of Parkinson's disease (PD). To investigate the effect of proteasome dysfunction on dopaminergic degeneration, we systematically examined the neurotoxicity of MG-132, a reversible proteasome inhibitor, in a dopaminergic neuronal cell line (N27 cells) and in animal models. Exposure to 5 μM MG-132 resulted in the time-dependent mitochondrial depolarization and mitochondrial

release of proapoptotic proteins, cytochrome *C* and Smac. The functional consequence of the mitochondrial release of apoptotic factors was demonstrated by the significant activation of caspase-9 and -3 and by PKCδ proteolytic cleavage, indicating activation of the intrinsic apoptotic pathway. No obvious ROS generation was observed in N27 cells exposed to MG-132, and pretreatment with the SOD mimetic MnTBAP showed no effect on MG-132-induced caspase-3 activation. Thus, proteasome inhibition appears to trigger the mitochondrial-mediated apoptotic cascade independent of ROS generation in this dopaminergic neuronal cell model. To further examine the neurotoxic effect of MG-132 on the nigral dopaminergic system in animal models, we stereotaxically injected MG-132 in the substantia nigra and then examined for neurochemical and histological changes. MG-132 (one dose, 0.1 μg in 4 μL) was injected into the right substantia nigra compacta (SNc) in C57 black mice, and vehicle injected in the left sides served as the control. Neurochemical analysis showed significant depletion of striatal dopamine and its metabolite DOPAC in the MG-132-injected SNc as compared to the vehicle-injected SNc. Also, we observed a dramatic decrease in the number of TH positive neurons in MG-132-injected substantia nigra compared to the vehicle-injected sides. These results suggest proteasomal inhibition by MG-132 induces mitochondrial-mediated apoptotic cell death, which possibly contributes to neurochemical depletion and nigral dopaminergic degeneration in both cell culture and animal models. Together, our results demonstrate that proteasomal dysfunction can induce nigral dopaminergic degeneration similar to that in Parkinson's disease. Keywords: Proteasome inhibitor, Apoptosis, Parkinson's disease

P-131

HYPERTENSIVE AND TACHYCARDIC RESPONSES TO ORAL TOLUENE IN THE RAT. Gordon, C.J., Oshiro, W., Samsam, T., Becker, P., Mack, C., Bushnell, P., *Neurotoxicology Division, National Health Effects and Environmental Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA*

Little is known regarding the effects of toluene and other volatile organic compounds on autonomic processes. Such studies should be performed in unrestrained and undisturbed animals to avoid the effects of handling stress on processes regulated by the autonomic nervous system. This study determined the effects of oral toluene on blood pressure (BP), heart rate (HR), core temperature (T_c), and motor activity (MA) in unrestrained rats. Male, Long-Evans rats were surgically implanted with radiotransmitters (Data Sciences, St. Paul, MN) to monitor BP, HR, T_c, and MA. Telemetry data were recorded continuously in undisturbed rats housed individually at an ambient temperature of 22 °C while allowed to drink and feed ad libitum. Toluene in a corn oil vehicle was administered by gavage at doses of 0, 0.4, 0.8, and 1.2 g/kg at 11:30 h while the telemetry data were monitored over 48 h. Dosing with corn oil led to transient elevations in BP, HR, T_c, and MA that were attributed to the stress of handling. All doses of toluene led to transient increases in BP and HR that exceeded the control response during the first 30–60 min after dosing. Toluene elicited elevations in BP and HR that persisted for several hours after dosing. BP was elevated by 20 mmHg over controls at 4 h after dosing with 1.2 g/kg toluene. HR remained elevated above controls by approximately 25 and 50 b/min in the 0.8 and 1.2 g/kg groups, respectively. T_c increased above controls for several hours after treatment with 1.2 g/kg. MA increased above control levels during the first hr after dosing. There was gradual recovery followed with a secondary rise in MA in rats dosed with 1.2 g/kg. Overall, toluene at oral doses of 0.4–1.2 g/kg affected the autonomic control of blood pressure and body temperature. The effects of oral dosing are likely to be comparable to that of inhalation exposures. The highest toluene dose of 1.2 g/kg induced a marked tachycardia, hypertension, hyperactivity, and mild hyperthermia. These autonomic responses are important for the development of PBPK models for toluene and other volatile organic compounds. Keywords: Toluene, Blood pressure, Temperature. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

P-132 ***Pre-Doctoral Student (Group 4)***

ASSOCIATION OF CELL CYCLE REGULATORY PROTEINS WITH CELL CYCLE EXIT AND DIFFERENTIATION IN MOUSE EMBRYONIC MIDBRAIN NEURONAL PRECURSOR CELLS. EJ Gribble, S Hong, XZ Yu, EM Faustman, *Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA*

Culturing and differentiating multipotent neuronal precursor cells is of great interest for the understanding of both normal and perturbed neuronal differentiation and the potential treatment of neurodegenerative diseases. Furthermore, thorough characterization of the behavior of these cells in culture should allow for the study of neurodevelopmental toxicant interference with the basic processes of neuronal proliferation, cell cycle exit, and differentiation. We have previously demonstrated alterations in cell cycle kinetics and cell cycle regulator expression following metal (As, Cd, and MeHg) exposure in mouse embryonic fibroblasts. As this same class of cell cycle regulatory proteins has been shown to facilitate developmental neuronal cell cycle exit and differentiation, we examined expression of p21, p27, and p53 protein concurrent with changes in proliferation and differentiation status in mouse embryonic midbrain neuronal precursor cells (NPCs) across 9 days in culture. Cellular proliferation remained high for several days in culture as indicated by cell counts, BrdU uptake, and the expression of proliferative markers ki-67 and PCNA. Neuronal differentiation, observed through

morphological changes, hematoxylin staining, Western blotting and immunocytochemical visualization of nestin, Sox11, GAP-43, and NF-L, steadily increased with time in culture, and was temporally associated with increases in p27 and p53 but not p21 protein expression. Three neuronal phenotypes were identified in our cultures via expression of tyrosine hydroxylase, tritiated GABA uptake, and acetylcholinesterase activity for dopaminergic, GABAergic, and cholinergic neurons respectively. Cultures were negative for GFAP, a marker for glial cells. This work presents a newly characterized model system in order to make cross-species comparisons of neurodevelopmental processes and toxicant susceptibility and to utilize transgenic mice to determine molecular mechanisms of neurodevelopmental toxicants on proliferation and differentiation. The p53 and p27 proteins appear to play a dual role in effecting normal physiological cell cycle exit and in the stress response in midbrain NPCs. Keywords: Neuronal precursor cells, Cell cycle, Differentiation. *This work was supported by the following sources: NIEHS grant 5 R01 ES10613, the Center for Child Environmental Health Risks Research through NIEHS grant P01ES09601 and EPA grant R 826886010, the Center for Ecogenetics and Environmental Health through NIEHS grant P30 ES07033, the Environmental Pathology Training grant (T32ES07032), and the NIC-HID, P30 HD02274*.

P-133 ***Pre-Doctoral Student (Group 4)***

RISKS ASSOCIATED WITH ATTENTION DEFICIT DISORDERS: ARE PARENTS OF CHILDREN WITH AD/HD MORE SUSCEPTIBLE TO ENVIRONMENTAL EXPOSURES THAN CONTROLS? LP Heilbrun, CS Miller, JL Perkins, *Department of Family and Community Medicine, University of Texas School of Medicine, San Antonio, TX, USA*

Recently, it has become evident that genes can affect an individual's ability to detoxify environmental chemicals and that the genes they inherit can serve as underlying risk factors for neurological impairment. Specific genetic polymorphisms for critical detoxification genes, including PON-1, CYP2D6, and NAT2, have been shown to underlie chemical intolerance-the inability to tolerate a wide variety of structurally unrelated chemicals. To date, no studies have examined the role of pre- and post-natal environmental exposure histories in the development of Attention Deficit Disorders (AD/HD), or the effect of parental vulnerability to chemical exposures on the likelihood that their offspring may develop AD/HD. Objectives: (1) to compare the prevalence of chemical intolerance in mothers of children with AD/HD with that of mothers whose children do not manifest AD/HD, (2) to determine if children born to parents with higher intolerance scores will have a higher prevalence of co-morbid mental, learning/behavioral, and motor coordination disorders than controls and (3) to identify potential risk factors for children with AD/HD. Approach: intolerance in mothers and the frequency of AD/HD in their children will be evaluated with an on-line survey using a secure website. The Quick Environmental Exposure and Sensitivity Inventory (QEESI©), a validated instrument, will be used to gather chemical intolerance scores for each mother. Participants will also complete a comprehensive environmental exposure history emphasizing potential neurotoxic exposures. The estimated time to complete the survey is 30–45 min. Cases will be mothers of children 5 years of age or older who have an AD/HD diagnosis. Mothers of children with AD/HD will be asked to identify and recruit another mother whose child is matched for age and sex to their child, but who does not have physician-diagnosed AD/HD. Researchers believe that environmental neurotoxins are one of the etiological factors responsible for AD/HD. It is, herefore, imperative that preventable risk factors are identified for future generations of susceptible children. Keywords: Attention deficit disorder, Chemical intolerance, Learning disabilities

P-134

THE GAP BETWEEN NEUROTOXICOLOGY AND PUBLIC POLICY: CASE STUDIES OF ENVIRONMENTAL TOXINS AND NEURODEVELOPMENTAL DISORDERS. Roger D. Masters, *Dartmouth College, Hanover, NH, USA*

It is increasingly evident that a dangerous gap exists between growing knowledge linking environmental toxins to neurodevelopmental disorders (illustrated by the presentations to this conference) and the reasoning involved in public policy debates in the United States. This presentation summarizes a number of cases illustrating how legislative and administrative decisions are divorced from theories and data linking neurotoxicology to human behavior. For example, sociologists and criminologists paid no attention to evidence linking lead to violent crime when confronting the sharp drop in U.S. rates of violent crime following 1991. Although annual sales of leaded gas were not correlated with the same year's violent crime rates, their correlation with violent crime rates 17–19 years later was consistently over $r = .90$ (with an even stronger relation to rates of violent crime by young offenders), suggesting prenatal exposure to fumes of leaded gasoline as a causal factor in violence. Contemporary policy implications of this finding for the use of manganese gasoline additives are paralleled by the failure to consider the effects of lead on cognition, ADHD, autism, or asthma. Similarly, debates over water treatment chemistry have continued to ignore evidence that some chemical compounds (such as H2SiF6 or Na2SiF6) enhance the uptake of lead as well as behavioral deficiencies linked to this neurotoxin. These failures to consider neurotoxicology and its applicability to policy decisions can be linked to the broader resistance of social scientists to developments in the life sciences, suggesting the need for new paradigms of research and the development of social science courses open to insights from biological fields including neurotoxicology. Keywords: Neurotoxins, Behavior, Public policy